DEEP BRAIN STIMULATION AND THE REGULATION OF AUTONOMIC FUNCTIONS, INCLUDING MICTURITION

Holly A. Roy

The Queen's College and Nuffield Department of Surgical Sciences

Supervisors

Mr. Alexander L Green
Dr. Ricarda AL Menke
Dr. Mkael Symmonds

ABBREVIATIONS

ACC Anterior cingulate cortex

ALE Activation likelihood estimate

ANS Autonomic nervous system

BOLD Blood oxygen level dependent

CAN Central autonomic network

$CMRO_2$ Cerebral metabolic rate of oxygen consumption

CSF Cerebrospinal fluid

CT Computed tomography

dACC Dorsal anterior cingulate cortex

DBS Deep brain stimulation

DTI Diffusion tensor imaging

FA Fractional anisotropy

FEV1 Forced expiratory volume in 1 second

FVC Forced vital capacity

EEG Electroencephalography

FMRIB Centre for Functional Magnetic Resonance Imaging of the Brain

HRF Haemodynamic response function

GPi Globus pallidus interna

LFP Local field potential

MD Mean diffusivity

MNI Montreal Neurological Institute

MRI Magnetic resonance imaging

MSA Multiple system atrophy

NAO Negative autonomic outcome

NIRS Near Infra Red Spectroscopy

NTS Nucleus of the solitary tract

PAO Positive autonomic outcome

PCC Posterior cingulate cortex

PAG Periaqueductal grey area

PD Parkinson's disease

PET Positron Emission Tomography

PMC Pontine micturition centre

PPN Pedunculopontine nucleus

PTSD Post-traumatic stress disorder

PVG Periventricular grey

SMA Supplementary motor area

SO Successful outcome

STN Subthalamic nucleus

UO Unsuccessful outcome

VAT Volume of activated tissue

VPL Ventral posterolateral thalamic nucleus

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PRIZES, PRESENTATIONS AND PUBLICATIONS ARISING FROM DPHIL

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"Subcortical local field potential characteristics during micturition and related tasks"
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"Modulation of autonomic nervous system function by deep brain stimulation of the subthalamic nucleus: comparison between outcome groups using a structural fingerprint technique"
Travel bursary awarded to the top ten UK submitted abstracts to the joint German-British Neurosurgical Meeting

Presentations

"Subcortical structural correlates of bladder dysfunction in Parkinson's disease"
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"Structural MRI correlates of autonomic dysfunction in Parkinson's disease."
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"Subcortical local field potential characteristics during micturition and related tasks"
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"Modulation of autonomic nervous system function by deep brain stimulation of the subthalamic nucleus: comparison between outcome groups using a structural fingerprint technique"
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Publications

Beta Oscillations and Urinary Voiding in Parkinson's Disease. **Roy HA**, Aziz TZ, FitzGerald JJ, Green AL. *Neurology* (in press)

Effects of Pedunculopontine Nucleus Stimulation on Human Bladder Function. **Roy HA**, Roy C, Pond D, Forrow B, Zrinzo L, Foltynie T, Akram H, Fitzgerald JJ, Aziz TZ, Green AL. *Neurourology and urodynamics* Jun 12. doi: 10.1002/nau.23321

State of the art: novel applications for deep brain stimulation. **Roy HA**, Green AL, Aziz TZ. *Neuromodulation*. 2017 May 17. doi: 10.1111/ner.12604.

Deep brain stimulation and multiple sclerosis: Therapeutic applications. **Roy HA**, Aziz TZ. *Multiple sclerosis and related disorders* (2014) 3: 431-439

EXTENDED ABSTRACT

Describing the anatomy and behaviour of neural structures that support physiological functions, and understanding their susceptibility to injury through disease processes, is one of the goals of neuroscience research today. The control of autonomic function, including micturition, involves the interaction of highly distributed neural systems to maintain homeostatic balance within the internal milieu of the body and to optimise survival within the hostile environment of the world. Autonomic control is relevant to many diseases of the peripheral and central nervous system and also appears to have implications for cognitive functions, both in the sense that impaired autonomic performance can result in temporary or permanent cognitive impairment due to poor perfusion of the brain, but also in the sense that autonomic input has a major role in our emotional processing and decision making. Better understanding the organisation of this system, then, will have relevance for clinical medicine as well as cognitive neuroscience and physiological science.

In this thesis I use three different approaches to better understand the organisation of autonomic and bladder control, all based loosely around the specific insights that can be gained from working with a certain clinical population (patients undergoing deep brain stimulation (DBS)). In the early chapters, I use brain imaging techniques to investigate correlations between white and grey matter structural changes and autonomic symptoms in patients with Parkinson's disease (PD) and other movement disorders. These analyses identify the left hippocampus as a key grey matter structure relevant to autonomic dysfunction in Parkinson's disease, and suggest that white matter structural changes associated with autonomic dysfunction are more widespread

and less specific than grey matter changes. In addition to this, I investigate whether connectivity to any particular brain regions is associated with improvements or deterioration in autonomic symptoms in PD patients following DBS surgery of the subthalamic nucleus. Significant regions are identified and discussed.

In the second main part of the thesis, the focus turns to the control of the urinary bladder. Here, I use local field potential (LFP) analysis to investigate the changes in oscillatory activity of small populations of neurons in the vicinity of implanted DBS electrodes associated with bladder related behaviours. In the first LFP chapter I describe correlations between oscillatory signatures in the basal ganglia and lower urinary tract symptoms in patients with Parkinson's disease, demonstrating that the beta oscillation may be linked to dysfunctional voiding in this patient population, and that voiding dysfunction is likely to be distinct from the other urinary problems experienced by Parkinson's disease sufferers. In the second LFP chapter I describe two case studies focussing on the role of alpha oscillations in the periaqueductal grey area (PAG) and ventral posterolateral thalamic nucleus (VPL) during bladder filling, urinary voiding and other bladder-related tasks.

Finally, moving to a more translational approach, in the last chapter I investigate the effect of deep brain stimulation of the pedunculopontine nucleus (PPN) on bladder filling capacity in a series of patients with Parkinson's disease, demonstrating that stimulation at this nucleus increases bladder filling capacity. This chapter is appropriately placed at the end of the thesis, to represent the hope that study of the neural control of the bladder, both at a structural level using MRI techniques, but also critically at a more local level using local field potential and allied

electrophysiological techniques, could lead to interventions such as neuromodulation that improve urinary symptoms in patients with bladder dysfunction.

CHAPTER 1: INTRODUCTION

The autonomic nervous system (ANS) is involved in the regulation of physiologic and homeostatic parameters relating particularly to the visceral organs and the co-ordination of physiological responses to threat. Blood pressure and heart rate, respiration, pupillomotor reactivity, sexual function, gastrointestinal secretions and motility, and urine storage and micturition are all under a degree of autonomic nervous system control. Furthermore, there is close integration between the autonomic nervous system and other neural functions such as emotion and cognition (Critchley and Garfinkel, (2015)), and thus brain regions that are known to be important for autonomic control are also implicated in emotional functions, such as the amygdala in fear conditioning and PTSD.

Many autonomic functions are maintained via peripheral nervous system feedback loops, often with a central nervous system component. Collectively, the network of brain areas involved in maintaining and regulating the autonomic nervous system is known as the 'central autonomic network' (CAN) and important progress in our understanding of the anatomy and function of the CAN in the human have been made in recent years due to developments in neuroimaging techniques (Hyam *et al*. (2012), Macey *et al*. (2016)). Modulation of activity within the CAN at specifically selected anatomic targets, using deep brain stimulation (DBS), offers a possible means of altering or modifying autonomic functions, and carefully observed studies of autonomic side effects arising from DBS for other indications, have made it possible to experimentally test the effects of DBS on the autonomic nervous system (Lovick (2014)). However, these results have to be interpreted in the light of the underlying

pathology of the patients in which the electrodes have been implanted- for example, many of the observations will be described here based on data from STN DBS in patients with PD- it is not clear whether STN DBS would have similar effects in healthy control subjects and it is highly likely that the reported effects are in some way modulated by the disease state. Nevertheless, emerging patterns of autonomic changes following DBS have implications for sufferers of a range of organ-specific cardiovascular, respiratory, gastrointestinal and genitourinary disorders that implicate ANS functioning, in addition to patients with primary dysautonomias, and conditions such as Parkinson's disease (PD) and multiple system atrophy (MSA) that are associated with dysautonomia. The scope is vast and for patients with severe and life-threatening or life-impairing disturbances of the autonomic nervous system, the risks of deep brain stimulation may be acceptable when weighed against the symptoms and implications of their condition.

The purpose of this thesis is to further develop scientific understanding about the interaction between DBS and the autonomic nervous system, first looking at autonomic nervous system function in general, and then examining more closely the role of DBS in the function of one particular system under a degree of autonomic control: the micturition system. In this introduction, I begin by describing the neural organisation of the central autonomic network, including insights from structural and functional imaging studies, and discuss evidence regarding the effect of DBS on the function of the autonomic nervous system. I then outline the main purpose of the first part of the thesis on general autonomic function. Following this, I then review the state of knowledge about the neural control of the bladder, including functional imaging studies but in this case using formal meta-analytic techniques to identify

statistically significant brain regions involved in bladder control based on the published functional MRI evidence to date. Using this as a basis, I then discuss the body of evidence concerning the effect of DBS on bladder control and subsequently outline the lines of investigation followed in this thesis. The focus on evidence from functional and structural neuroimaging studies is because I will use this modality as a tool in my own research described later.

Supraspinal neural organisation of autonomic control

Regulation of autonomic function occurs via the interaction of various brain structures, which contribute to the "central autonomic network" or CAN. Functions of the CAN include regulating the balance of sympathetic and parasympathetic activity at any one time. This regulation can occur as part of feedback loops that are specific to selected organ systems, or secondary to central command mechanisms. These central command mechanisms are not driven by end organ feedback but instead are generated by higher cortical areas, for example, as occurs in preparation for exercise (Basnayake *et al*. (2012)). Important subcortical nuclei include the nucleus of the solitary tract (NTS) in the medulla, which receives afferent input from the sympathetic and parasympathetic systems, and from baroreceptors and chemoreceptors. Brainstem sites that are important for autonomic outflow include the locus coeruleus, the parabrachial nucleus of the pons (Napadow *et al.* (2008), Loewy *et al*. (1991), and various nuclei within the medulla including sympathetic outflow pathways in the ventral medulla, the caudal and rostroventral medulla (Loewy *et al.* (1982), Gianaros *et al*. (2012) and parasympathetic outflow nuclei including the nucleus ambiguus and the dorsal motor nucleus (Gianaros *et al.* (2012)). The

interaction between midbrain, diencephalic and forebrain structures and the brainstem autonomic nuclei are extensive. The role of these regions has been extensively studied in animal recording and lesion studies as well as human cases of stroke and other lesions. Now, autonomic challenges can be applied during functional magnetic resonance imaging to study neural changes associated with autonomic activation in the human. Autonomic challenges include the Valsalva manoeuvere and handgrip challenges (Saito *et al*. 1986), forehead, foot or hand cold pressor tests (Macey *et al.* 2014) and end-expiratory breath hold challenges, which produce sympathetic excitation (Zubin Maslov *et al.* 2014). These tasks typically induce global changes in blood oxygenation and blood flow in addition to local changes related to increased or decreased neural activation, thus, the global changes are typically excluded from analyses as confounding factors (Macey *et al*. 2016).

The insular cortex is buried within the lateral sulcus, and is overlain by and intimately related to, the parietal opercula, whose gyri and sulci interdigitate with those of the insula (Ture *et al.* (1999)). The insula is involved in the higher response to autonomic challenges (Al-Otaibi *et al*. (2010), Macey *et al*. (2016), Critchley *et al*. (2015) Napadow *et al*. (2008)) and may affect autonomic outflow via connections to thalamic, limbic and brainstem regions, including the hypothalamus (Allen *et al*. (1991)). Certain fMRI paradigms, using a foot cold pressor challenge, handgrip task (both with predominantly sympathetic components) and Valsalva manoeuvre (activating both sympathetic and parasympathetic pathways) have demonstrated a topographical organisation of autonomic response within the insula, with an anteroposterior and lateralised differentiation of responses over the 5 gyri of the

insula and the two brain hemispheres (Macey *et al.* (2012)) and a unique pattern of activation associated with each task.

The cingulate cortex has also been established as a key region for autonomic response with anterior, mid and posterior cingulate cortex showing differential involvement. For example, posterior cingulate cortex activity has been associated with a withdrawal or reduction in parasympathetic activity (O'Connor *et al*. (2007), Wong *et al.* (2007)), while anterior cingulate cortex activity has been correlated with sympathetic nervous system activity using pupillary dilatation and skin conductance to infer sympathetic activation (Critchley *et al*. (2005), Nagai *et al*. (2004)). Stressor-evoked suppression of baroreflex sensitivity was found to co-vary with signal in the perigenual anterior cingulate cortex, dorsal anterior cingulate cortex and posterior cingulate cortex (Gianaros *et al*. (2012)), as well as producing increased functional connectivity between the perigenual ACC and the amygdala, periaqueductal grey area and pons.

The hippocampus and amygdala both respond to changes related to activation of the sympathetic nervous system, such as blood pressure challenges (Harper *et al*. (2000)) and tasks evoking effortful stress (Critchley *et al*. (2000)) and also have been shown to correlate with changes in a heart rate variability derived high frequency regressor, which is thought to provide an indication of parasympathetic output to the heart (Napadow *et al*. (2008)). Both amygdala and hippocampus have been activated in tasks involving the Valsalva manoeuver (Henderson *et al.* (2002), Harper *et al*. (1998)); the amygdala during hypoxic breathing challenge (Critchley *et al.* (2015)), and the hippocampus in tasks involving inspiratory resistance (Faull *et al*. (2016)). Interestingly, feedback from the autonomic nervous system modulates activation of

these regions in response to an emotional fear task: both amygdala and hippocampus demonstrated a significant main effect of cardiac cycle (systole/diastole) in their response to fearful stimuli (Garfinkel *et al*. (2014)).

Other cortical regions that have been implicated in autonomic function include the ventromedial prefrontal cortex (Veberne *et al*. (1997)), and sensorimotor cortices (pre- and post- central gyrus). Autonomic activation of sensorimotor cortex is difficult to dissociate from task based motor and sensory activation, however, Nowak *et al*. (1999) demonstrated increased activation of post-central gyrus with a hand grip exercise that persisted after the application of local anaesthesia, supposedly ruling out a direct sensory input to the region, and activation of the post-central gyrus has also been described during heart rate changes in a task designed to elicit baroreceptor activation alone independent of motor or cognitive effort by inducing baroreflex responses using lower body negative pressure (Kimmerly *et al*. (2005)). Ventromedial prefrontal activity has been show to vary with skin conductance level (Nagai *et al.* (2004)) and stimulation of muscle sensory afferents (Goswami *et al*. (2011)) and it has been suggested that its activity is related to hippocampal activation.

Finally, the cerebellum appears to have an important role in autonomic control. The cerebellum responds to blood pressure changes that occur in a variety of tasks including Valsalva (Henderson *et al*. (2002), Harper *et al*. (1998)), ventilatory challenges of 5% and 10% CO2 (Harper *et al*. (1998)), changes in minute ventilation (Critchley *et al*. 2015b), heart rate changes (Gray *et al.* (2007)) forehead and hand cold pressor tasks (Harper *et al*. (1998)), a moderate intensity handgrip task (Wong *et al*. (2007)), and emotional arousal (O'Connor *et al*. (2007). Activation of the

cerebellar vermis specifically has been reported during effortful tasks designed to elicit a stress response (Critchley *et al*. 2000) as well as emotional arousal (O'Connor *et al*. (2007)), moderate intensity handgrip (Wong *et al.* (2007)) and blood pressure increases (Critchley *et al*. (2015)).

Generalised autonomic changes following DBS

(i) Subthalamic nucleus (STN) DBS

Deep brain stimulation of the subthalamic nucleus (STN) in Parkinson's disease has been shown to have measurable effects on autonomic nervous system activity, both intra-operatively and following implantation. Thornton *et al*. (2002) showed that high frequency stimulation of the STN produces a physiological effect similar to that during exercise. According to a microstimulation study by Benedetti *et al.* (2004), consistent autonomic effects were achieved with dorsal STN stimulation regardless of whether the patient was aware of, or blinded to, the onset of stimulation. By contrast, stimulation of the ventral STN produced variable autonomic effects that were modulated by patient awareness of stimulation, and on three occasions, was accompanied by an additional emotional response. The authors hypothesise that the dorsal STN autonomic effect may be directly related to central command, while the ventral STN effects may be inter-related with emotional and associative responses via the limbic system. In one study, which investigated motor and non-motor sequelae during intra-operative exploration of the STN in subjects with PD (Sauleau *et al*. (2005)), autonomic effects were described at 19.6% of target points evaluated, occurring in 15 of the 17 patients included in the study. The authors described two

groups of symptoms which occurred in association with one another: (1) those of a subjective character, producing feelings of stress, anguish or anxiety; chest congestion, faintness or abdominal discomfort; and a sensation of warmth or cold, and (2) those of a more objective nature, including mydriasis, sweating and flushing, either unilateral or bilateral; and tachycardia and increased blood pressure. There was no lateralisation associated with these symptoms, however, they were commonly evoked when the stimulating electrode was well-placed for improving motor symptoms, and the effects increased with increasing voltage. The authors suggested that the effects could have been caused by current spread to local autonomic structures connected with the hypothalamus, such as the median forebrain bundle, zona incerta or Forel's fields; or by stimulation of the limbic subdivision of the STN, thought to be connected with other autonomic structures including the anterior cingulate cortex.

Similarly, studies in patients with PD to investigate post-operative effects of STN DBS on autonomic variables have demonstrated various autonomic effects resulting from STN stimulation. A study of 19 PD subjects found improvements in symptoms of dyshidrosis based on a semi-structured questionnaire, but no change in the sympathetic skin response recorded from the palm and the sole of the foot before and after DBS (Trachani *et al*. (2009). Halim *et al.* (2011) carried out structured questionnaires in 11 PD subjects with STN DBS. 3 out of 11 subjects reported improvements in autonomic symptoms following DBS; improvements in sweating were reported by all three subjects, urinary symptoms by two and bowel symptoms by one. All 3 subjects had early onset PD, while the 8 subjects who did not report autonomic improvement had late onset PD.

STN DBS has been shown to produce symptomatic improvements in gastrointestinal symptoms associated with PD, including constipation, sialorrhoea, dysphagia and difficulty with defecation, and also leads to normalisation of recorded electrophysiological signals as recorded by electrogastrography (Krygowszko-Wajs *et al.* (2016)), which may well be due to changes in the autonomic nervous system in general.

Although generalised autonomic changes appear to follow STN DBS, the evidence is less clear as to whether cardiovascular autonomic variables improve in patients who have had STN DBS. Holmberg *et al*. (2005) compared changes in cardiac autonomic reflexes over a 1-year period between a group of PD subjects receiving pharmacological treatment only and another group who had undergone STN DBS. However, they found no significant difference in the cardiovascular autonomic score change relative to baseline between the two groups, and moreover in the STN group, there was an increase in the number of patients displaying pathological responses to tilt testing. However, in a more recent study which compared heart rate variability during sleep in the 'OFF' and 'ON' stimulation states in 8 patients (Liu *et al*. (2013)), STN DBS significantly increased the sympathetic component of the autonomic response, with DBS 'ON' compared with DBS 'OFF'. Furthermore, cerebrovascular reactivity, which is thought to be linked to orthostatic hypotension and is reduced in PD as a result of impaired sympathetic innervation, was shown to improve with STN DBS (Ratsep *et al*. (2012)).

(ii) Periaqueductal grey area (PAG) DBS

The periaqueductal grey area is a horseshoe-shaped grey-matter structure, which encircles the cerebral aqueduct in the midbrain (Carrive & Morgan (2011)). It is arranged into longitudinal columns that represent functionally distinct regions (Bandler and Shipley (1994)). Stimulation of the lateral PAG with excitatory amino acids evokes defensive ('fight or flight') reactions associated with tachycardia, raised arterial blood pressure and distribution of peripheral blood flow towards the limbs (Depaulis *et al*. (1992)), while stimulation of the ventrolateral PAG column elicits quiescence, as would be expected after social defeat, associated with reduced arterial blood pressure and bradycardia (Zhang *et al*. (1990), Depaulis *et al*. (1994)). Similar effects have been reproduced in stimulation experiments in humans with implanted deep brain stimulation systems (Green *et al*. (2006)). While eliciting a passive coping response or a 'fight or flight' response is not necessarily desirable or useful therapeutically, the ability to reliably modulate blood pressure using PAG stimulation may have important therapeutic benefits for patients with refractory hypertension. Reduction in visual analogue pain score with rostral PAG DBS correlated with the reduction of blood pressure achieved by PAG stimulation during 9 six-minute testing sessions (Green *et al*. (2006)), and a case report describes a subject treated with PAG stimulation for chronic pain whose blood pressure dropped so significantly that his anti-hypertensive medications could be stopped (Patel *et al*. (2011)). PAG DBS has also been shown to oppose the postural drop in blood pressure associated with standing from sitting in patients with chronic pain and clinical orthostatic hypotension or orthostatic intolerance (Green *et al*. (2006)) and to modulate the balance of parasympathetic and sympathetic nervous system activity as measured by heart rate

variability (Green *et al*. (2010)).

Like cardiovascular function, the effects of STN DBS on respiratory function are also unclear. In a group of 10 subjects with STN DBS, there was a significant increase in peak expiratory flow rate with DBS turned on, which correlated with the overall energy product (a numerical value based on the product of pulse width, voltage and frequency of stimulation), with no effect of DBS on FEV1 or FVC (Hyam *et al*. (2012)). However, this positive effect on respiratory function should be compared with the findings of Chalif *et al.*, who found that patients with STN DBS reported symptoms of dyspnoea following chronic stimulation (Chalif *et al*. (2014)), suggesting that the long-term symptomatic changes following STN DBS may be different to the short term ON/OFF effects.

PAG DBS also has an effect on respiratory function. In a group of ten patients with PAG DBS, peak expiratory flow rate increased by a mean 13.4% with PAG stimulation ON vs. OFF (Hyam *et al.* (2012)), although there was no significant change in forced vital capacity (FVC) or FEV1/FVC ratio in the same group of subjects. Different anatomical columns of the PAG region are differentially involved in the response to respiratory challenge, with the lateral PAG responsible for the sensorimotor aspects of breathlessness and the ventrolateral PAG activated during the anticipation of a breathing challenge (Faull *et al.* (2016)). Column-specific targeting may therefore be necessary if PVG/PAG stimulation is to prove effective in the management of respiratory disorders such as asthma.

Structural imaging as a complement to functional imaging

Most of the evidence in humans that we have about the central autonomic network has been derived from functional MRI studies, as discussed earlier in this chapter. fMRI provides information about neural activation within grey matter brain regions and therefore allows researchers to define functional correlates of autonomic performance. However, brain structural analyses are important to complement functional studies, particularly in diseases such as Parkinson's disease, where changes in white or grey matter structure, either due to the primary pathology or secondary effects, can cause changes in functional responses. Non-invasive mapping of structural white matter connectivity has been made possible using diffusion tensor imaging (DTI) and tractography. Diffusion tensor imaging is carried out by modifying the standard T2 MRI sequence with the addition of diffusion-sensitizing gradients. The rate of water diffusion in the direction of the gradient can be quantified. If at least 6 diffusion sensitive data sets can be collected, this allows the diffusion tensor to be constructed. The diffusion tensor is a matrix of vectors which describes the degree of diffusion anisotropy in white matter tracts (see Mukherjee *et al.* (2008) for theoretic underpinnings). Various metrics derived from the diffusion tensor can be used to describe microstructural features of the white matter within a single voxel, including longitudinal diffusivity (the "primary eigenvector") (λ_1) which represents the rate of diffusion in the direction of the white matter fibres, and λ_2 and λ_3, the mean of which is radial diffusivity, and describes diffusion perpendicular to the fibre direction. Mean diffusivity (MD) provides an average of the eigenvectors and gives a measure of the total diffusivity of water within a voxel, while fractional anisotropy (FA) is an indicator of the directional diffusion within a voxel. These quantitative DTI metrics

can be used to identify white matter microstructural changes in diseases such as schizophrenia (Jones *et al.* (2006)), motor neuron disease (Menke *et al.* (2014)) and brain tumours (Roberts *et al*. 2005) and pinpoint the anatomical location of abnormalities (Menke *et al.* (2014)). Changes within the white matter can commonly occur in neurodegenerative processes and while conventional clinical imaging techniques are not able to detect subtle white matter abnormalities, diffusion tensor imaging (DTI) is sensitive to detect microstructural changes in white matter regions (DeBoy *et al.* (2007), Douaud *et al.* (2009)). Reduced FA and increased MD have been demonstrated in neurodegeneration (Pierpaoli *et al.* (1996)) and can result from axonal degradation or demyelination, (Beaulieu 2002). Reduced MD has been reported in pre-symptomatic familial Alzheimer's disease, where it was interpreted as reflecting early neuropathological changes such as microglial activation or neuronal and glial swelling, which could hinder diffusion in extracellular spaces (Ryan *et al*. (2013)). In addition, a rodent study of cerebral infarction directly related decreased MD to markers of acute cell injury (Tuor *et al*. (2014)).

The diffusion tensor can also be used to predict the course of white matter fibers using fibre tracking, based on the principle that the direction of the fibres within a given voxel is parallel to the primary eigenvector. When the propagated fibre track (or "streamline") reaches the boundary of its current voxel, the direction changes to the principle direction of the next voxel. User-determined constraints based on anatomical knowledge can be imposed to ensure that only realistic streamlines are built (Mukherjee *et al.* (2008)). Diffusion tensor imaging can be used to describe white matter tracts that propagate from any defined seed voxel and it is therefore useful in

the context of deep brain stimulation for mapping white matter connectivity with the DBS electrode (Owen *et al*. (2007); Boccard *et al*. (2016), Fernandes *et al*. (2015).

The implementation of advanced structural MRI techniques to understand autonomic and bladder symptoms has been slow and there are very few studies, particularly which relate autonomic or bladder disorders to changes within the white matter (Indelicato *et al*. (2015), Smith *et al.* (2015), Sakakibara *et al.* (2015), Wehrberger *et al*. (2014)). However, structural analysis holds promise in identifying mechanisms of disease, biomarkers and loci for intervention. Thus, in chapter 4, I focus on the use of quantitative structural MRI to evaluate white and grey matter correlates of bladder and autonomic dysfunction in PD.

Supraspinal neural control of the bladder: review of evidence and meta-analysis of fMRI evidence

The central nervous system plays a critical role in the regulation of the urinary bladder. Bladder control involves various autonomic, cognitive and sensorimotor operations, and establishing an understanding about the functional role of different brain regions in bladder circuitry, and how these regions interact with each other and with the peripheral nervous system, has been a key goal of neuro-urological research. Animal studies, using tract tracing, electrical stimulation, lesioning and neural recording techniques, were important in establishing the involvement of key subcortical brain areas in the processes of urinary storage and bladder voiding. These included the pontine micturition centre (PMC), (Loewy *et al.* (1979), Holstege *et al*. (1986), Blok *et al*. (1997)), pontine continence centre (Holstege *et al*. (1986),

Griffiths *et al*. (1990)), periaqueductal grey area (PAG) (Noto *et al*. (1991), Blok *et al*. (1995), Liu *et al*. (2004)), and the globus pallidus (Lewin & Porter (1965), Porter *et al.* (1971)). Early insights into bladder control networks in humans were inferred through studies of patients with brain lesions that impaired urinary function (Andrew & Nathan (1964)). Important areas identified by this approach include the frontal lobes in general (Maurice-Williams (1974)), and brainstem regions including the PAG (Yaguchi *et al*. (2004)). More recently, alterations in bladder function have been reported following implantation of deep brain stimulation (DBS) electrodes within the thalamus (Kessler *et al*. (2008), subthalamic nucleus (STN; Seif *et al*. (2004), Herzog *et al*. (2006), (2008)), internal globus pallidus (GPi) (Mordasini *et al*. (2015)), PAG (Green *et al*. (2012)) and pedunculopontine nucleus (PPN; Aviles-Olmos *et al.* 2011), implicating all of these regions in some aspect of human urinary control. However, both techniques have two main limitations: they are both limited to small regions of interest (either the site of the lesion or the site of the stimulating electrode) and neither technique yields direct insights about neural mechanisms for bladder control in healthy individuals.

Developments in functional brain imaging have facilitated a controlled, systematic, experimental whole-brain approach to investigate the neural control of the bladder in both healthy human subjects and those with bladder disorders. Functional imaging experiments are able to identify patterns of neural activation that occur in response to specific bladder-related tasks or states. Such ‘states’ include urinary voiding, bladder filling or the subjective experience of certain bladder-related sensations, such as urinary urgency. Although a range of functional imaging methodologies have been used including positron emission tomography (PET; e.g. Nour *et al*. (2000), Blok *et*

al. (1997), functional magnetic resonance imaging (fMRI; e.g. Griffiths *et al*. (2005), Mehnert *et al.* (2008)) and near infrared spectroscopy (Matsumoto *et al*. 2009), fMRI, as a relatively safe and non-invasive technique, with good spatiotemporal resolution, is rapidly becoming the dominant modality. Functional MRI is not a direct measure of neuronal activity, but instead uses the blood oxygen level dependent (BOLD) signal, to infer underlying neuronal activation. The increased BOLD signal following neuronal activation is thought to be a result of changes in intravascular magnetic susceptibility weighting due to increased oxygenated haemoglobin travelling to areas of activity (Ogawa *et al*. (1990)). However, changes in the BOLD signal can be produced by other factors that change intravascular magnetic susceptibility, such as changes in cerebral blood flow and cerebral blood volume (Logothetis *et al*. (2001); Shmuel *et al*. (2008)) and these must be taken into account during data analysis and interpretation.

The most common type of paradigm used for brain-bladder fMRI is an 'infusion withdrawal' paradigm (see Griffiths *et al*. 2005), whereby many repetitions of infusion and withdrawal of small volumes of fluid into the bladder are carried out, usually at a relatively empty bladder and a relatively full bladder (in many cases, the bladder is filled to provoke the sensation of urgency). The contrast (infusion-withdrawal) is averaged over multiple trials, due to the inherently low signal-to-noise of fMRI, and is thought to reveal brain activations associated with bladder filling or the experience of urinary urgency (depending on the exact details of the trial). Other approaches using fMRI to study brain-bladder activity include measuring the BOLD signal during or immediately preceding urinary voiding (Krhut *et al.* (2012), Shy *et al*. (2014) and, more recently, the use of resting state fMRI (Nardos *et al*. 2014).

A great deal of novel information about important activations and deactivations associated with bladder state and bladder sensation in healthy subjects and those with different bladder diseases has been provided by fMRI studies. However, participant numbers within individual studies are typically low due to the fact that most task-based fMRI studies require the subject to be catheterised and are therefore difficult to recruit to. The low participant numbers raises questions about the generalizability of individual study findings. At the time of writing this chapter, there has been no quantitative meta-analytic approach applied to multiple datasets to generate a more robust estimate of key brain-bladder control areas. Generating such estimates is of relevance clinically, to provide some preliminary guidance to those who may be treating or diagnosing conditions affecting brain regions putatively implicated in bladder control, and also for researchers within the brain-bladder neuroimaging field, in order to effectively guide region-of-interest analyses of functional or structural imaging data.

As part of the introduction to this thesis, and to set the stage for further investigations regarding the neural control of the bladder, I performed a meta-analysis of functional brain imaging studies using MRI to elucidate urinary control networks. I selected only task-based fMRI experiments and included data collected from both healthy subjects and those with bladder dysfunction. The primary aim was to identify areas of consistent, statistically robust, activation across studies, which therefore represent reliable targets for further investigation and could be labelled in a putative "bladder control map" of significance to clinicians. A secondary aim was to assess the differences in brain activation associated with bladder filling and urinary voiding

tasks. Although my thesis does not include fMRI experiments, fMRI is currently the dominant technique in the field and by summarising what has been shown using this modality, I felt that I would be in the best position to plan my own experiments in such a way as to incorporate existing knowledge and complement these current findings. Thus final aim of the meta-analysis, relevant to this thesis, was to identify regions of the brain that appear to be important for bladder control in order to aid the interpretation of results from DTI and LFP analyses which are described later in the thesis.

Primary literature search

A literature search was carried out using Scopus and Web of Science (including Medline and Pubmed). I specified that included papers must have been published between 1st January 2000 and 22nd March 2016, to improve consistency of imaging methodology. In order to identify relevant task-related fMRI studies of bladder control, combinations of four medical subject heading terms were used ("bladder", "micturition", "voiding", "continence") and two key words ("fMRI", "neuroimag*"). The total number of records obtained was 1417, with 951 remaining after removal of duplicates. 71 studies were selected after screening for eligibility and after accessing the full text article, 10 studies met the eligibility criteria (figure 1.1). These 10 studies were used for the meta-analysis. Studies presenting novel analysis of data that had been published in a previous article were not eligible for inclusion; only the results of the original analyses were included.

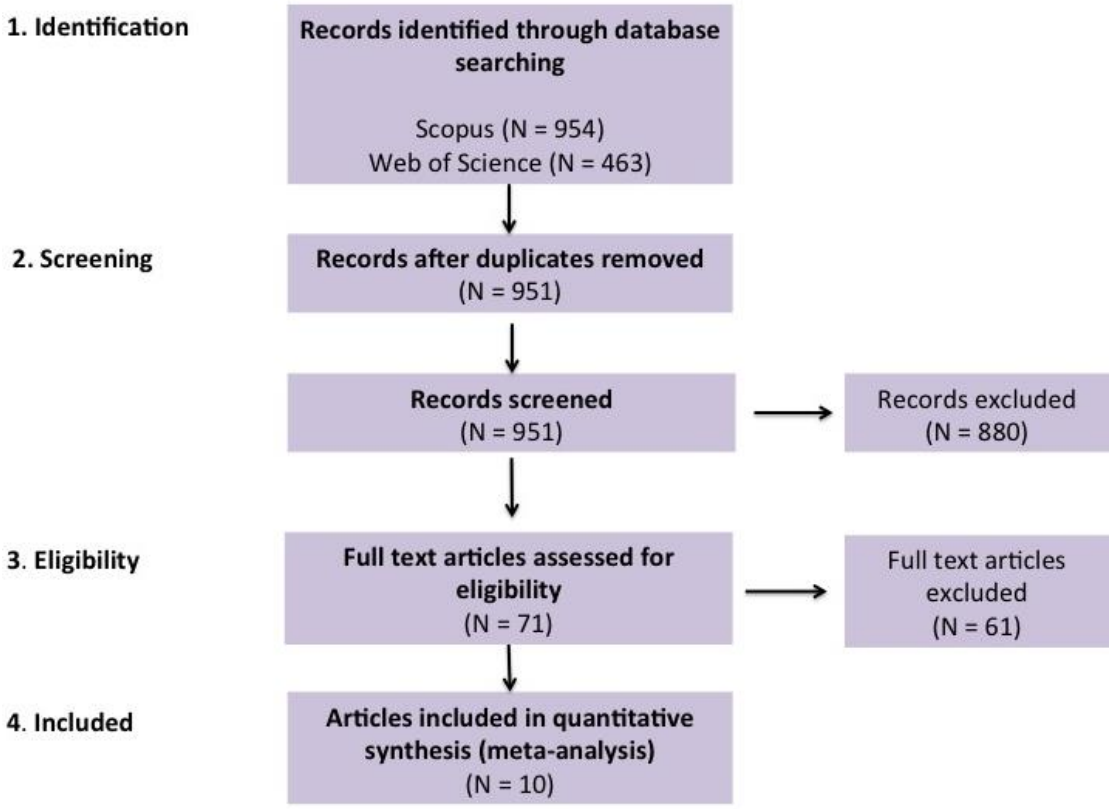


Figure 1.1. PRISMA flow-chart of the literature search and selection process.

Eligibility criteria

Eligibility criteria were as follows: (1) studies were original reports of task-dependent fMRI; (2) the neuroimaging task involved bladder filling or urinary voiding; (3) stereotactic coordinates of neural activation were made available (in Talairach or Montreal Neurological Institute, MNI, coordinates). Authors were contacted to request data if the co-ordinates were not published. Studies were rejected based upon the following exclusion criteria: (1) review articles only, containing no novel experimental results; (2) data from the same subjects were reported in another paper already included in this review; (3) assessment of pelvic floor muscle contraction only; (4) PET or NIRS experiments (5) experiments depended upon thinking about or

imagining bladder filling or voiding only without any physiological change in bladder state imposed by the task.

Data identification

For eligible papers, the following information was identified in the paper: (1) number of subjects used in the final analysis or analysis of interest, (2) whether study participants were healthy subjects or had a bladder control disorder, and what disorder this was, (3) the nature of the fMRI task (filling or voiding), (4) whether study participants were male or female, or whether both male and female participants were included in a single study. In studies in which there were further tasks in addition to the baseline bladder paradigm (such as neuromodulation or pelvic floor contractions) only data from conditions which examined exclusively bladder-related tasks were extracted.

Analysis

Foci that were reported as reaching statistical significance were recorded from each study. If required, foci were converted from Talairach co-ordinates to MNI space using the Lancaster transform (icbm2tal) in GingerALE (www.brainmap.org/). The Lancaster transform is recommended over the Brett transform because it has been shown to reduce the MNI/Talairach co-ordinate bias associated with reference frame and scale. An activation likelihood estimation approach was used to carry out the meta-analyses. This analysis was performed using the GingerALE tool via BrainMap (ALE 2.3.5; http://www.brainmap.org; Eickhoff *et al*. (2009)). GingerALE is a meta-

analysis tool which determines anatomical concordance between fMRI studies by comparing spatial co-ordinates extracted from each study. The co-ordinates from each study are then weighted according to the number of subjects in the study and used to determine the likelihood of activation of each voxel during bladder related tasks, assigning an activation likelihood estimate (ALE) score to each voxel. Permutation testing using cluster level inference ($p < 0.05$ (FWE)) can then employed to determine the statistical significance of ALE scores. Interpretation of small volume clusters should be carried out with caution as no minimum cluster size of small volume clusters was set. All analyses were whole-brain only.

Two main meta-analyses were performed: firstly using all eligible studies, and secondly running the analysis for "bladder filling studies" and "voiding studies" separately, with and without data obtained from subjects with bladder dysfunction. There were not enough eligible studies identified by the literature search to perform a direct contrast between filling and voiding studies.

Outcome of literature search and analysis

Primary literature

There were 10 task-based fMRI studies included in the meta-analysis with a total of 140 subjects and 156 foci (see table 1.1). Of these, 101 subjects were healthy and contributed 82 foci, while 39 patients had a disease or disorder affecting bladder function, representing 74 foci. The vast majority of subjects were female (136 female,

20 male) and the majority of studies (7 out of 10) were based on a bladder filling paradigm (91 subjects, 108 foci).

Authors	Date	Task paradigm	Disease/healthy (+ disease type if applicable)	Number of subjects and sex
Shy et al.	2014	Voiding	Healthy	10, F
Krhut et al.	2014	Bladder filling	Healthy	23, F
Tadic et al.	2013	Bladder filling	Healthy	11, F
Krhut et al.	2012	Voiding	Healthy	6, F
Tadic et al.	2010	Bladder filling	Disease, urge urinary incontinence	14, F
Kavia et al.	2010	Bladder filling	Disease, Fowler's syndrome	6, F
Kuhtz-Buschbeck et al.*	2009	Full bladder, conscious evocation or suppression of bladder	Healthy[1]	17 F, 16 M

[1] * included in whole group analysis but not in the filling/voiding sub-analysis as it is not strictly a filling or a voiding study

		sensation		
Griffiths et al.	2007	Bladder filling	Healthy and disease (urge urinary incontinence)	21 F (10 healthy, 11 with bladder disease)
Zemplini et al.	2010	Bladder filling	Disease (incomplete spinal cord injury)	4 F, 4 M
Mehnert et al.	2008	Bladder filling	Healthy	8 F

Table 1.1. All studies included in final meta-analysis

Bladder fMRI: all studies

The meta-analysis including all bladder tasks in all subjects (healthy and patients) revealed 4 clusters of activation, including the right and left supplementary motor area, the right mid cingulate gyrus, the right inferior frontal operculum, the left insula and the cerebellar vermis (see figure 1.2 and table 1.2).

Cluster number	Volume (mm^3)	ALE	MNI co-ordinates			Side, region
			X	Y	Z	
1	3952	0.01787	4	22	46	Right supplementary

						motor area
		0.01231	-6	12	38	Left supplementary motor area
		0.0945	0	4	44	Right supplementary motor area
		0.00781	8	18	32	Right mid cingulate gyrus
2	2416	0.01745	40	12	4	Right inferior frontal operculum
3	1432	0.01611	-38	12	2	Left insula
4	760	0.0097	2	-68	-22	Cerebellar vermis VI

Table 1.2: activations shown in meta-analysis of all bladder fMRI studies in all subjects, where ALE represents the estimate of the activation likelihood.

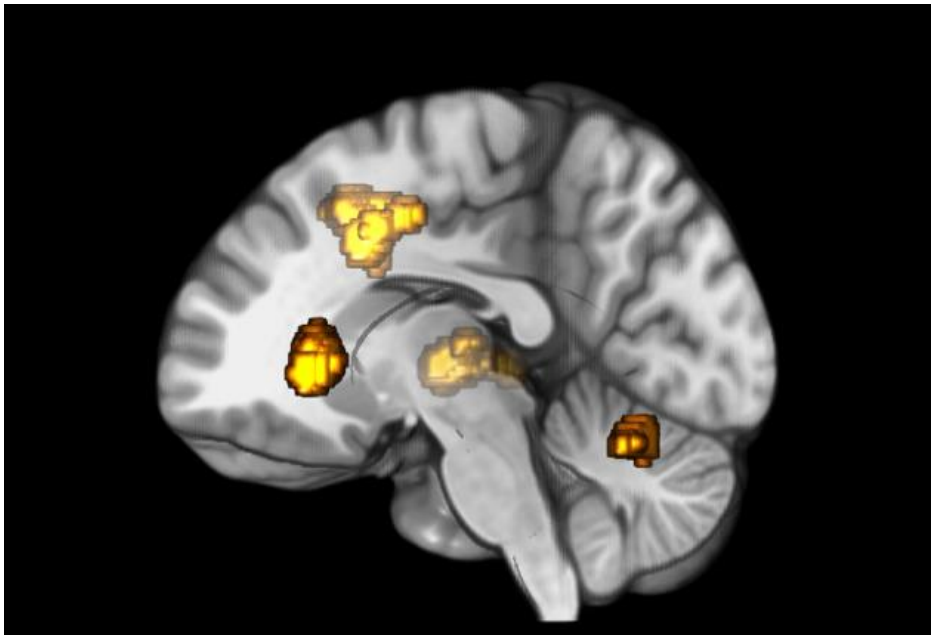


Figure 1.2: Regions of significant activation related to bladder-related tasks in all subjects.

Filling only studies

Including only bladder filling/withdrawal studies revealed 5 activation clusters including the right inferior frontal operculum and left insula, the left olfactory cortex, right supplementary motor area and cerebellar vermis (table 1.3, figure 1.3A). Excluding any datasets obtained from subjects with bladder disorders, bladder filling elicited activity in the right frontal inferior operculum, left superior parietal and paracentral cortex, left olfactory cortex, bilateral thalamus and bilateral midbrain (table 1.4, figure 1.3B).

Cluster number	Volume (mm^3)	ALE	MNI co-ordinates			Side, region
			X	Y	Z	
1	3152	0.01744	40	12	4	Right inferior frontal operculum
2	1336	0.0097	2	-68	-22	Cerebellar vermis VI
3	1264	0.0139	-38	12	2	Left insula
4	1152	0.01112	2	16	44	Right supplementary motor area
5	576	0.00926	-6	20	-10	Left olfactory cortex

Table 1.3: activations shown in meta-analysis including only bladder filling studies, for all subjects (healthy and "bladder disorder", in analysis), where ALE represents the estimate of the activation likelihood;

Cluster number	Volume (mm^3)	ALE	MNI co-ordinates			Side, region
			X	Y	Z	
1	1008	0.00967	40	14	4	Right frontal inferior operculum
2	480	0.00841	-5	20	-10	Left olfactory lobe
3	480	0.00841	-14	-20	-9	Left midbrain
4	480	0.00841	14	-20	-9	Right midbrain
5	480	0.00841	-10	-16	3	Left thalamus
6	480	0.00841	10	-16	3	Right thalamus
7	480	0.00841	-25	-60	52	Left superior parietal lobule
8	448	0.00814	-4	-34	60	Left paracentral lobule

Table 1.4: activations shown through meta-analysis including only bladder filling studies, for healthy subjects only, where ALE represents the estimate of the activation likelihood;

Voiding only studies

When the analysis was re-run with voiding studies only (all subjects were healthy), significant activations occurred bilaterally in the anterior cingulate cortex and

supplementary motor area, with further activation in the left orbital and left frontal cortex (table 1.5, figure 1.3C).

Cluster number	Volume (mm^3)	ALE	MNI co-ordinates			Side, region
			X	Y	Z	
1	920	0.00639	-16	16	36	Left superior frontal cortex
			-8	14	38	Left supplementary motor area
2	512	0.0049557	-10	37	-6	Left anterior cingulate cortex
		0.00484	-11	43	-12	Left middle orbitofrontal cortex
3	512	0.00496	10	37	-6	Right anterior cingulate
		0.00484	11	43	-12	Right anterior cingulate
4	512	0.00658	8	22	44	Right supplementary motor area

Table 1.5: activations including only voiding studies, healthy subjects only (which is all the voiding studies), in the analysis

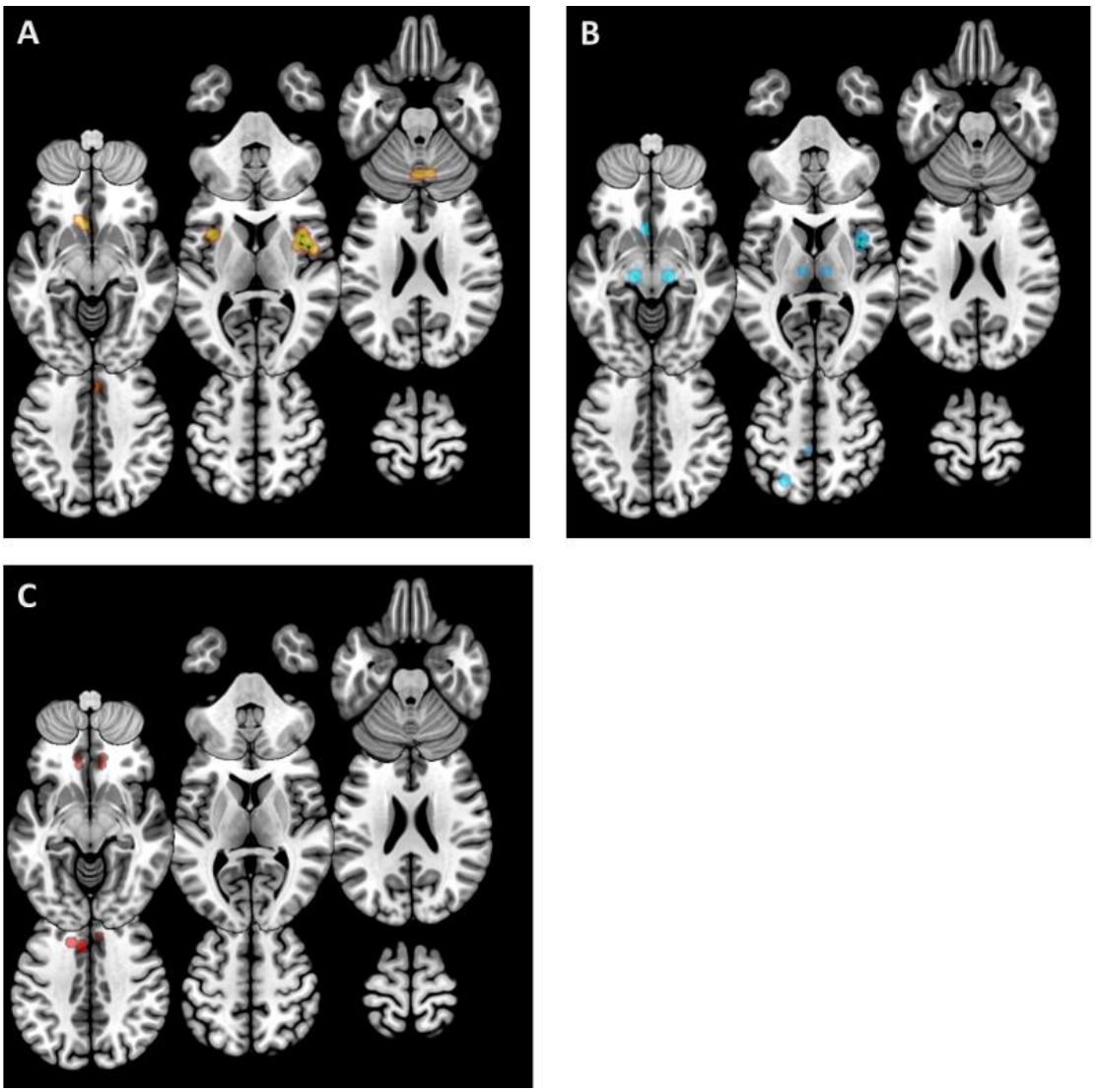


Figure 1.3. All brain images are shown in axial section with significant activations in colour ($p < 0.05$ (FWE)). Sections have been chosen to best represent the activations. A) Brain regions with significant activation related to bladder filling (red-yellow). All subjects, including healthy subjects and those with "bladder disorders" are included. B) Brain regions of significant activation related to bladder filling in healthy subjects only with normal bladder function (pale blue). C) Regions of significant activation related to voiding in healthy subjects with normal bladder function (red).

Discussion of findings

All-task, all-subject meta-analysis

Meta-analysis of whole brain studies across all bladder related tasks revealed significant task-correlated changes in BOLD signal in the bilateral supplementary motor area (SMA), left insula, right mid-cingulate cortex, right inferior frontal operculum and cerebellar vermis (table 1.2). This pattern of activation associated with bladder-related tasks is generally supported by the results of PET studies during bladder filling (e.g. Blok *et al*. (1997), Athwal *et al.* (2001), Herzog *et al*. (2008)), however, activation of the insula, cingulate cortex, frontal cortex, SMA and cerebellar vermis has also been reported during pelvic floor contract/relax tasks in subjects with a full bladder (Seseke *et al*. (2006), Seseke *et al.* (2008)), so it is not entirely clear whether their activity is related to sensations arising from a full bladder, activity of muscles involved in maintenance of continence or a combination of the two. Furthermore, activity in the cingulate, cerebellum and medial frontal gyrus has been associated with urinary voiding in fMRI experiments (Shy *et al*. (2014)). In an attempt to disentangle responses to bladder filling from activations related to urinary voiding, we next analysed data from these two different paradigm types separately. Regarding the SMA, an "SMA syndrome" has been described, which typically includes contralateral hemiparesis or motor apraxia with or without a speech deficit (Abel *et al*. (2015)), initially described by Laplace (Laplace (1977)). Bladder dysfunction is not specifically mentioned in this patient group, implying that SMA lesions alone are not sufficient to impair bladder control, despite a possible role for

the SMA in sensorimotor aspects of bladder awareness and voiding. Connections between the SMA and the basal ganglia and cerebellum (via pontine nuclei) could provide pathways for its involvement.

Bladder filling

The functional response to bladder filling was identified first for "all subjects" (table 1.3, figure 1.2A) and then for "healthy subjects only" (table 1.4, figure 1.2B). The "all subject" analysis revealed significant filling related changes in signal within the left insula, the right inferior frontal operculum, the right SMA and the cerebellum. A recent paper proposed that brain control of the bladder could be summarised in terms of 3 separate 'circuits' of brain areas, which converge on a central hub (Griffiths *et al*. (2015)). Circuit 1 involves the thalamus, insula and lateral prefrontal cortex (connecting to the medial prefrontal cortex which regulates voiding) and is modelled as the normal circuitry for handling bladder sensation. Circuit 2 involves the dorsal anterior cingulate cortex (dACC) and supplementary motor area (SMA), important in creating a sensation of urgency and controlling pelvic floor muscles to prevent accidental urinary leakage in patients with urge urinary incontinence. Circuit 3 is thought to involve the parahippocampal gyrus although its precise functional role is not described in detail. The insula is thought to be the primary cortical area for the representation of visceral interoception, receiving afferent information from Aδ and C fibres and creating a subjective awareness of an individual's homeostatic state (Craig *et al*. (2003). The insula or the nearby frontal inferior operculum, was significantly activated in response to bladder filling for the whole group analysis and healthy

subjects only (though only bilaterally activated in the whole group analysis). It is likely that there are subtle differences in insular activation between subjects who have bladder disorders and those with normal bladder function, but it is clear that this area performs a role in both groups. It also is interesting to note that bladder filling (both in the whole group analysis and the healthy subjects only) activated the left olfactory (or piriform) cortex. This cortical region is closely linked anatomically to the amygdala and parahippocampal gyrus and its activation supports the proposed involvement of the parahippocampal gyrus and associated regions in some aspects of bladder control and highlights it as an area for further investigation. Although the cerebellum does not form part of the model proposed by Griffiths *et al.*, we found significant cerebellar activation in our "all subject" analysis. Cerebellar activation is frequently reported during bladder filling in PET and fMRI (e.g. Athwal *et al*. (2001), Matsuura *et al*. (2002), Nour *et al*. (2000)) and it is thought functionally to be part of a network which includes the anterior insula and cingulate cortex, along with other frontal and parietal regions, and has a possible attentional role (Jarrahi *et al*. (2015)). Thus, its activation in response to bladder filling in the "all subjects" group is unsurprising.

Subcortical regions including the thalamus and midbrain, which are known to be implicated in bladder control through other-modality studies (see Griffiths & Fowler (2013) for a review) were identified in the bladder-filling analysis in healthy subjects only. This suggests that in certain subjects with bladder disorders, the normal subcortical networks responsible for bladder control may be impaired. Typically, brain-bladder imaging studies have focused on cortical regions, however, an in-depth study of subcortical responses to bladder filling in healthy subjects and those with neuro-urological disorders could yield important insights into disease

pathophysiology. Furthermore, significant cerebellar and SMA activation were *not* apparent in the "healthy subject only" analysis raising the possibility that the involvement of these areas might play a more prominent role in urinary control in subjects with bladder disorders than those with normal bladder function.

Urinary voiding

Voiding-related activity occurred predominantly within the bilateral cingulate cortex and bilateral medial frontal cortex (i.e. SMA), table 1.5, figure 1.2C. The left superior frontal cortex and the left middle orbitofrontal cortex also emerged as having significant changes in activity related to voiding tasks. Involvement of the cingulate cortex fits with the view that the ACC is the brain's homeostatic motor area (Craig *et al.* 2003), regulating motor and autonomic responses to maintain internal balance (Critchley *et al*. 2003), including, perhaps, regulating urinary voiding when necessary. The SMA is important for pelvic floor muscle control (Seseke *et al.* (2006), Zhang *et al*. (2005)) and as it is primarily involved in motor planning, it may have a role in preparing for and co-ordinating the various motor changes associated with voiding. The orbitofrontal cortex is generally associated with processing of pleasure (Berridge & Kringelbach (2015)), which might in this case be related to the pleasant sensation of bladder voiding. These results are also consistent with the three circuit model proposed by Griffiths *et al*. (2015), where circuit one is involved in the normal response to bladder filling (and therefore not substantially involved in the voiding process), whereas circuit two (SMA/ACC) is activated during bladder filling in

subjects with urge urinary incontinence because these areas are linked to the motor control of the pelvic floor.

The consistency of the imaging data, even from a small number of studies, such that significant clusters have emerged from the meta-analysis, shows that fMRI during bladder-tasks produces reproducible brain effects, engaging both cortical and subcortical regions. fMRI has provided the first significant opportunity to gain spatiotemporally specific insights into the functional anatomy of neural bladder control networks in healthy subjects, and to visualise neural networks for bladder control at a whole brain level. The benefits of this technique, along with reproducibility of findings, makes it clear that further developments should be pursued using this modality, particularly to contrast healthy and "bladder dysfunction" patients in a single study with the aim of gaining clinically relevant insights into the mechanism of neuro-urological disorders.

Limitations of the meta-analysis

The main limitations of this study are the relatively small number of brain-bladder fMRI studies and the small sample sizes in those studies that do exist (the mean sample size in the studies included here is 14). Moreover, the task paradigm varied between studies, and the 'bladder disorder' group was very heterogeneous. Low numbers are likely to be in part due to invasive nature of the task paradigms and therefore difficulty recruiting subjects, but also to other factors, including the technically challenging nature of experiments, and perhaps also a general stigma associated with the study of incontinence due to fear of contamination of MRI

equipment or inconvenience to other MRI users. We note that in this field, resting state fMRI may offer an acceptable non-invasive alternative to task based fMRI, and that early resting state fMRI studies have been described (Nardos *et al*. (2014), Kilpatrick *et al*. (2014)) and could be included in a future meta-analysis.

General limitations of fMRI methodology in understanding the neural basis of bladder control & discussion of alternative techniques

There are a number of general limitations of functional MRI in the evaluation of the central control of the bladder. fMRI attempts to investigate brain activity correlated with physiological or cognitive function in an artificial, non-naturalistic setting (Drake & Tannenbaum (2010)). This may be particularly problematic in the investigation of the neural control of the bladder because many postural and environmental features involved in normal voiding or bladder storage are absent from the experimental paradigm. Furthermore, the low signal-to-noise necessitates multiple repetitions of a given task, again making the experimental conditions less similar to real-life scenarios and also more difficult for the subject to tolerate. Combining modalities, for example, EEG and fMRI, may allow fMRI & EEG data collected in the scanner to be referenced against ambulatory EEG signals, allowing us to evaluate to what extent the fMRI signal is representative of brain activity associated with naturalistic urinary control.

There are also a number of limitations inherent to the fMRI technique itself, not specific to brain-bladder imaging. fMRI is an indirect measure of neuronal activity, and is thought to more closely reflect the inputs and processing within an area than

the spiking outputs from the area (Logothetis *et al*. (2001), Viswanathan & Freeman (2007)). The BOLD signal influenced by multiple factors including the cerebral metabolic rate of oxygen consumption ($CMRO_2$), as well as cerebral blood flow and cerebral blood volume, which can complicate the interpretation of results (Shmuel *et al.* (2008), Magri *et al*. (2012)). Various other physiological and non-physiological factors can also lead to signal changes, including subject movement, scanner noise, thermal noise, changes in end tidal CO_2 (due to the vasodilatory influence of CO_2) and movement of the chest during respiration (Birn *et al*. 2006). The influence of cardiac and respiratory noise is particularly marked in the brainstem, where changes in blood and CSF flow during the cardiac cycle have a large effect (Van de Moortele *et al*. 2002, Friese *et al*. 2004). Moreover, although not strictly a limitation, there is a characteristic dip in the BOLD response immediately after stimulus presentation, followed by an increase to a peak at about 6 seconds. This is known as the haemodynamic response function (HRF) and should be taken into account when interpreting fMRI data.

Local field potentials as an alternative to fMRI for investigating the neural control of the bladder

The local field potential (LFP) is a weighted neural signal typically recorded from an intracranial extracellular electrode. It represents the averaged contribution of a neuronal population within 1-2 mm of the electrode tip (Logothetis *et al*. (2001)) including events such as voltage-gated membrane oscillations, population synaptic potentials and synchronised dendro-somatic activity (Magri *et al*. 2012, Belitski *et al*. 2008). Unlike multi-unit activity (MUA), which is high frequency (400-3000 Hz) and

provides information about neuronal outputs, the LFP reflects input information and local integrative processing signals and is more closely related to the BOLD signal (Logothetis *et al*. (2001)). For example, it has been suggested that dendritic membrane potential changes that do not necessarily result in neuronal firing but reflect subthreshold information processing which may be responsible for generating the beta-frequency LFP signal that is characteristic of Parkinson's disease (Weinberger *et al*. (2006)). Sustained LFP changes that correspond to stimulus duration are typical, whereas MUA tends to respond to stimulus onset and then die away (Logothetis *et al*. (2001)). LFPs have a better temporal resolution than fMRI, and although they can only provide information about a single region of interest in the vicinity of the electrode, unlike fMRI which can generate whole brain spatial maps, resolution of activity within small nuclei and those which are difficult to image by fMRI is possible. LFP activity is normally viewed and interpreted in the frequency domain. Frequency bands have traditionally been classified as delta, (2-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (15-30 Hz), low gamma (30-80 Hz), and high gamma (80-150 Hz). Although the boundary between bands is not precise, the grouping is not entirely arbitrary and reflects features of neural oscillatory dynamics. At the same time, it should be kept in mind that the classification is not based on any absolute factor or physiological variable. Different frequency bands of the LFP signal have been shown to correlate with various behavioural or cognitive states and in some cases may also represent a pathophysiological signature, such as the beta band in Parkinson's disease (Oswal *et al*. (2013). Nevertheless, some analysis of LFP signals does not take into account these partitions and analyses the signal at the level of each individual frequency (e.g Belitski *et al*. (2008)). The functional significance of different frequency bands may vary according to brain region. For example, in the

monkey visual cortex, low frequency (1-5 Hz) and high frequency (72-76 Hz), but not mid-frequency (28-32 Hz) waveforms were informative about the visual stimulus. In the globus pallidus, gamma band (60-80 Hz) power correlates with contralateral hand movement amplitude and velocity (Brucke *et al*. (2012)) and STN theta- and beta-band oscillations have been linked with response inhibition (Zavala *et al*. (2015)) while gamma oscillations may be related to motor effort (Tan *et al.* (2013), (2015)). Thus, at present, LFP frequency domain correlates of function tend to be nucleus specific, though this may change as our understanding of LFPs develop and further recording sites are sampled. Interestingly, spontaneous fluctuations in the BOLD signal have been shown to correlate with neuronal activity in the gamma band and higher frequencies (Shmuel *et al*. 2008). Magri *et al*. (2012) found that LFP signal in the gamma frequency band was the most informative about the BOLD signal, followed by beta and alpha frequency bands (Magri *et al*. 2012). In this study, Magri *et al*. described a positive correlation for all bands, meaning that an increase in LFP power correlated with an increase in the BOLD signal.

There have been no studies to investigate the LFP signature associated with bladder filling or voiding. Indeed, most LFP studies focus on cognitive or motor tasks; very few if any investigate visceral sensation or the function of visceral organs (certainly, it has not been possible to identify any). Investigating the changes in the LFP profile during bladder related tasks is likely to be useful from a number of different angles. Firstly, it will be informative about the role of specific deep nuclei in the control of the bladder, complementing what is already known through functional imaging. Secondly, it may reveal signals that could be used as input for a brain machine interface system for voiding or continence- such as signals associated with the strong

urge to void or a full bladder. Finally, studying the LFP associated with bladder control in specific disease states could help to understand the mechanism of bladder dysfunction in the condition in question, as the LFP may reflect certain pathophysiological features. In chapter 5 and 6 of this thesis I explore the use of LFP recordings to investigate bladder control in human subjects.

DBS studies as the bridge between neuroimaging and neurotherapeutics

Brain stimulation experiments using DBS are in some ways the ultimate test of a brain region's functional role. Insights from neuroimaging and LFP studies are of greatest practical value if they can be utilised to identify sites for intervention to modify performance within a given neural system, and if a region is identified as active during fMRI of a particular task, but stimulation of the brain region does not elicit task-related responses, the interpretation of the fMRI experiment should be questioned. There is a growing literature describing the effects of DBS on bladder function, adding new insights to the knowledge derived from fMRI, as well as raising further questions and highlighting therapeutic opportunities.

As with LFPs, DBS stimulation data provides insights about the role of a highly specific brain area on bladder function, and can be complementary to whole-brain functional neuroimaging. Furthermore, while fMRI studies are excellent at identifying activity within cortical regions, DBS targets are often subcortical. There is a surprisingly rich literature regarding the effects of DBS on bladder function, and this holds great potential for exploitation as a tool to treat incontinence or enable the development of brain machine interfaces for bladder control in spinal cord injury and

other related conditions, particularly if DBS stimulation results are interpreted in the light of an overall understanding of the central control of voiding as derived from neuroimaging and neurophysiology.

Improvements in lower urinary tract symptoms in PD patients after STN DBS have been shown in a number of studies. In a small study with five participants STN DBS was shown to increase bladder volume at normal desire to void, overall bladder capacity, and the bladder volume threshold for detrusor overactivity (Finazzi-Agro *et al*. 2003). The majority of subsequent studies corroborate this early observation, finding that STN stimulation in human subjects with PD, at clinically therapeutic stimulation parameters, increases sensory thresholds during bladder filling (Seif *et al*. (2004); Herzog *et al*. (2006), Herzog *et al*. (2008)), facilitating a larger bladder capacity and enabling greater volume to be held in the bladder before the onset of detrusor overactivity. At an extreme end of the spectrum, two patients were reported to have gone into urinary retention after STN DBS implantation (Fritsche *et al*. (2009)). The only exception is a report by Winge *et al*. (2007), where no urodynamic changes were reported with STN DBS ON compared with STN DBS OFF, and the reason for this difference is not clear, although it may be that differences stimulation parameters or electrode location (not published in all studies), or patient factors, influenced the results. A PET study demonstrated that STN DBS appeared to modulate afferent bladder information transmission from the periaqueductal grey area to the posterior thalamus and this was proposed as a possible mechanism of action for the improved urinary storage following STN DBS (Herzog *et al*. (2008)).

Other DBS targets for bladder control have also been investigated. The PAG, which is well recognised to be an important link in the brain-bladder system, receiving afferent information about bladder fullness and contextual information from forebrain regions, may act as a switch to change between voiding and storage modes (see Griffiths & Fowler 2013 for a review). In the human, it has been shown that DBS of the PAG increases maximal bladder capacity but not bladder volumes at earlier stages of filling, consistent with the theory that PAG activity is critical at the storage-voiding switch (Green *et al*. (2012)). Perhaps for patients with urgency incontinence, a DBS system which stimulated this region intermittently, when subjects experienced a sensation of urinary urgency, could prevent leakage and allow the patient enough time to reach a toilet facility- for this a "closed loop" type model of DBS would be the optimal design, if appropriate physiological signals within the PAG could be identified as consistent with urinary urgency.

In a single subject with Parkinson's disease, pedunculopontine nucleus (PPN) DBS produced new onset detrusor overactivity after electrode implantation (Aviles-Olmos *et al*. (2011)). Since the PPN is located close to the brainstem pontine micturition and storage centres, it is probable that both effects reported were due to different activity at one of these critical bladder centres. The effects of thalamic DBS, tested in patients with essential tremor (ET), reduced bladder capacity and sensory thresholds during bladder filling (Kessler *et al*. (2008)). Pallidal DBS in dystonia sufferers reduced detrusor overactivity, reduced the urine flow rate and increased the post-void residual, without affecting bladder filling parameters (Kessler *et al.* (2014)), suggesting that the pallidum may have a role in co-ordinating urinary voiding. Interestingly, one patient with traumatic brain injury who received NAcc/IC DBS with the goal of improving

frontal lobe functioning to reduce overall disability caused by TBI was able to stop taking tolterodine for urinary urgency because he became able to "self-initiate toileting" (Rezai *et al*. (2016)). It is hopeful that these early studies may lead to new treatment options for patients with severe incontinence or voiding difficulties, either as an independent neuromodulation device, or, for individuals with bladder disorders secondary to peripheral nerve or spinal injuries, as part of a system coupled to a functional electrical stimulation device to link brain signals to bladder contractions.

Some aspects of these DBS observations fit well with what is already known about bladder control through neuroimaging and animal studies. In particular, the involvement of the PAG in bladder filling and voiding has been well documented (Athwal *et al*. (2001), Shy *et al*. (2014), Holstege & Blok (1994)). The role of the GPi in the urine storage and voiding phases has been described in cats (Porter *et al*. (1971)) and a handful of neuroimaging experiments (e.g. Shy *et al*. 2014), whereas the STN, which has been the most carefully investigated of all the DBS sites in the human, is not normally mentioned in fMRI reports. This may be due to the small size of the nucleus or because its efficacy demonstrated by DBS is specific to its role in PD rather than a unique bladder related function.

CHAPTER 2: METHODS

Plan for investigation

Autonomic control is becoming recognised as increasingly important in a number of diseases. For example, impaired autonomic control in obstructive sleep apnoea (Macey *et al*. (2012)) and orthostatic hypotension (Udow *et al.* 2016) can result in brief periods of hypoperfusion of the brain with cognitive conseqences. Furthermore, autonomic changes are important for creating states of feeling and emotion (Damasio (1994)) and for influencing decision-making (Bechara *et al*. (1997)). Nevertheless, subtle changes in autonomic nervous system activity may go un-noticed, particularly in the context of a disease with prominent symptoms, such as Parkinson's disease and other motor disorders, where the motor symptoms may mask or detract from autonomic problems. In the first part of this thesis, I will investigate the effect of deep brain stimulation on autonomic symptoms in Parkinson's disease (PD); in Chapter 3, by analysing the structural connectivity patterns associated with (i) improvements and (ii) worsening of autonomic symptoms after STN DBS and in Chapter 4 by describing an analysis of structural changes within the grey and white matter regions of the brain that are associated with autonomic symptoms in PD. I will consider how these structural correlates of autonomic symptoms fit with existing knowledge about the central autonomic network, and also how they relate to the findings of Chapter 2 concerning the effects of DBS on autonomic function.

In the second part of the thesis, my aim is to experiment with alternative (i.e. non-fMRI techniques) to understand the neural control of the bladder. Firstly, I attempt to

investigate whether LFP recordings can provide information about the role of specific brain nuclei in bladder control, or act as a biomarker for bladder dysfunction in disease (chapters 5, 6). I then use DBS as a tool to further investigate bladder control by investigating the effect of PPN DBS on bladder control in a series of cases. I selected the PPN with the aim of further investigating the findings of the case report published by Aviles-Olmos *et al*. (2011) demonstrating new onset bladder disturbance after PPN implantation (Chapter 7).

Ethical approvals and consent

All patients gave consent for their information to be used for research purposes. All parts of this study were carried out in accordance with the Declaration of Helsinki and received approval from the Oxfordshire Research Ethics Committee B study 09/H0605/62 (bladder) and study 05/Q1605/47 (autonomic questionnaire).

Questionnaires

An autonomic symptom questionnaire was carried out pre-operatively and post-operatively in consecutive DBS patients (see Appendix 1 for questionnaire). DBS patients admitted to the functional neurosurgery department were approached on the pre-operative evening or in a pre-operative clinic, or if not possible, shortly after surgery, for completion of the pre-operative questionnaire. If the subject completed the questionnaire in the post-operative week, they were asked to report symptoms as they had been in the six months prior to surgery, ignoring any new changes that had developed since surgery. Subjects were given the choice as to whether to complete the

questionnaire themselves or with the researcher. The post-operative questionnaire was either mailed out to subjects 6 months after surgery or completed over the phone or during a clinic visit- depending on the subject's preference. If the patient requested that the questionnaire was filled in before 6 months had passed (usually due to clinic attendance) this was done, and the date of completion recorded. Similarly, if follow-up was not possible at 6 months due to lack of patient availability, repeated attempts at follow-up were continued over subsequent months. However, if a patient expressed the wish not to complete the follow-up questionnaire, they were not contacted after this point.

The COMPASS-31 questionnaire is a refined and internally consistent abbreviation of the well-established and validated "autonomic symptom profile" and "composite autonomic score" (COMPASS) (Suarez *et al*. (1999)). The autonomic symptom profile and COMPASS questionnaire were originally validated in a population of patients with autonomic failure (including those with Parkinsonism) and the correlation between COMPASS questionnaire measures and clinical scores based on the results of autonomic tests were established (Suarez *et al*. (1999)). (Autonomic tests included orthostatic blood pressure and heart rate responses to tilt, heart rate response to deep breathing, quantitative sudomotor axon testing at three upper limb sites, and beat-to-beat blood pressure measurements during the Valsalva manoeuvre, tilt and deep breathing.) The COMPASS-31 questionnaire was subsequently created as an abbreviated version of the COMPASS questionnaire (Sletten *et al*. (2012)). It assesses autonomic function across six domains, namely orthostatic intolerance, vasomotor symptoms, secretomotor symptoms, gastrointestinal (upper and lower), bladder and pupillomotor. Comparisons between questions selected for the

COMPASS-31 questionnaire and the original COMPASS questionnaire were made by calculating the Cronbach α coefficient as a measure of internal consistency for items in each domain. The threshold for acceptability of the Cronbach α coefficient was set at 0.70 or higher. Factor analysis was then used to re-define symptom domains within the selected COMPASS-31 questions, and a Cronbach α coefficient was re-calculated to evaluate internal consistency within the finalised questionnaire tool.

The autonomic questionnaire used in this thesis was structured around the COMPASS-31 autonomic questionnaire (Appendix 1) (Sletten *et al*. (2012)) with supplementary questionnaires interspersed; these included the St George Respiratory Questionnaire (Jones *et al.* (1992)), the King's Health Questionnaire (Kelleher *et al*. (1997) and the Birmingham IBS symptom questionnaire (Roalfe *et al*. 2008). The supplementary questionnaires were generally only answered if patients reported relevant symptoms in the core 'COMPASS-31' section of the questionnaire. For example, if a patient reported having bladder problems, they were asked to complete the King's Health Questionnaire. Overall, the questionnaire took approximately 45 minutes to complete.

For the purposes of analysis, the autonomic questionnaire was used to generate two separate "scores" for use in subsequent analysis. First, the published algorithm (Slettern *et al*. 2012) was used for creating an autonomic symptom score from the autonomic questionnaire, for each patient (see Appendix 1 for algorithm). The bladder component of the COMPASS-31 score was also noted for each patient (= COMPASS bladder score).

Data from the autonomic questionnaire is used in chapter 3 (autonomic fingerprint study) and chapter 4 (autonomic and bladder structural MRI study).

Subjects

Study one subjects (chapter 3): autonomic fingerprint

8 subjects with subthalamic nucleus DBS were included within the autonomic fingerprint chapter).

Subjects who had completed the autonomic COMPASS-31 questionnaire and met the following criteria were selected for the fingerprint study. The eligibility criteria were that the subject:

(i) must have competed a pre-operative and post-operative questionnaire

(ii) must have completed the post-operative questionnaire no more than 24 months after surgery

(iii) should have had no subsequent DBS electrodes implanted at new sites between pre-operative and follow-up questionnaire

(iv) should have available a pre-operative DTI and T1 weighted MRI and a post-operative CT for electrode localisation

A total of 14 subjects initially met the inclusion criteria. Of these, 10 had Medtronic electrode systems and 4 had alternative (either Boston Scientific of St Jude Medical)

systems. It was decided that for consistency and to avoid confounding factors, only the subjects with Medtronic systems would be included in the analysis. One of the 10 subjects with Medtronic systems was excluded due to poor quality DTI images (subject number 124). A second subject was excluded because he only had left sided DBS (subject number 45). The analysis was therefore conducted in a group of 8 subjects. For the purposes of the analysis, the subjects were split into two groups: a "successful autonomic outcome" group (SO) (improvement in autonomic symptoms by more than 5% of baseline, n =3) and an "unsuccessful autonomic outcome" group (UO) (less than 5% improvement, or worsening in autonomic scores, n =5).

Study two subjects (chapter 4): autonomic TBSS study

38 PD subjects who had completed a pre-operative autonomic symptom questionnaire and 19 non-PD movement disorder subjects who had also completed a pre-operative autonomic symptom questionnaire were recruited for the PD/disease-control structural study. Eligibility criteria consisted of:

- PD or movement disorder diagnosis
- Completed pre-operative autonomic symptom questionnaire
- Had diffusion-weighted MRI scans of suitable quality. Subjects lacking a T1-weighted MRI scan were excluded from the grey matter structural part of the study.

Subjects who reported a diagnosis of upper or lower urinary tract malignancy were excluded from the analysis.

Study three subjects (chapter 5): voiding LFP study

7 subjects with GPi DBS (all had PD), 7 subjects with STN DBS (all had PD) and 1 subject with PAG/VPL DBS (chronic pain) were recruited and provided informed consent for participating. Data was analysed from all subjects who were able to void urine successfully and whose voiding recordings were intact and suitable for analysis: GPi (n = 6), STN (n = 4), PAG/VPL (n = 1)

6 subjects with motor thalamus DBS (2 = PD, 3 = essential tremor, 1 = dystonic tremor) were also recruited and tested but their results have not been included within this thesis.

Study four subjects (chapter 6): filling LFP study

1 subject with PAG/VPL DBS was included within the bladder filling study. He had DBS for chronic pain and his case details are included in chapter 6.

Study five subjects (chapter 7): PPN DBS bladder study

7 patients with DBS electrodes targeted to the PPN were recruited to undergo urodynamic testing, which took place in a hospital clinic in Oxford. 6 subjects were recruited from the Oxford Functional Neurosurgery Department and 1 subject was recruited from the National Hospital for Neurology and Neurosurgery.

Operative procedures in DBS patients

Patients were generally admitted to hospital the night before their operation. For patients with Parkinson's disease, medications were withheld on the morning of surgery in order to accentuate symptoms to aid intra-operative testing. After infiltrating the pin sites with local anaesthetic, a Cosman-Roberts-Wells (CRW) frame (Radionics Inc., Burlington, Massachusetts) base ring was fitted to the patient's skull, oriented roughly parallel to the AC-PC line. Subsequently a whole brain stereotactic CT was acquired with the base ring in place (1mm slice thickness) and image fusion software (Radionics Image Fusion and Stereoplan) were used to fuse the newly acquired stereotactic CT with a high-resolution T1-weighted MRI scan that had been taken during a previous pre-operative scanning session. This enabled target selection as detailed below for each nucleus. Electrode trajectory was planned in such a way as to avoid venous lacunae, sulci and the cerebral ventricles.

Targeting

Subthalamic nucleus DBS

Targeting the STN was carried out based on direct visualization of the STN on a T2 or FLAIR sequence. It is possible to identify the red nucleus as a round hypointense structure, and subsequently to identify a high intensity structure 2-4mm anterolateral to the red nucleus which is the STN (Liu *et al*. 2001). A dorsolateral region within the STN is selected as the target.

Globus pallidus interna DBS

The surgical target is the posteroventral globus pallidus interna (GPi). The GPi is identified visually using MRI and the tip of the electrode is directed to lie just above the optic tract.

Pedunculopontine nucleus DBS

For the subjects with pedunculopontine nucleus (PPN) electrodes, the surgical procedure was carried out at the John Radcliffe Hospital for patients 1-6 and at the National Hospital for Neurology and Neurosurgery for patient 7. For the Oxford patients, the electrode trajectory was based on visual identification of landmarks on a pre-operative MRI; target placement of the electrode was dorsolateral to the superior cerebellar peduncular decussation and medial to the medial lemniscus. DTI was also used to identify the target as it is sited near fibres of decussation, lemniscus and motor tracts which are all in different orientations and therefore could be displayed in different colours. An example of rostral and caudal electrode placement in the axial plane, along with a sagittal view of the electrode trajectory, can be seen in figure 2.1. For the London patient, electrode placement was guided by stereotactic proton density MRI sequences that were also used to confirm accurate lead placement (Avilés-Olmos *et al*. (2011)).

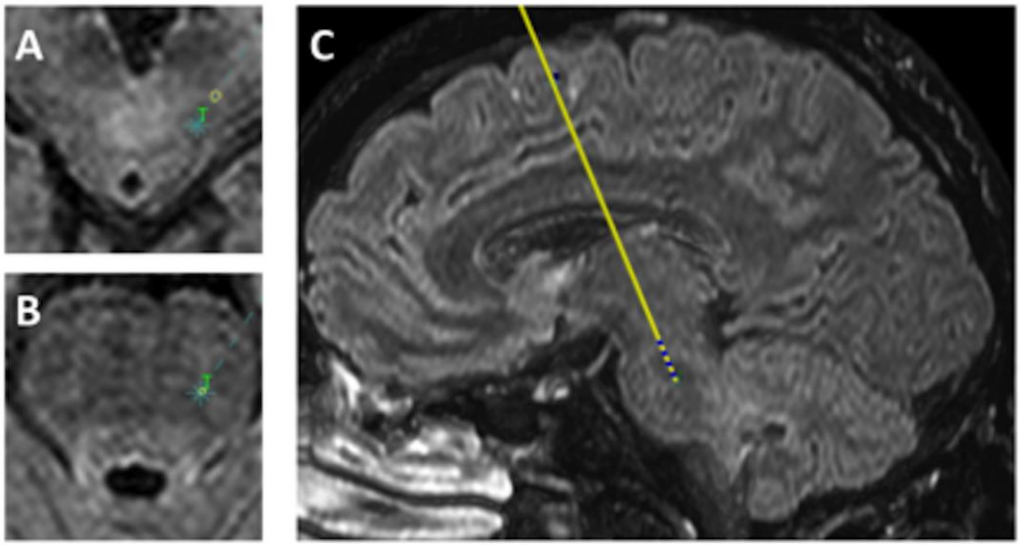


Figure 2.1: Figure to show location of PPN using stereotactic planning software. T1 weighted MRI, with PPN rostral (A) and caudal (B) electrode targeting in the axial plane. The symbol 'T' indicates the target point. Sagittal view of electrode trajectory (C).

Periaqueductal grey area DBS

Images were used to target the electrode to the PAG based on desired co-ordinates for the deepest electrode as follows: 10mm below the AC-PC line; between the dorsal part of the red nucleus and superior colliculus in the AP plane; and approximately 3mm lateral to the lateral boundary of the aqueduct and third ventricle. The VPL was located from 5mm above and up to 5mm below the AC-PC line, 5-8mm posterior to the mid-commissural point, and approximately 12-14mm lateral. Electrodes (Medtronic 3387, Medtronic, Minneapolis, MN, USA) consisted of 4 cylindrical contacts of 1.5 mm diameter spaced at 1.5 mm intervals. Electrodes were tunnelled laterally, fixed to the skull with a titanium bioplate^{TM} and externalised for 7 days of testing; if clinical effect was shown, electrodes were internalized

and connected to a Kinetra implantable pulse generator (Medtronic Minneapolis, MN, USA).

Surgery

Stage 1 surgery was generally performed under local anaesthetic, with the exception of GPi DBS for dystonia, which was done under GA. Intravenous antibiotics were given 30 minutes prior to incision. After planning the electrode trajectory, pre-calculated stereotactic co-ordinates were applied to the frame and a 2.7mm twist drill craniostomy made at the entry site. A macroelectrode (TM®, Radionics) electrode was then passed along the pre-planned trajectory to the target. Impedance monitoring as the electrode is being inserted can facilitate accurate placement: white matter impedance is higher than grey matter. Once electrode was sited, macroelectrode test stimulation was performed to assess the clinical effect. When the desired target was been established, the rigid electrode was removed and the narrower DBS electrode was inserted and secured using mini-plates attached to the skull at the entry site. The subject then underwent a second stereotactic CT for confirmation of electrode position. For most subjects, the DBS electrode is then secured and externalised for a period of trialling (usually 5-6 days) before it is attached to an internal pulse generator (IPG) in a second procedure under general anaesthesia. In some cases, the entire stage 1 and stage 2 procedure is completed in one day under general anaesthesia. (Pereira *et al*. 2008; Holl *et al*. 2010).

Electrode types

Medtronic 3389 electrodes are used for PPN and STN DBS - the four circumferential contacts are 1.27mm diameter and 1.5 mm in length and are 0.5 mm apart. Stimulation is either bipolar or monopolar in configuration. Medtronic 3387 electrodes are used for GPi and thalamus- the four circumferential contacts are 1.27mm diameter and 1.5 mm in length and are 1.5 mm apart. For both 3387 and 3389, each of the four contacts is platinum-iridium and cylindrical in dimension. One subject (subject 39, LFP study) had an eight-contact Boston Scientific electrode implanted. The electrode dimensions are as follows: 8 contacts of 1.5 mm length and 0.5 mm spacing. See figure 2.2 for example pictures of an electrode.

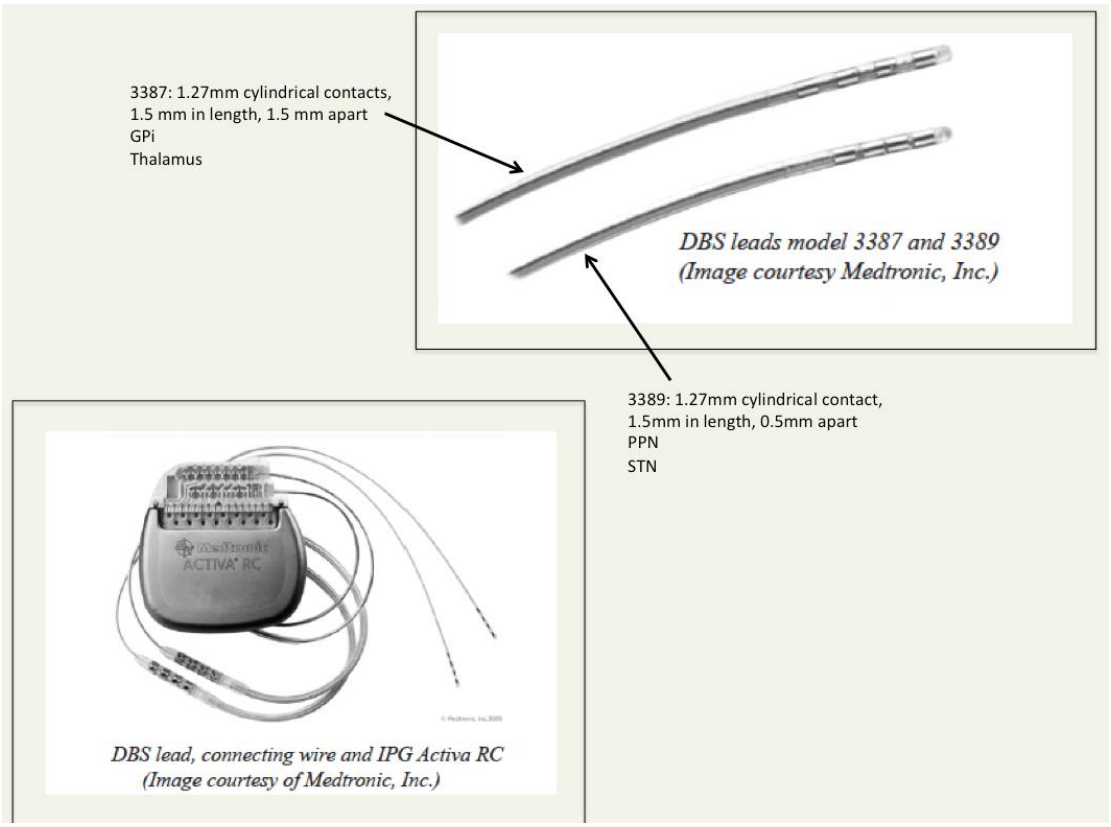


Figure 2.2: DBS electrodes, demonstrating dimensions and connectivity to IPG (courtesy of Medtronic, Inc.)

Image acquisition

Imaging

Subjects underwent MRI on a 1.5 Tesla Phillips Achieva magnet. Diffusion-weighted data was acquired using a single-shot echo planar imaging sequence (176 x 176 reconstructed matrix, voxel size = 1.8 x 1.8 x 2 mm, repetition time = 9390 ms, echo time = 65 ms, and slice thickness, 2mm). DTI data were acquired with 33 directions, (b value = 1200 s/mm^2) and one image without diffusion weighting was obtained (b = 0)). Furthermore, a high-resolution T1-weighted structural image (voxel size 1x1x1 mm^3) was acquired to check for gross structural abnormalities and used here for registration and structural analysis purposes, with a three-dimensional 'FLASH' sequence (TR = 12 ms, TE = 5.6ms, flip angle = 19^o, with elliptical sampling of *k*-space, giving a voxel size of 1 x 1 x 1 mm^3 in 5.05 min). Post-operative CT scans were also obtained where necessary to determine the location of the electrodes.

Image analysis

Images were analysed using the FMRIB Software Library (FSL), Release 5.0 © 2012 (Smith *et al*. (2004)).

DTI fingerprint study (chapter 3)

See chapter for specific methods

DTI TBSS study (chapter 4)

Structural MRI pre-processing (grey matter analyses)

The T1-weighted data was analysed with FSL-VBM (Douaud *et al*. (2007)), an optimised voxel-based morphometry protocol (Good *et al*. (2001); Smith *et al*. (2004)) carried out with FSL tools (Smith *et al*. (2004)).

T1-weighted images were placed into a dedicated FSL-VBM directory for each study. Each image was visually inspected to check for gross abnormalities. The design matrix files (design.mat and design.con) were then created using the GLM gui. The images were skull-stripped and moved into a new directory. A study-specific template was then created. In studies where there were different numbers of patients and controls (e.g. the PD/disease-control study in study 5) the same number of patients and controls were used to create the template, using non-linear registration. Finally, each grey matter image was registered to the template, concatenated to create a 4D image and smoothed using a Gaussian kernel of sigma = 3mm.

FMRIB's Integrated Registration and Segmentation Tool (FIRST) (Patenaude *et al*. 2011) was then used to carry out structural segmentation of selected grey matter structures (see individual analysis for description of which structures were segmented). In order to scale the volume of each subcortical structure according to the head size of the individual patient, SIENAX was run for each subject (Smith *et al*. 2002). SIENAX produced a volumetric scaling factor by which each subcortical

region-of-interest volume was multiplied to provide an estimate of region-of-interest volume corrected for head size. Pearson's Product Moment Correlation between subcortical grey matter volume corrected for head size and bladder score was calculated using IBM SPSS Statistics for Mac (version 20). Results of partial correlations corrected for age were considered significant for $p < 0.05$ after applying Bonferroni correction for multiple comparisons.

DTI pre-processing

Each subject's diffusion-weighted scans were individually inspected for gross distortions, corrected for head motion and eddy currents and brain extracted using BET (Smith *et al*. (2002b)). Fractional anisotropy, mean diffusivity, axial diffusivity (eigenvector 1) and L2 and L3 images were created by fitting a diffusion tensor model to the diffusion data using DTIFIT (Behrens *et al*. (2003)) within the FMRIB diffusion toolbox. Radial diffusivity images were then created by averaging the L2 and L3 maps using the 'fslmaths' function within the FSL toolbox (radial diffusivity = (L2 +L3)/2) (Menke *et al*. (2014)).

Tract-based spatial statistics (TBSS) pre-processing

Individual FA images were aligned to every other FA image, and the "most representative" result was used as a target image to which all the original FA images were non-linearly registered. The target image was then affine-aligned into MNI152 standard space and each individual image transformed into 1x1x1mm MNI152 space

by combining the nonlinear transform to the FA image with the affine transform from the target image to MNI152 space. Images were then averaged to create a mean FA image, which was thinned to create a mean FA skeleton (Smith *et al*. (2006)). This FA skeleton represented the centres of all tracts common to the group. Each subject's aligned fractional anisotropy data were then projected onto this skeleton. The same operations used to register the individual FA images to the study specific template and project FA values onto a study specific template were applied to the mean diffusivity, axial diffusivity and radial diffusivity maps (Menke *et al*. (2014)).

Statistical analyses

Randomise (permutation testing) was implemented in FSL for all analyses unless otherwise stated. In the TBSS analysis, for the comparison of DTI metrics (FA, MD, L1 and RD), correlation between clinical score and individual metrics were carried out using permutation-based non-parametric testing applied using a voxel-wise general linear model (GLM), carried out using the whole brain white matter skeleton with age as a covariate of no interest. Results were considered significant for $p < 0.05$ ***after correction for multiple comparisons*** (family wise error, FWE), using the threshold free cluster enhancement (TFCE) approach (Smith *et al*. (2009)). Similarly in the VBM analysis, analyses were performed using a voxelwise general linear model with permutation-based non-parametric testing, correcting for multiple comparisons across space.

Further information about individual statistical analyses can be found in chapter 4.

Limitations of DTI

Although DTI has clear advantages as a non-invasive technique for white matter tract reconstructions, there are limitations. In particular, anatomical accuracy is lost in the process of transformations (e.g. the transformation from CT to MRI space, or subject-MRI to MNI space). Furthermore, DTI is notoriously weak in predicting path trajectories in areas of crossing fibres, such as the brainstem. Ideally, DTI maps should be compared with results of histological studies in order to evaluate the plausibility of the findings.

LFP analysis (chapter 5 and 6)

Experimental paradigms are outlined within each individual chapter. Below are the basic recording and analysis methods.

Local field potential (LFP) recording

All patients apart from one (subject 39) had a quadripolar macroelectrode implanted. Subject 39 had a Boston Scientific eight-contact electrode implanted. Recordings were made 3-6 days post-operatively in all subjects unless otherwise stated. LFPs were recorded from the externalised DBS electrode(s) using a portable physiological measuring system (Porti 7, TMS International, Netherlands) and sampled at 2048 Hz. A ground electrode was placed on the dorsum of the patient's wrist and a common average reference was used.

Electrodes were either within the subthalamic nucleus (STN), the globus pallidus (GPi), the periaqueductal grey (PAG) or the ventroposterolateral thalamic nucleus (VPL).

Signal analysis

Signals were analysed off line using Spike2 software and the NSPL toolbox in Matlab (Mathworks). First, bipolar channels were created, by subtracting the signal in adjacent channels, to create two bipolar channels per electrode. (In subject 39, four bipolar channels were created by subtracting the signal in adjacent channels, and the middle two channels were used for analysis.)

The signal was then downsampled to 1000 Hz and exported to a custom software package called NSPL, provided by Shouyan Wang. Bipolar signals were low pass filtered at 200 Hz, high pass filtered at 2 Hz, and bandstop filtered at 50 Hz using a Butterworth filter to exclude mains artefact.

Power spectral density analysis was performed on 10 second data segments using a 1.5 second Hanning window and the power in the following frequency bands was computed using an algorithm within the NSPL program which integrated the power in the spectra within each of the following frequency bands: 4-8 Hz, 8-12 Hz, 12-25 Hz, 25-60 Hz, 60-90 Hz, 90-150 Hz. Power was averaged across all available contact pairs for each nucleus and was normalized to rest. Relative power was used for

analysis due to variability in absolute power due to factors such as local tissue properties (Neumann *et al*. (2016)).

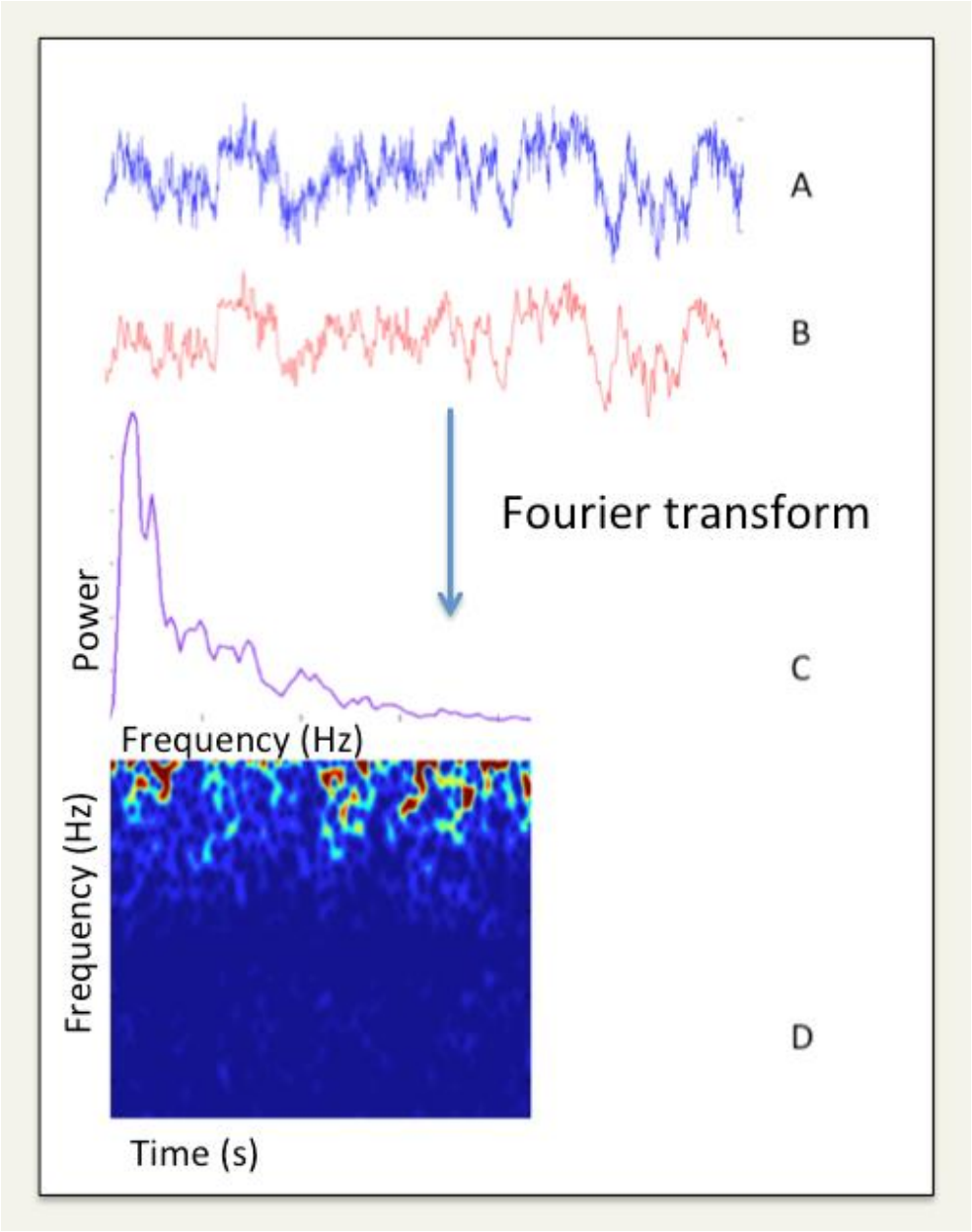


Figure 2.3: Schematic to illustrate filtering and manipulation of the LFP signal. A: the raw bipolar signal prior to filtering. B: the same signal after low pass, high pass and line noise filtering. C: power spectral analysis of the same signal. D: time-frequency plot of the signal, where time is on the *x* axis and frequency on the *y* axis.

Urodynamics (chapter 7)

Urodynamics was carried in accordance with International Continence society guidelines. The majority of urodynamics testing was done in the Urology Outpatients Department, Churchill Hospital, Oxford, with a urodynamics specialist nurse operating the urodynamics machine.

Subjects were asked to attend for testing with a full bladder. On arrival and completion of a consent form, participants were asked to void urine in order to fully empty their bladder and urinalysis was performed to exclude a urinary tract infection. If the sample was free from white cells and nitrites, the subject was given 50mg nitrofurantoin as prophylaxis against infection and we proceeded with the urodynamics. If there were clear signs of infection on the urinalysis, the testing session was terminated and the subject was contacted at a later date for re-scheduling.

All patients within the urodynamics group had their Parkinson's disease medications withheld for 12 hours prior to testing, with the exception of one patient (patient 7) who took a quarter of the normal dose of medication. Participants then underwent bladder catheterisation with a dual lumen catheter (8 Fr), and a rectal catheter (4.5 Fr) was inserted to measure abdominal pressure. The bladder was infused with a sterile solution of isotonic saline at an average rate of 30ml min^{-1}. During infusion of saline into the bladder, participants were asked to report when they first experienced the following sensations; 1. Initial sensation of bladder filling, 2. Initial desire to void, 3. Normal desire to void, 4. Strong desire to void, 5. Maximal capacity (point at which they could tolerate no further filling). When the patient reported reaching maximal

capacity, the saline infusion was stopped immediately. If detrusor overactivity occurred, filling was stopped at this point, which was taken to be the maximum bladder capacity. The bladder was emptied via the catheter using a syringe and filling was repeated for a further 3 trials to give a total of 4 bladder fills.

For each patient, bladder filling was carried out twice with PPN DBS turned 'ON' and twice with PPN DBS turned 'OFF'. The ON/OFF order was pseudorandomised and allocated prior to the patient attending for the study. At least ten minutes was left between changing the stimulation and starting the next bladder infusion, to ensure that the effects of stimulation being ON or OFF had reached a steady state.

CHAPTER 3: STN FINGERPRINT

Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has been shown to modulate autonomic nervous system activity both intra- and post-operatively, as discussed in the introduction (Chapter 1). Effects range from intra-operative changes in cardiovascular variables (Sauleau *et al*. (2005), Benedetti *et al*. (2004)) to more subtle but clinically beneficial changes such as improvements in symptoms of autonomic dysfunction including orthostatic hypotension and dyshidrosis (Trachani *et al*. (2010), Halim *et al*. (2010)). Furthermore, physiological and symptomatic improvements are reported in bowel function (Halim *et al.* (2010), Krygowszko-Wajs *et al*. (2016)) and urinary function (Seif *et al*. (2004), Winge *et al.* (2007), Dafsari *et al*. (2016)), which are under partial autonomic regulation. Intentionally harnessing these autonomic effects could have clinical benefits for Parkinson's disease sufferers, however, the mechanism responsible for the changes remains unclear.

Common autonomic symptoms in Parkinson's disease include orthostatic hypotension swallowing difficulties, sweating, constipation, diarrhoea and urinary storage symptoms including frequency, urgency and nocturia (Jain *et al*. (2011), Chaudhury *et al*. (2006), Sakakibara *et al*. (2001)). Brain structural abnormalities that underlie these common autonomic symptoms in Parkinson's disease have not been conclusively investigated, although Chen *et al*. (2016) described reduced baroreflex sensitivity and reduced grey matter in the left hippocampus, right amygdala, bilateral insula, bilateral cerebellum, bilateral caudate, right fusiform and left middle frontal gyrus in patients with Parkinson's disease (Chen *et al*. 2016). Hypothetically, it might be expected that structural alterations within the central autonomic network (CAN), such as the ventromedial medulla, hippocampus, insula, cingulate cortex,

ventromedial prefrontal cortex and cerebellum (Macey *et al*. 2016) could be implicated. In addition, brain structures that have been linked with specific autonomic syndromes in non-PD populations might be expected to be involved. For example, postural tachycardia syndrome sufferers have reduced grey matter volume in the left anterior insula, right middle frontal gyrus and right cingulate gyrus, and reduced white matter in various frontal regions including below the right pre- and post- central gyri (Umeda *et al*. 2015). Urge incontinence in older women is linked with changes in activation of the medial prefrontal cortex (Griffiths *et al*. (2009)) and anterior cingulate gyrus (Griffiths *et al*. (2007)); while altered resting state functional connectivity has been described in patients with irritable bowel syndrome in the postcentral gyrus, thalamus, anterior cingulate cortex and prefrontal cortex (Ke *et al*. (2015)).

The STN itself is not currently considered to be part of the CAN (Hyam *et al*. (2012)) however autonomic effects of STN DBS may arise if stimulation of the STN modulates activity within local CAN regions via direct current spread, or within anatomically distant CAN nuclei via stimulation of white matter pathways connecting these regions as part of a functional network. CAN regions local to the STN include the zona incerta and the Forel's Fields (Sauleau *et al*. (2003)). Activation of these regions would produce autonomic effects such as tachycardia and mydriasis, which would be noticed at the time of surgery. It could be argued that the more subtle changes in autonomic control that have been reported including improvements in symptoms of dysautonomia, are more likely to occur via long-range structural connections of the STN to higher-order CAN regions which are able to orchestrate more complex autonomic responses. For example, neural regions that have been

linked with postural tachycardia syndrome include the left anterior insula, right middle frontal gyrus and right cingulate gyrus, and various frontal regions including below the right pre- and post- central gyri (Umeda *et al*. (2015)); STN DBS may improve symptoms of postural hypotension by modulating function within some or all of these areas.

There is support for a model of internal functional subdivisions within the STN, based on a tripartite system of division described in the animal literature, with specific motor, associative and limbic zones (Karachi *et al.* (2005)). In primates, the motor region of the STN is dorsally placed; the associative STN is more medioventrally placed; and a small limbic region is located in the anterior medioventral portion of the STN. Similarly in humans, a recent tractography study demonstrated that dorsal DBS contacts within the STN were more strongly connected to the supplementary motor cortex, superior, middle and inferior frontal gyrus, regions involved in motor control, while moving more ventrally, contacts were more strongly connected to the amygdala, hippocampus and post-central gyrus, typically associated with limbic function (Accolla *et al*. (2016)). The associative region in humans is believed to represent an intermediate zone, with some motor and some limbic connections (Lambert *et al*. (2012)). In terms of autonomic responses, an intra-operative stimulation study reported autonomic effects following stimulation of both the dorsal and ventral STN (Benedetti *et al*. (2004)). Dorsal stimulation evoked constant autonomic effects as assessed by changes in heart rate and heart rate variability, while ventral stimulation produced autonomic effects that were dependent on whether or not the patient was blinded to the stimulation. Furthermore, ventral stimulation also produced an emotional response that was dependent on blinding. This is consistent

with the view that ventral stimulation modulated autonomic function via an indirect pathway that was connected with higher limbic/autonomic centres, whereas dorsal stimulation produced autonomic effects via direct stimulation of local autonomic structures, with less potential for modulation by higher centres.

The aim of this study was to assess the connectivity profile associated with improvements in autonomic symptoms following STN DBS compared with the connectivity profile associated with an absence of improvement. Specifically, we wanted to understand whether the cluster of structural connections associated with improved autonomic symptoms represented regions known to be implicated in autonomic function or regions named as part of the "motor", "associative" or "limbic" profile of connections. In order to achieve this aim, a series of PD patients was followed prospectively, and autonomic symptoms were evaluated pre and post STN DBS. Assessment of autonomic symptoms was carried out using the "COMPASS-31" questionnaire completed pre-operatively and 9 months post-operatively. The COMPASS-31 questionnaire is a refined and internally consistent abbreviation of the well-established and validated "autonomic symptom profile" (Suarez *et al*. (1999)) and "composite autonomic score" (Suarez *et al*. (1999)). The COMPASS-31 questionnaire assesses autonomic function across six domains, namely orthostatic intolerance, vasomotor symptoms, secretomotor symptoms, gastrointestinal (upper and lower), bladder and pupillomotor (see methods for more details and Appendix 1 for the questionnaire).

Diffusion-weighted MRI (DWI) is an imaging technique that allows microstructural properties of white matter to be defined on the basis of diffusion of water molecules.

Diffusion tractography uses DWI measurements of diffusion characteristics to reconstruct white matter tracts (Basser *et al*. (1996), Beaulieu *et al* (2002), Johansen-Berg *et al*. (2009)). In this study, diffusion tractography was combined with whole-brain anatomical parcellation to describe the connectivity 'fingerprint' associated with each patient's active DBS contacts, and to identify significant features of the connectivity profile associated with improvements in autonomic symptoms. Anatomical parcellation was carried out with the automated anatomical labelling (AAL) parcellation (Tzourio-Mazoyer *et al*. (2002)), which includes 116 cortical, subcortical and cerebellar regions (including the vermis).

Methods

Patient selection

15 subjects completed pre- and post-operative questionnaires and had undergone T1-weighted and diffusion-weighted MRI scans. From this group of 15 subjects, one subject was excluded due to an excessively long follow up interval, two were excluded because the stimulation was unilateral, and one was excluded due to poor quality of the DTI scan. From this final group of 11 subjects, the 8 subjects with Medtronic electrodes (model 3389) were selected for final analysis (see figure 3.1). It was decided to include only subjects with Medtronic 3389 electrodes in order to keep every between-subject factor constant apart from subtle differences in electrode localisation and individual anatomy. Thus, the final analysis was carried out using data from 8 subjects.

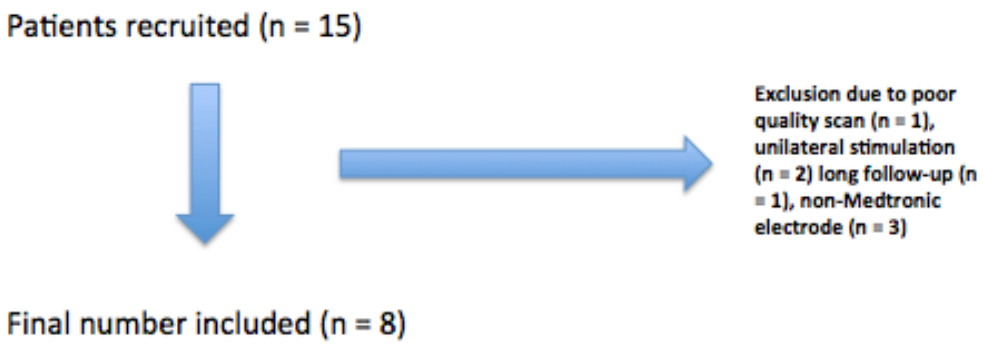


Figure 3.1. Flow chart to show inclusion and exclusion of patients

Imaging

A diffusion-weighted MRI and a T1-weighted MRI were obtained for each patient. See general methods (chapter 2) for details of the imaging parameters.

Localisation of the DBS electrode and simulation of the volume of activated tissue

The post-operative CT was skull-stripped and used to locate the DBS electrode contacts. A single-voxel binary mask was then created for each of the four contacts on each electrode. The post-operative CT and the DTI b0 volume were co-registered to T1 space using the FLIRT tool (FMRIB, Oxford) with a 3D rigid-body transformation (6 degrees of freedom). This used a nearest-neighbour method of interpolation. The resulting transformation matrix from DTI to T1 space was used to transform the DBS contact masks from T1 space to DTI space with a trilinear interpolation method. With the transformed electrode contact mask in DTI space, a mask of the voxel of

maximum intensity was made. This mask was taken to represent the voxel within DTI space with the maximum probability of containing the electrode contact.

Modelling volume of activated tissue (VAT)

A previously described method for modelling the electric field distribution, or volume of activated tissue (VAT) around the active electrode contacts was used (Fernandes *et al*. (2015)). The method is applicable for voltage and tissue impedance between ranges of 1-10V and 500-1000Ω respectively and was initially validated in the context of movement disorders. The formula produces an estimated spherical electric field distribution around the electrode contact, given a particular voltage and impedance, assuming monopolar stimulation. In cases of bipolar stimulation, a modification to the formula was used, as reported in Fernandes *et al.* (2015), whereby an elongated sphere was produced encircling the active contacts and the intermediate contacts, simulating the application 3/5 of the bipolar voltage at each of the contacts and their intermediate contacts.

Defining network nodes

In order to quantify and compare connectivity profiles and their relative strengths, the brain was parcellated into 116 cortical, subcortical and cerebellar regions based on the automated anatomical labelling (AAL) atlas; each of the 116 regions then represented a network node to which connectivity strength could be assessed.

The AAL template (originally in MNI space) was transformed into the DTI space of each individual patient using the existing T1 to DTI transformation matrix obtained in a previous step (see above), concatenated with the transformation matrix derived from linearly co-registering the ICBM152 in MNI space into the T1 space of the structural image with an affine registration (12 degrees of freedom) using a nearest neighbour interpolation.

Tractography

Each subject's diffusion-weighted image was individually inspected for gross distortions, corrected for head motion and eddy currents and brain extracted using BET (Smith *et al*. (2002)). A Markov Chain Monte Carlo sampling algorithm was used to estimate the local probability of fibre direction in each voxel, using an automated estimation of two fibre directions within each voxel. Using the estimated VAT as a seed region, probabilistic tractography was applied at a single voxel level, sampling 5000 streamlines per voxel. Connectivity strength was first calculated at a voxel level, then extended to a regional level. Connectivity strength at a voxel level was defined as the proportion of fibres passing through voxel *x* that reach voxel *y*. Extended to a regional level, connectivity strength between region *x* and region *y* is defined as the number of fibres in region *y* that are also present in region *x*, divided by (5000*(number of voxels in region *x*). The result was a connectivity matrix for each patient representing connectivity from each VAT (right and left) to each of the 116 regions of the AAL template.

Statistical analysis

Connectivity profiles from the right and left electrodes were concatenated to produce global connectivity estimates of bilateral STN stimulation in each patient. Individual connectivity measures were then used in the group analysis, as described in Fernandes *et al.* (2015). Two groups were created based on change in autonomic symptoms post-operatively; a "successful autonomic outcome" group (SO) (improvement in autonomic symptoms by more than 5% of baseline) and an "unsuccessful autonomic outcome" group (UO) (less than 5% improvement, or worsening in autonomic scores). Using permutation-based t-tests, the average connectivity strength in the two groups (SO and UO) were compared with a $p < 0.05$ criterion for significance. A second, more conservative approach was also employed to compare groups and identify group-exclusive target areas. Nodes which survived a threshold of 5% of the maximum connectivity strength for each individual and were found to be common to all subjects within the group were identified and taken to represent core regions of intrinsic group connectivity.

Results

Patient information

8 subjects were included in the analysis, 5 male and 3 female. The mean age at the time of DBS surgery was 61 years. Mean follow up duration was 9 months (range 5-24 months). Autonomic score decreased in 3 subjects (signifying improvement),

increased in 3 subjects (signifying deterioration) and remained the same in 2 subjects (see table 3.1).

Patient	1 (UO)	2 (UO)	3 (SO)	4 (UO)	5 (PO)	6 (UO)	7 (SO)	8 (UO)
Age (years)	65	56	58	66	69	62	55	58
Gender	M	M	F	F	M	M	M	F
% change in autonomic score	-3%	+28%	-64%	+507%	0%	+41%	-22%	-28%
Follow up duration (months)	7	6	24	5	9	9	11	6
Stimulation voltage (R L) (V)	2.6, 3	2.5, 3.3	3.3, 3.4	2, 2.5	2.5, 2.5	2.2, 3.5	2.8, 2.5	2.9, 1.2
Stimulation configuration (monopolar/bipolar) (R L)	M M	M M	B B	B B	B B	M B	B B	M M
% change in overall UPDRS score (off medications)	-28%	-24%	-33%	-21%	-13%	-54%	-27%	-45%

Table 3.1. Table to illustrate subject demographics, stimulation parameters, change in autonomic score and change in UPDRS following surgery. SO = successful outcome, UO = unsuccessful outcome

Relationship between change in autonomic score and UPDRS

There was no significant correlation between change in autonomic score and change in UPDRS following surgery (Pearson correlation, two-tailed).

Connectivity profiles

Connectivity profiles were identified for all 8 subjects (see Appendix 3 for individual connectivity profiles). The single subject data was used to produce a global structural connectivity fingerprint for each group (SO and UO) (figure 3.2).

Comparison between successful and unsuccessful outcomes

The SO group (subjects whose autonomic score improved by more than 5% after surgery) had significantly stronger connectivity with the left rolandic operculum, right post-central gyrus, right supramarginal gyrus, and right cerebellar vermis than the UO group, as shown in figure 3.3 and table 3.2. The UO group (those whose autonomic symptoms worsened after surgery or changed by less than 5%) displayed stronger connections with the right cerebellum (AAL cerebellar area 9) as shown in figure 3.3. Bar charts representing the difference in connectivity strength between the VAT and these significant areas for the successful and unsuccessful areas are shown in figure 3.3B and in enlarged form in Appendix 3. The analysis was re-run removing the subjects whose scores did not actually worsen from the unsuccessful outcome group (i.e. subjects whose autonomic score improved by less than 5%) thus creating a direct

comparison between patients whose scores improved and those whose worsened. This did not affect the results.

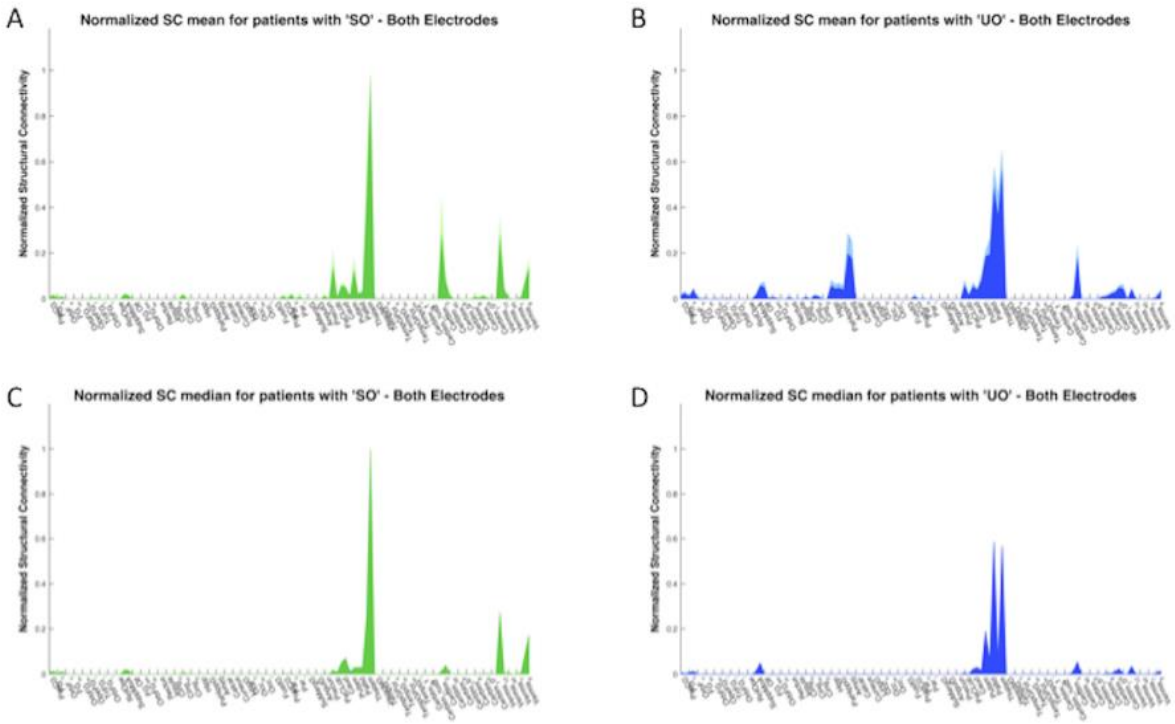


Figure 3.2. Group statistics, demonstrating group patterns of anatomical connections for the successful (SO) and unsuccessful (UO) autonomic outcome groups, with each of the 116 AAL areas listed on the *x* axis. A chronological list of these areas is given in Appendix 2 to aid interpretation of the diagram. Data is displayed as mean connectivity strength (A, B) and median connectivity strength (C, D). SC = structural connectivity.

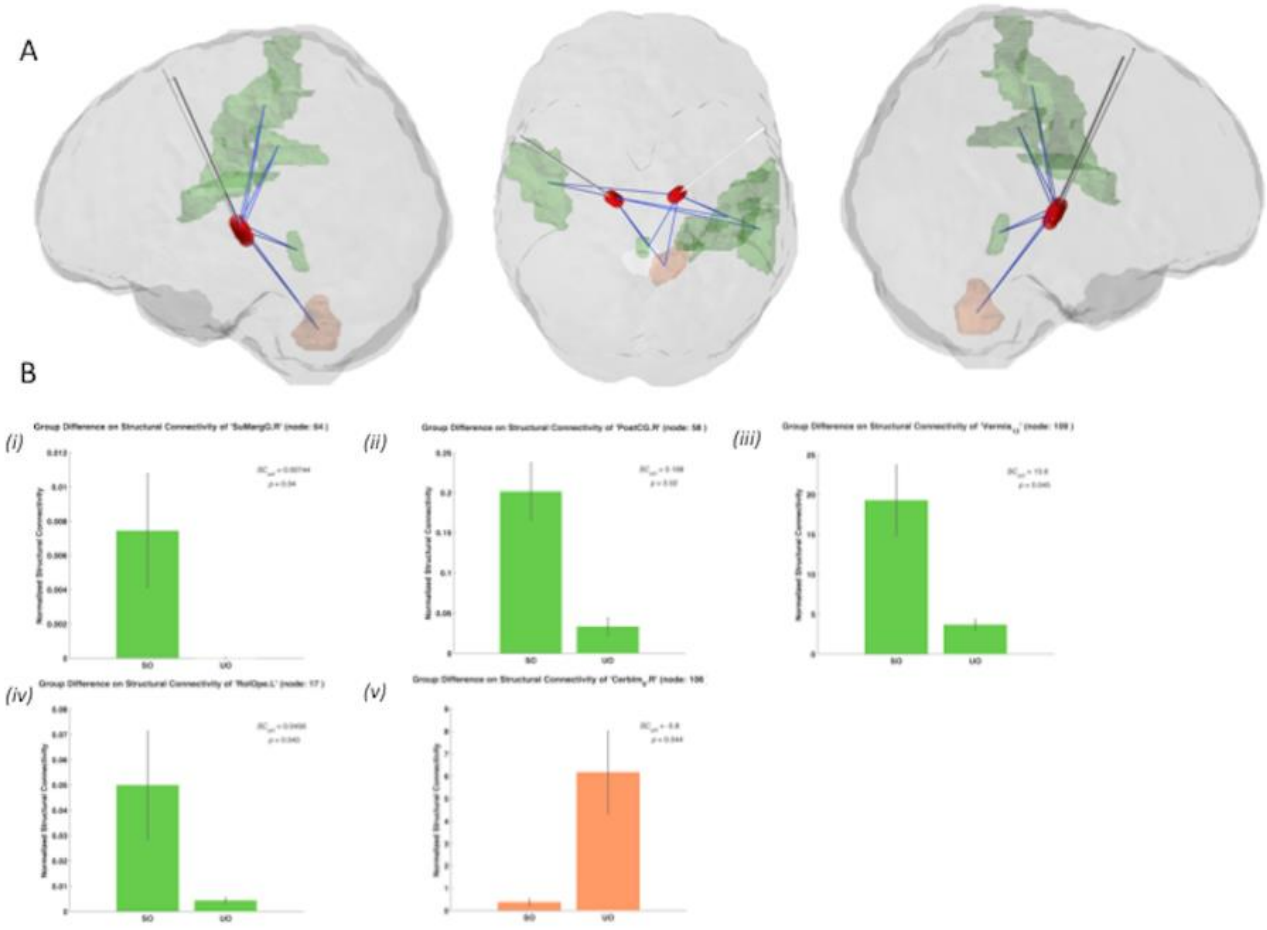


Figure 3.3A: Areas associated with stronger connectivity to the stimulation source in SO (green) and UO (red) groups (left view, top view, right view). B: Plots showing differences in connection strength between each of the significant network nodes and the SO and UO groups (i) supramarginal gyrus (ii) postcentral gryrus (iii) cerebellar vermis (iv) left rolandic operculum (v) right cerebellum. Error bars represent standard deviation. See Appendix 3 for an enlarged version of the bar charts.

Node	**AAL region**	**SC_{SO}-SC_{UO}**	**p-value**	**Group**
17	Left rolandic operculum	0.0456	0.04	SO
58	Right post-central gyrus	0.168	0.024	SO
64	Right supramarginal gyrus	0.00744	0.04	SO
109	Right cerebellar vermis	15.6	0.041	SO
106	Right cerebellum	-5.8	0.043	UO

Table 3.2. Significant differences in structural connectivity between patients with SO and UO ($p < 0.05$), using a permutation-based paired t-test. The absolute value of the structural connectivity difference (SC_{SO}-SC_{UO}) and the p-value is included within the table. SC = structural connectivity, SO = successful outcome, UO = unsuccessful outcome

Group-exclusive target areas

Connections to the thalamus bilaterally and the left caudate survived a 5% threshold of the maximum connectivity strength of the network in each participant with an improvement in autonomic score, indicating that the bilateral thalamus and left

caudate are common intrinsic network nodes in the successful outcome group (table 3.3). There were no group-exclusive target areas in the negative autonomic outcome group.

Node	**AAL region**	**Group**
77	Left thalamus	SO
78	Right thalamus	SO
71	Left caudate	SO

Table 3.3. Brain areas common to all subjects of the same group after a 5% threshold was applied to the normalised fingerprints. SO = successful outcome, UO = unsuccessful outcome. See Appendix 3 for a graphical representation of these areas.

Discussion

Autonomic symptoms often occur earlier and can have a greater impact on quality of life than motor symptoms of PD according to some evidence (Jain *et al*. (2011)). Identifying a connectivity fingerprint associated with positive changes in autonomic symptoms after STN DBS could help to guide electrode placement to ensure benefits in motor and non-motor domains.

In this study, positive autonomic outcomes were associated with significantly stronger connections to a number of areas than negative autonomic outcomes. These areas included the left rolandic operculum, right post-central gyrus, right supramarginal gyrus, and right cerebellar vermis.

The pattern of connectivity associated with improvement/no improvement in autonomic functioning does not fit clearly within the "motor", "associative" and "limbic" subdivisions outlined in the introduction, although the regions highlighted by the analysis are not overtly motor and fit better within the "associative" or "limbic" profiles. On an individual basis, however, each region has been previously implicated in autonomic functioning as discussed below.

The left rolandic operculum directly overlies and interdigitates with insular cortex (see figure 3.4) (and in some regions encompasses insular cortex according to the Harvard-Oxford cortical atlas) (Ture *et al.* (1999)). The insula receives sensory visceral information from cardiovascular, gastrointestinal, respiratory and urinary organs (Cechetto and Saper (1987)) and also has efferent control over various autonomic reflexes including cardiovascular and gastric responses (Yasui *et al*. (1991)). The insula is consistently activated in fMRI studies of autonomic activity (Macey *et al*. (2016), Al-Otaibi *et al*. (2010), Allen *et al*. (1991)) and the involvement of a closely related cortical region in positive autonomic responses following DBS is unsurprising. It is perhaps surprising that the rolandic operculum rather than the insula itself was identified in this analysis. This may have been a result of various factors including misclassification during the segmentation and labelling process, idiosyncrasies of the AAL atlas, or the probabilistic nature of the tractography process. Also, this analysis only describes monosynaptic connections between the volume of activated tissue and each of the parcellated brain regions of the AAL atlas-connections to the insula may occur via the parietal operculum.

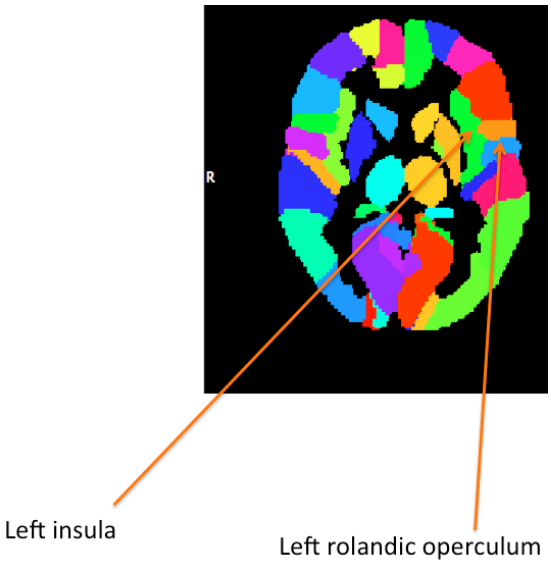


Figure 3.4: Illustration of the AAL segmentation to demonstrate the anatomical relationship of the insula and rolandic operculum.

The post-central gyrus has also been implicated in autonomic activation during hand-grip tasks (Nowak *et al*. (1999)), baroreceptor activation (Kimmerly *et al*. (2005)) and central cardiovascular control (Cechetto & Saper (1990)). Although some degree of activation may be related to the sensory changes related to these tasks, the post-central gyrus continues to demonstrate activity during the hand-grip task after the application of local anaesthesia, implying an involvement beyond its primary sensory function (Nowak *et al.* (1999)). The right supramarginal gyrus has been activated in a moderate intensity handgrip task, which produces a tachycardia representing withdrawal of parasympathetic activity (Wong *et al*. (2007)).

It is interesting to see that the cerebellar vermis was associated with positive autonomic outcomes and the right medial cerebellum (cerebellar area 9) with negative

autonomic outcomes. Although a recent tractography study did not identify any connectivity between STN and the cerebellum (Lambert *et al*. (2012)), an earlier tractography analysis did describe structural connections between the two regions (Aravamuthan *et al*. (2007)). The cerebellum is firmly established as an important autonomic region (Macey *et al*. (2016)) and activity is regularly seen in fMRI studies of autonomic control, including in the context of autonomic activity in response to social threat (Eisenbarth *et al*. 2016), pain-related fear learning in irritable bowel syndrome (Claassen *et al*. 2016, Timman *et al*. 2010), respiratory (Critchley *et al*. (2015)) and handgrip (Wong *et al*. (2007)) tasks. Furthermore, impaired classical conditioning of autonomic postural responses has been demonstrated in patients with cerebellar disorders (Kolb *et al*. (2004)). The cerebellar vermis is connected with the hypothalamus, brain stem and spinal cord, and is known to regulate cardiovascular, respiratory and gastrointestinal function (Timman *et al*. (2010), Snider (1950)), and has been shown in a PET study to play a role in autonomic cardiovascular control (Critchley *et al.* 2000). A role in autonomic function has also been demonstrated for the medial cerebellum, where we found that connectivity led to deterioration in autonomic symptoms. For example, while patients with lesions of the medial cerebellum do not show fear-conditioned bradycardia that is normal in healthy subjects (Maschke *et al*. 2002a), though there were no differences in fear related electrodermal responses between patients and controls. Thus, the improvements in autonomic function associated with STN connectivity are plausible in the light of what is already known about the cerebellar vermis, and although the mechanism of deterioration associated with medial cerebellar connectivity is not clear, its involvement in autonomic function is apparent.

Limitations

In this study, the autonomic nervous system was modelled as a unitary system. However, autonomic outputs to different organ systems do appear to have a degree of independence (Eisenbarth *et al*. (2016)). Thus, our model was perhaps over-simplistic, and focussing on a specific organ system may have yielded more accurate and meaningful results. Alternatively, use of a different model to represent the individual weighted contribution of each organ system to the result would enrich the data and interpretation, allowing the complexity of the neural control of autonomic functions to be better represented. Secondly, many brain areas responsible for autonomic control including the insula, hippocampus, cingulate and thalamus have internal functional organisation, with different regions having different autonomic responses (Cecetto & Saper (1987), Fanselow & Dong (2010)) or different connectivity patterns with subdivisions of the STN (Lambert *et al*. (2012)). Our study treated each structure as a unitary item, thus specific functional properties of small sub-regions will not have been identified.

It was interesting to note that some important autonomic structures such as the amygdala and hippocampus were not identified as being significantly connected in either the positive or negative outcome group, and that the parietal operculum, rather than the insula, was reported by the analysis as significant. One possible explanation for lack of significant connections with the amygdala is that the ventral amygdalofugal pathway passes through and around the STN without directly terminating (Lambert *et al*. (2012)) and therefore would not be detected by this

analysis technique as contacting the STN despite passing through and possibly affecting neural activity. Additionally, as discussed above, problems in the segmentation and labelling process might have led to inaccuracies. Although the high-resolution T1 image was used in the registration process to maximise accuracy, further improvements in accuracy and resolution could be achieved in future by increasing the number of diffusion directions and scanning at 3T.

Finally, I was limited in this study by small numbers of suitable participants and restriction to using only questionnaire-based analyses for quantifying changes in autonomic function. A future study incorporating physiological variables such as baroreflex sensitivity, tilt testing or respiratory changes, in a larger number of subjects, would allow more confident conclusions to be drawn about the neural fingerprint of autonomic symptomatic improvements following STN DBS.

CHAPTER 4: STRUCTURAL CORRELATES OF AUTONOMIC SYMPTOMS (AN MRI STUDY)

Introduction

Autonomic disturbances occur in as many as 70-80% of patients suffering from Parkinson's disease (PD) (Zesiewicz *et al.* (2003)) and also may feature in other movement disorders such as essential tremor (Lee *et al*. (2015), Sengul *et al*. (2015)) and dystonia, although autonomic symptoms in dystonia have been less well studied (Conte *et al*. 2016). Autonomic symptoms include cardiovascular, respiratory, gastrointestinal and urinary problems and can be linked to additional non-motor symptoms including cognitive impairment, due to changes in regulation of cerebral blood flow that can occur with autonomic dysfunction (Udow *et al*. (2016)). Structural brain changes associated with autonomic dysfunction in movement disorders have not been clearly defined, although a recent study showed a correlation between baroreflex sensitivity (BRS) and grey matter volume within the left hippocampus, left caudate and left cerebellum superior semilunar modules in PD patients (Chen *et al*. (2016)), demonstrating that degeneration within nodes of the recognised central autonomic network is indeed linked with autonomic symptoms in PD although causality is impossible to establish in this type of analysis. In this study, we aimed to find further evidence to support the association between grey matter atrophy within the central autonomic network and autonomic dysfunction in PD, and introduced a disease-comparison group consisting of age-matched subjects with a non-PD movement disorder, to determine whether the grey matter changes were specific to PD or common to other movement disorder patients.

There is less documented about the involvement of white matter pathways in autonomic dysfunction, despite the fact that white matter pathways connect the grey matter "nodes" of the CAN and are therefore also crucial network components. Using analysis of diffusion-weighted MRI (DWI) we sought to define regions of white matter microstructural change linked with autonomic symptoms. Diffusion-weighted MRI is a technique which allows microstructural integrity of white matter to be inferred in vivo (Lambert *et al*. (2012)) through the analysis of MRI derived metrics including fractional anisotropy (FA), a measure of directional diffusion of water molecules; mean diffusivity (MD), a measure of the total diffusion within a voxel; and axial (L1) and radial diffusivity. Given the growing interest in autonomic symptoms in non-PD movement disorders, a small comparison group made up of subjects with either essential tremor or dystonia was included. The null hypothesis being tested was that patterns of grey and white matter degeneration associated with autonomic dysfunction are not specific to PD but are present in other neurological conditions in which autonomic symptoms develop.

As a secondary analysis, and as a bridge to the second part of the thesis which focuses specifically on bladder symptoms, I sought to use the same approach to study grey and white matter changes associated with bladder dysfunction in PD and non-PD movement disorder subjects.

Methods

Subjects

38 subjects with PD and 19 subjects with a non-PD movement disorder (11 essential tremor/8 dystonia) who were admitted to the Oxford Functional Neurosurgery

Department for deep brain stimulation surgery completed a set of questionnaires including a validated autonomic questionnaire (COMPASS-31) (Sletten *et al*. (2012)). The groups did not differ significantly for age ($p = 0.282$, Mann-Whitney U Test). Mean age for non-PD group was 58.15 years and mean age for the PD group was 64.08 years. Of the PD subjects, there were 27 males and 11 females, and of the non-PD subjects, there were 11 males and 8 females.

Data acquisition: see general methods (chapter 2)

DTI, TBSS and structural image pre-processing: see general methods (chapter 2)

Statistical analysis

Randomise (permutation testing) was implemented in FSL for statistical analysis in all analyses described below unless specifically indicated.

TBSS correlations between measures of white matter microstructural integrity and clinical scores

Correlations between clinical score and FA, MD, L1 and radial diffusivity were determined using permutation-based non-parametric testing applied to voxel-wise general linear model, carried out using the whole brain white matter skeleton with age as a covariate of no interest. This was done first to look for correlations common to both disease groups, and subsequently to look for an interaction between the PD and non-PD group. Results were considered significant for $p < 0.05$, after correction for multiple comparisons (family wise error, FWE) within each region of interest, using the threshold-free cluster enhancement (TFCE) approach (Smith *et al*. (2009)).

Results were visualised using an algorithm that 'thickens' the skeletonised results by filling them out to occupy the local tracts seen in the mean FA map ('TBSS fill').

To test for group differences related to overall autonomic score and the bladder component of the autonomic score, and to assess clinical correlations within the PD and non-PD movement disorder groups separately, autonomic and bladder scores were regressed with the preprocessed TBSS images. A voxel-wise general linear model was applied using permutation-based non-parametric testing, implemented in FSL, with age as a covariate of no interest. For the group comparison, age was demeaned across both groups, but clinical scores were demeaned within each group. The comparisons were carried out for fractional anisotropy, mean diffusivity, radial and axial diffusivity maps for the whole brain white matter skeleton. Results were considered significant for $P < 0.05$, after correction for multiple comparisons within each region of interest using the threshold-free cluster enhancement approach (TFCE), (Smith *et al*. (2009)).

VBM statistical analysis

Correlations between storage symptom score and whole brain grey matter were assessed using permutation-based non-parametric testing via 'randomise' using the grey matter images and template generated by the VBM pipeline. This was run using the COMPASS-31 autonomic score and the bladder score component of the COMPASS-31 questionnaire.

Correlations between segmented grey matter structural volumes and clinical scores

Pearson's Product Moment Correlation between subcortical grey matter volume corrected for head size and clinical score was calculated using IBM SPSS Statistics for Mac (version 20). Results of partial correlations between subcortical structure volume and autonomic/bladder symptoms corrected for age were considered significant for $p < 0.05$ after applying Bonferroni correction for multiple comparisons.

Correlations between structural surface vertices and clinical scores

Additionally, vertex analysis was performed on the amygdala and hippocampus aiming to assess the correlation with autonomic score and bladder score on a per-vertex basis. The meshes were reconstructed in MNI space to remove variance due to differences in head size between individuals. Correlations were tested using the general linear model.

Results

Questionnaire results

There was no significant difference in overall autonomic score for the PD and the non-PD group ($p = 0.191$, independent samples t-test) although the PD scores tended to be slightly higher than the non-PD scores (mean score for PD group 19.5 ± 2.4, mean score for the non-PD group = 14.5 ± 2.9) (figure 4.1). There was no correlation between COMPASS score and UPDRS motor score (off medications) ($p = 0.748$, two-tailed).

We also looked at the difference between the PD and non-PD group for each of the subscores that made up the overall autonomic score. On assessment of the subscores,

there was a significantly higher bladder score for the PD group compared with the non-PD group (p = 0.013, Wilcoxon Signed Rank test, two-tailed); the mean score for the PD group was 1.58 ± 0.31, mean score for the non-PD group was 0.63 ± 0.23). Breaking down the bladder score into its 3 components, the only component of the bladder score which was significantly different between groups was the question about urinary leakage, where PD patients reported more severe symptoms than non-PD patients. Urinary leakage scores correlated with overall autonomic score for the PD group but not the non-PD group (r = 0.453, p = 0.004).

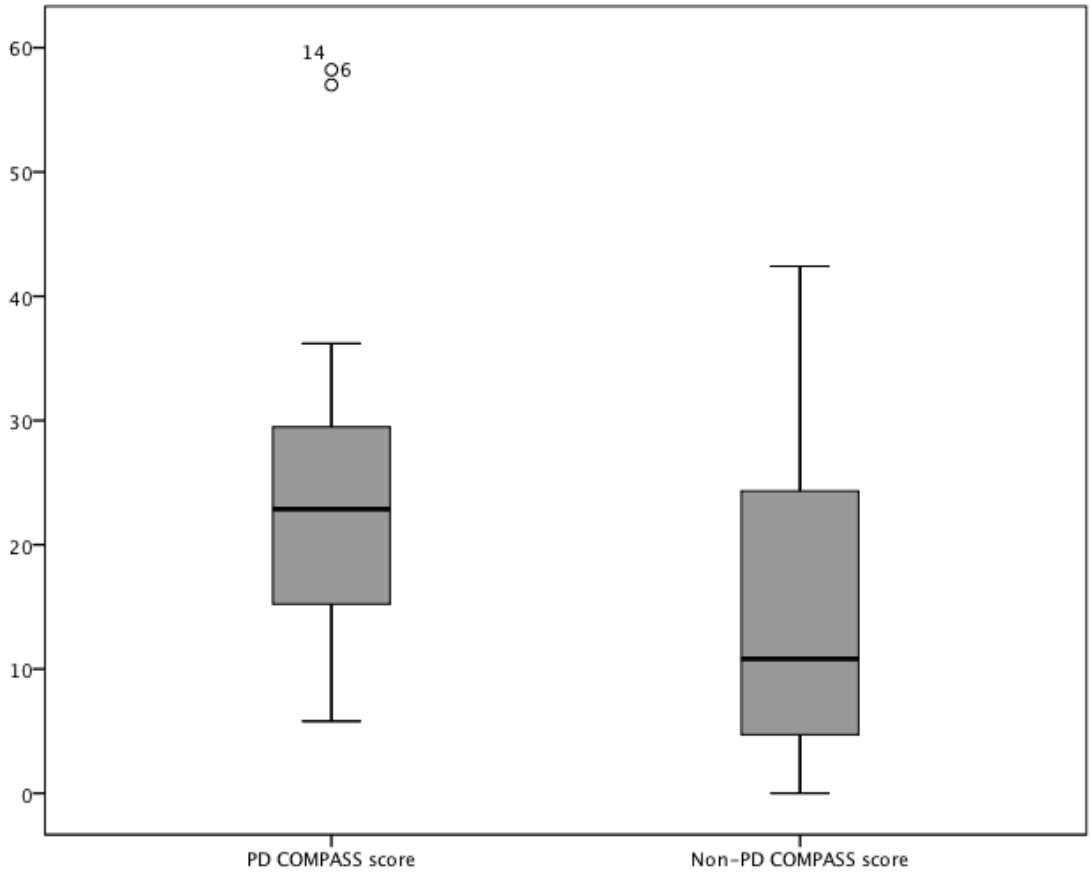


Figure 4.1. Overall autonomic score for the PD and non-PD groups

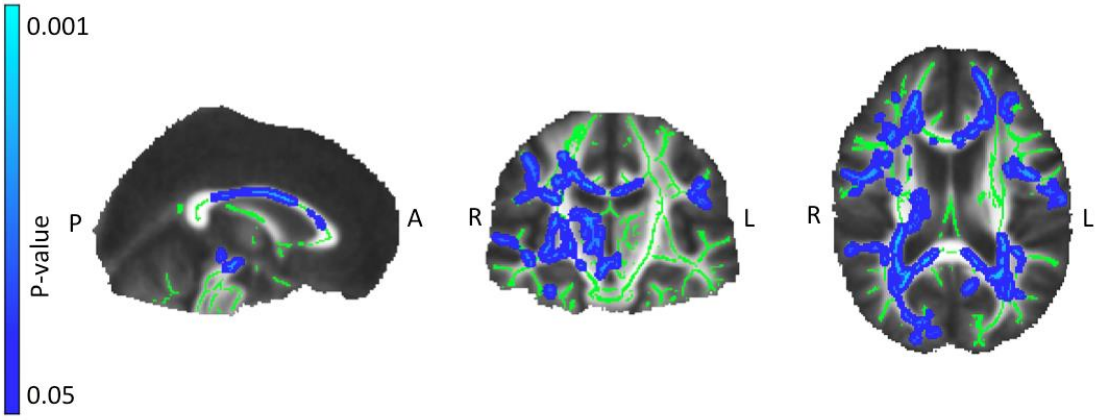
Autonomic score

White matter correlations with autonomic score

There were no significant correlations between COMPASS (autonomic) score and DTI metrics (FA, MD, L1 or radial diffusivity) for the PD group or for the non-PD group.

Combining the two groups into a single "movement disorder" group (n = 57) there were significant correlations between autonomic score and FA (figure 4.2), and autonomic score and radial diffusivity (figure 4.3).

Directly comparing correlations between autonomic score and DTI metrics in the two groups, there was also significant interaction in the correlation between autonomic score and MD (figure 4.4).



X = 90, Y = 108, Z = 90

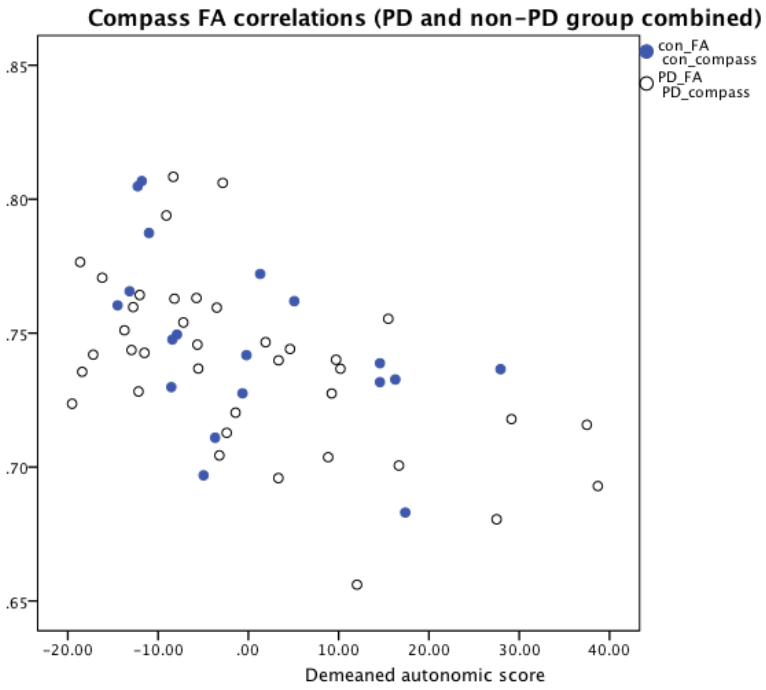
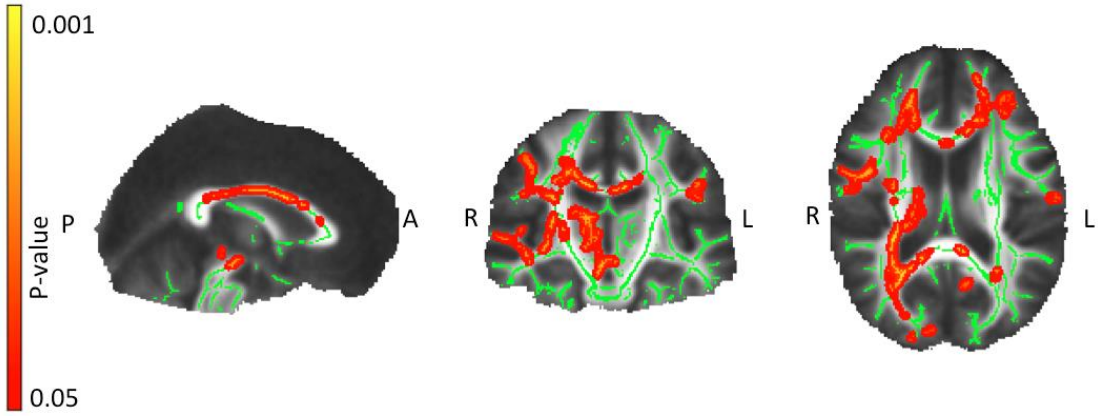


Figure 4.2. Above: areas of significant correlation between autonomic score and FA, revealed across the PD and non-PD groups (green: white matter skeleton, blue-light blue: areas of significant correlation between FA and autonomic score). Below: scatterplot, showing FA value and demeaned autonomic score for both PD (open circles) and non-PD group (blue filled circles).



X = 90, Y = 108, Z = 90

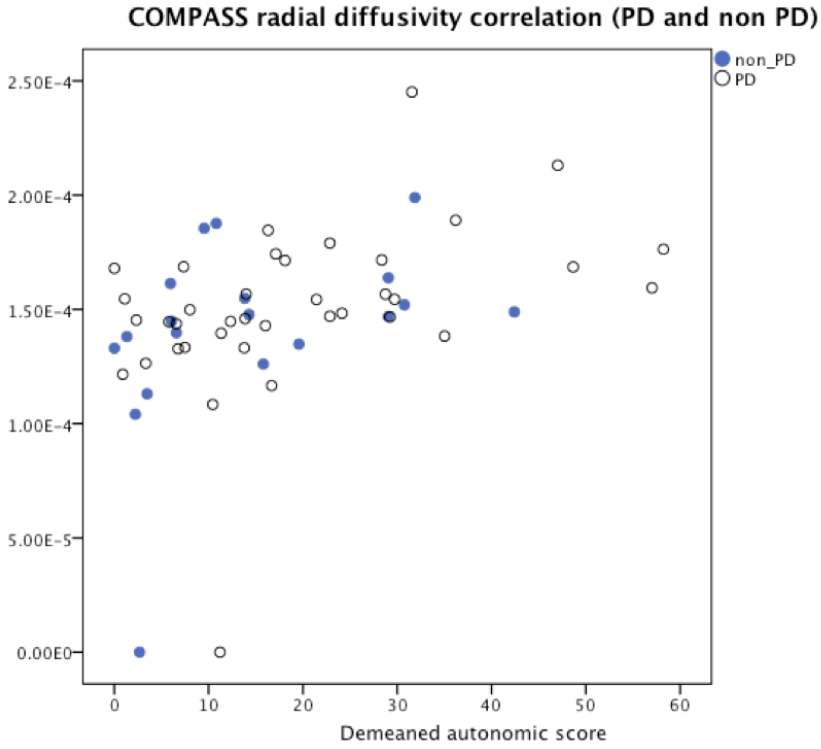


Figure 4.3. Above: Areas of significant correlation between radial diffusivity (RD) and autonomic score for the combined PD and non-PD subjects, (green: white matter skeleton, red-yellow: areas of significant correlation between RD and autonomic score). Below: scatterplot to illustrate relationship between RD and autonomic score in the significant regions, for PD subjects (open circles) and non-PD subjects (closed circles).

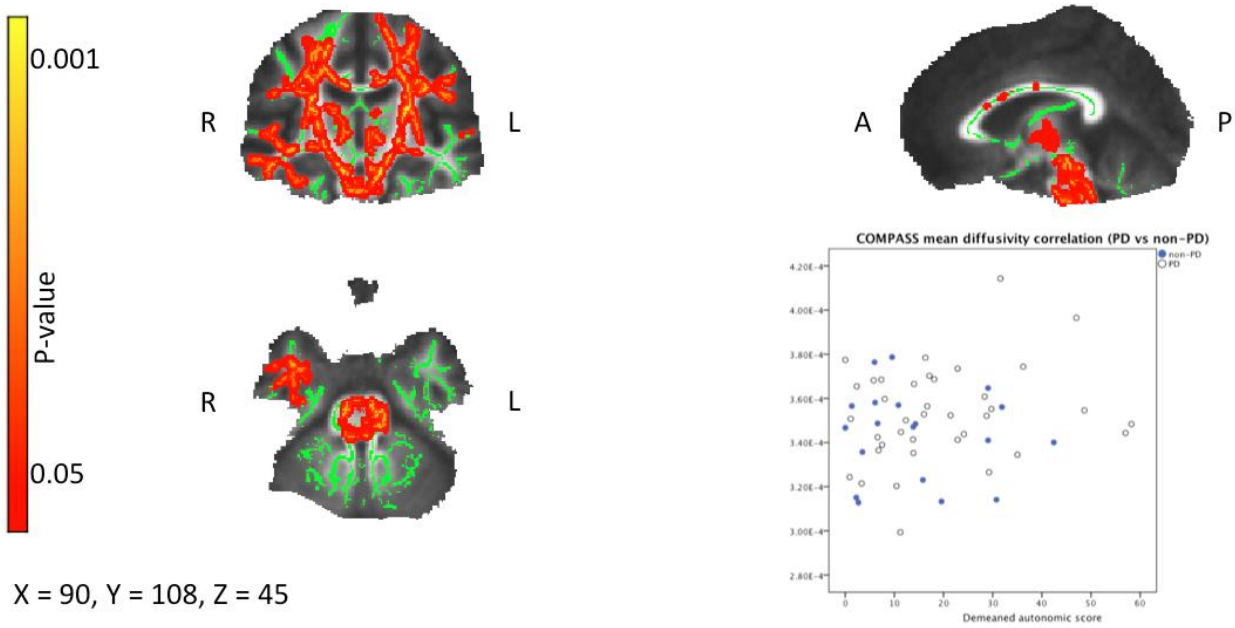


Figure 4.4. Areas of significant interaction between the PD and non-PD group in terms of relationship between mean diffusivity (MD) and autonomic score. Green: white matter skeleton; red-yellow areas of significant interaction. Scatterplot demonstrates interaction between PD (open circles) and non-PD (closed circles) group.

Group	FA	MD	L1	Radial diffusivity
PD	NS	NS	NS	NS
Non-PD	NS	NS	NS	NS
Combined	Significant correlation	NS	NS	Significant correlation
Interaction	NS	Significant correlation	NS	NS

Table 1. Significant correlations between autonomic score and DTI metrics for the two groups separately and combined

Grey matter correlations with autonomic score

Voxel-based morphometry did not reveal any significant correlations between grey matter volume and autonomic score for the PD group, the non-PD group or the combined groups.

Subcortical segmentation of grey matter regions did not reveal significant correlations between the volume of grey matter structures and autonomic score. However, plotting the values for left hippocampal volume and autonomic score for the PD group showed that those with a lower autonomic score appeared to have a larger hippocampal volume than those with a higher score. To investigate this relationship further, the PD subjects were then divided into two groups according to whether their autonomic score was above or below the mean score for the PD group (mean score = 20.08). The left hippocampal volume for the high score group (n = 15, range 21.43-58.23) was compared with the left hippocampal volume for the low score group (n = 21, range 0-18.1). This comparison demonstrated that subjects in the "higher score" group (mean left hippocampal volume = 4357 ± 653) had a reduced left hippocampal volume compared to those in the "lower score" group (mean = 4821 ± 105) $p = 0.02$, t-test, two-tailed (figure 4.5). Supporting this finding, shape analysis revealed a significant correlation between left hippocampal shape and autonomic score in the PD group. (figure 4.6). There was no significant relationship between left hippocampal volume and autonomic score in the non-PD group.

There was also a significant interaction between the PD and non-PD group for the correlation between autonomic score and left amygdala shape (figure 4.7).

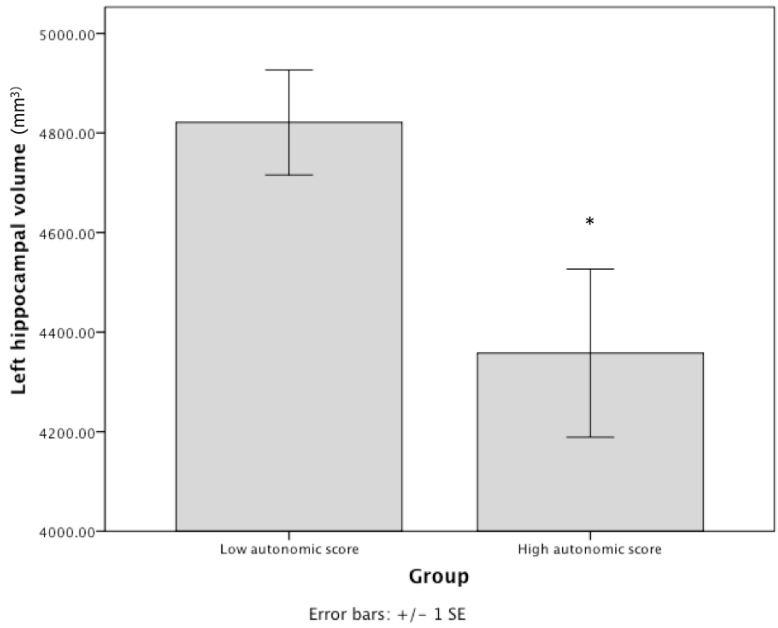
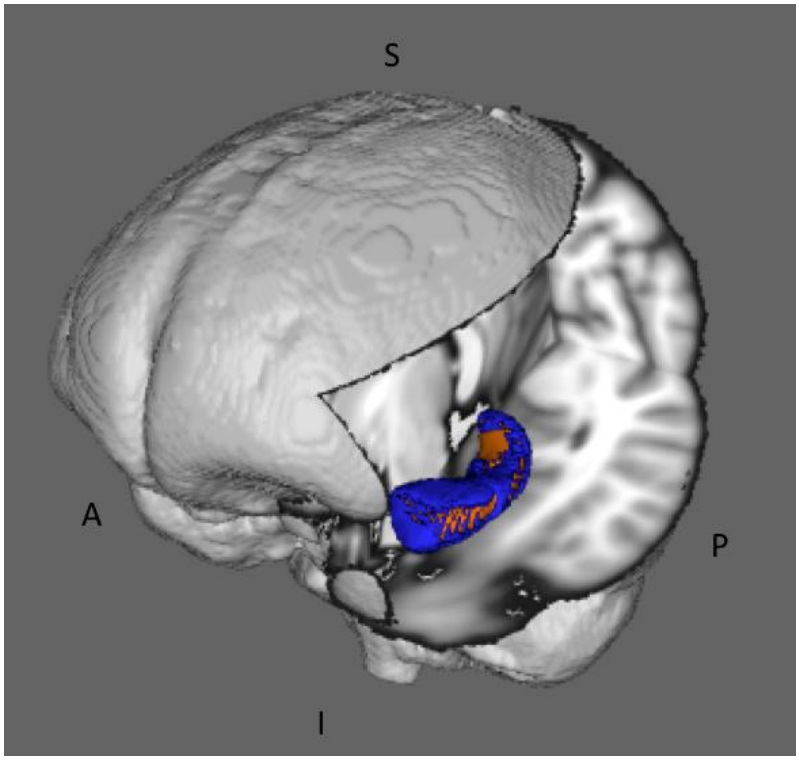


Figure 4.5. Mean left hippocampal volume in subjects with low autonomic score (score under 20) and high autonomic score (score over 20). * indicates significant difference between the two groups ($p < 0.05$). Error bars represent one standard error of the mean.



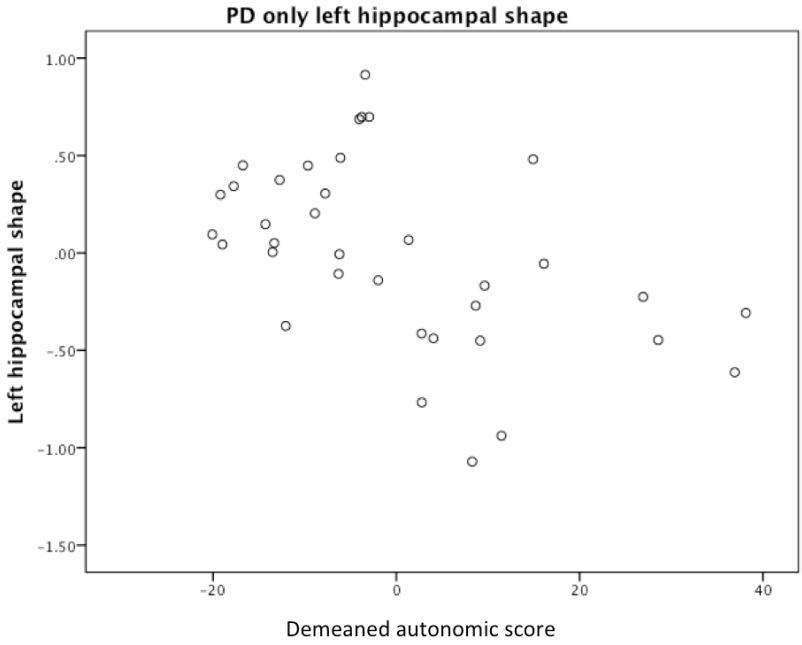
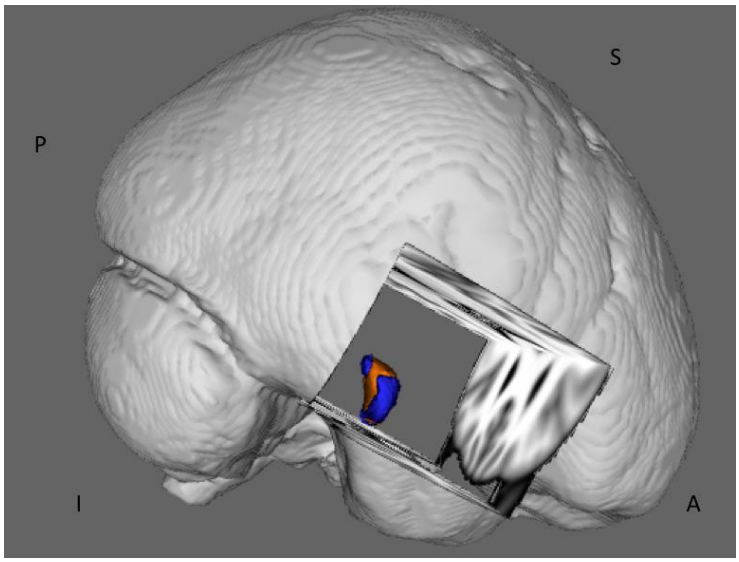


Figure 4.6. Above: 3-dimensional rendering of left hippocampus. Red regions represent areas of significant correlation between vertex dimension and autonomic score. Below: scatter plot to demonstrate correlation between hippocampal shape and autonomic score.



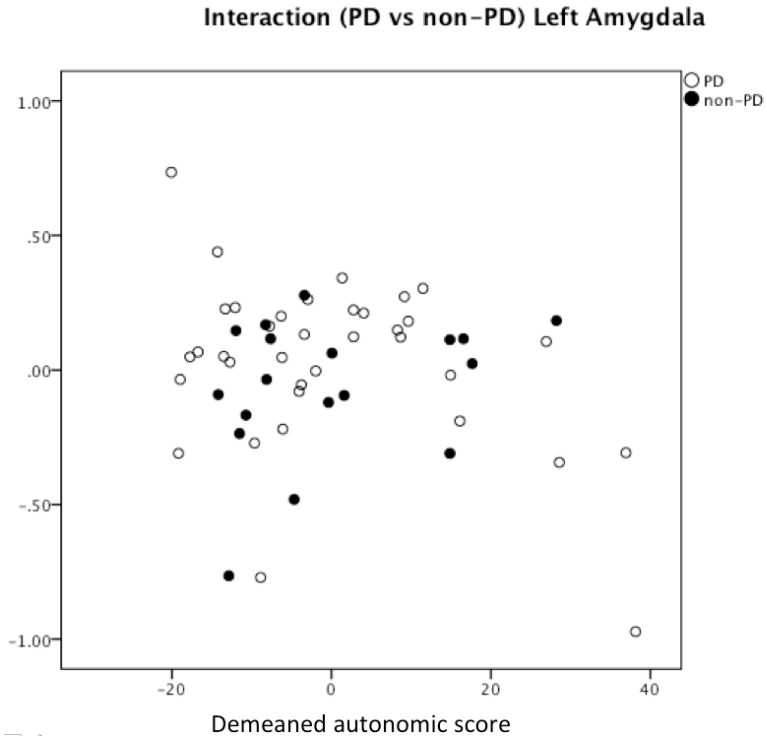


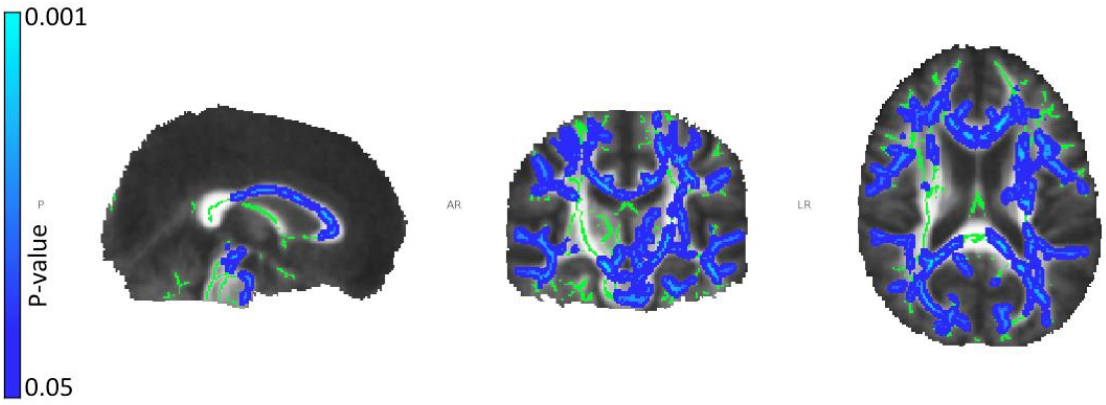
Figure 4.7. Above: 3-dimensional rendering of left amygdala. Red regions represent areas of significant interaction between the PD and non-PD group in terms of their relationship between vertex dimension and autonomic score. Below: scatter plot to demonstrate interaction between PD and non-PD groups with regard to left amygdala shape and autonomic score.

Bladder score

White matter correlations with bladder score

Given that the bladder symptom severity component of the autonomic score was scored more highly for the PD patients than the non-PD group, we analysed patterns of white and grey matter changes associated with bladder score.

Within the PD group alone, there was a significant correlation between bladder score and FA, MD and radial diffusivity (Figures 4.8-4.10).



X = 90, Y = 108, Z = 90

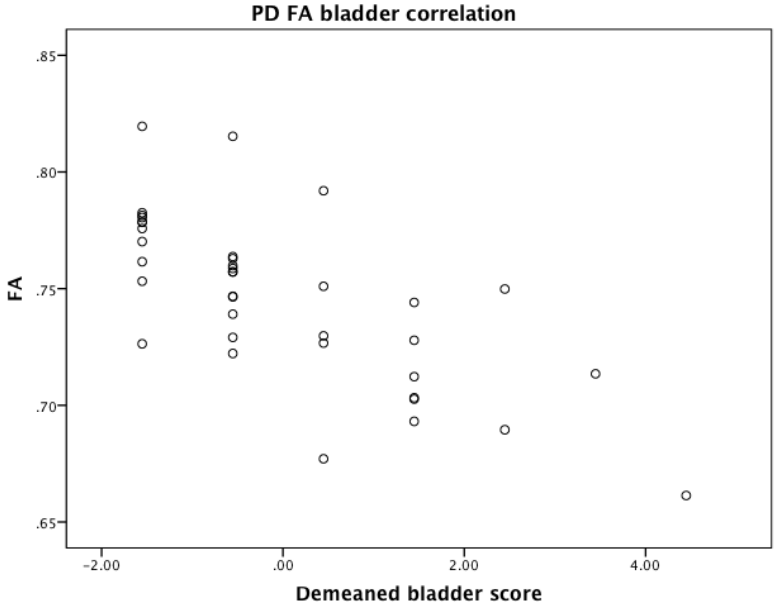
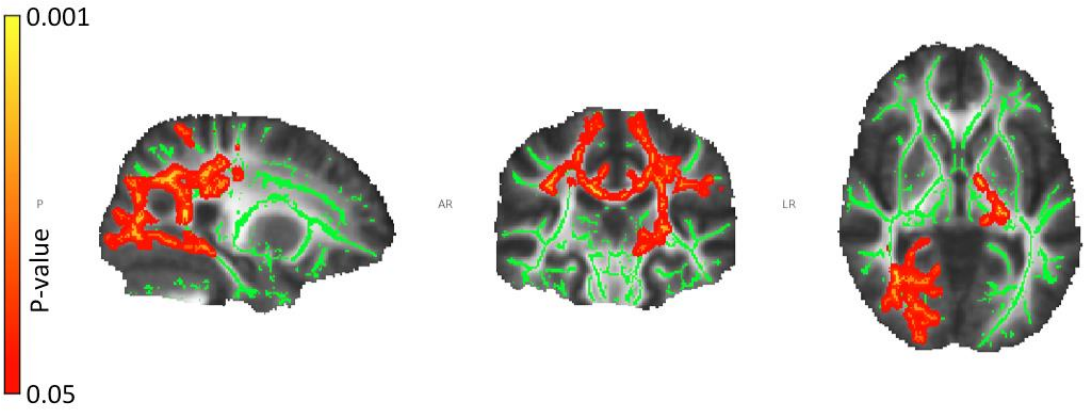


Figure 4.8. Above: areas of significant correlation between bladder score and FA for PD subjects only. Green: white matter skeleton, blue-light blue: areas of significant correlation between FA and bladder score. Below: scatterplot, showing FA value and demeaned bladder score for PD group



X = 90, Y = 108, Z = 45

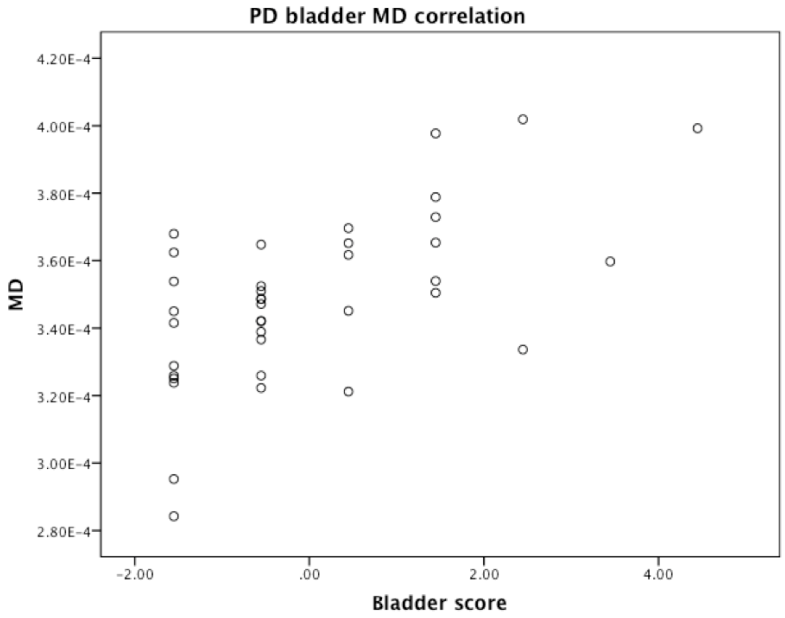
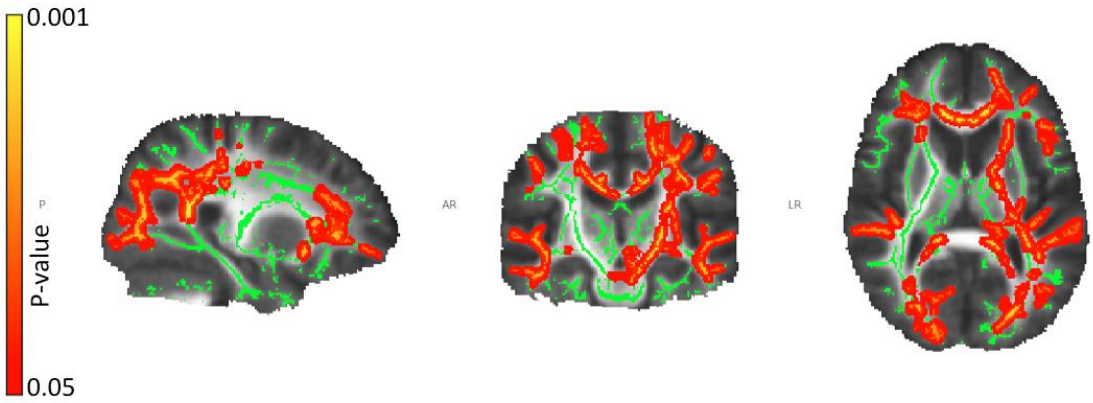


Figure 4.9. Above: areas of significant correlation between bladder score and MD for PD subjects only. Green: white matter skeleton, red-yellow: areas of significant correlation between MD and bladder score. Below: scatterplot, showing MD value and demeaned bladder score for PD group



X = 65, Y = 102, Z = 72

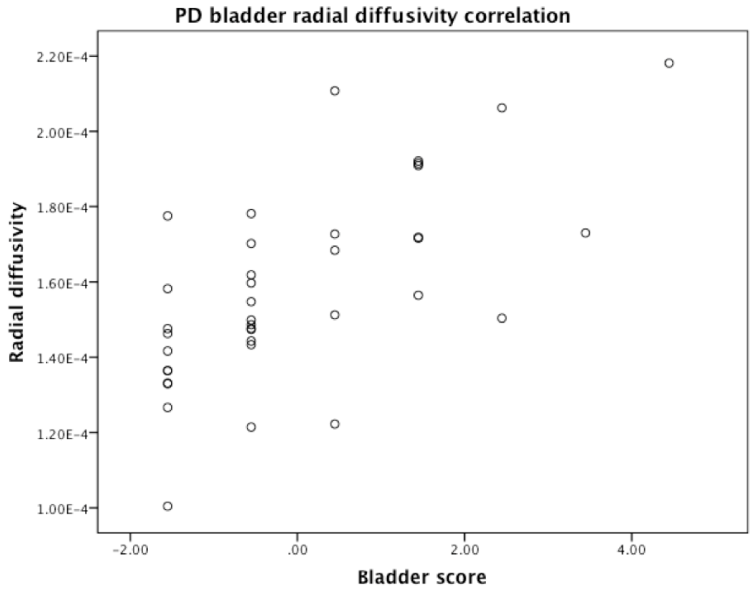
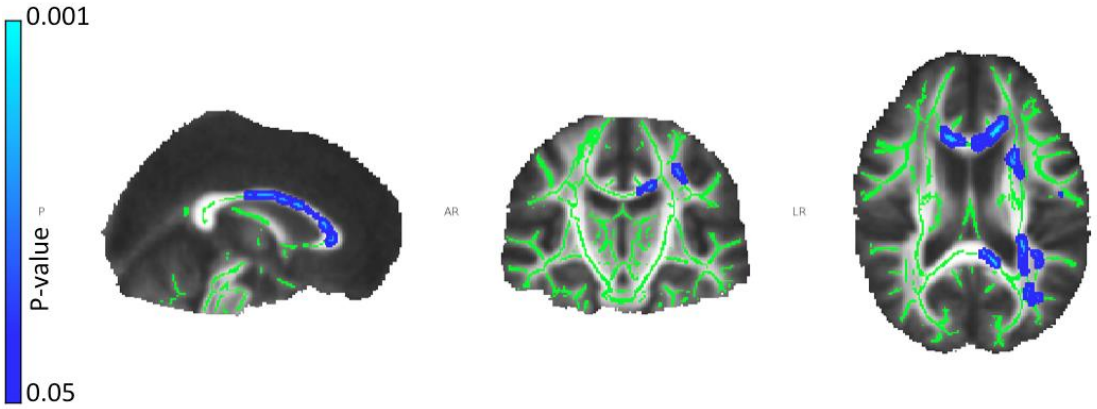


Figure 4.10. Above: areas of significant correlation between bladder score and radial diffusivity (RD) for PD subjects only. Green: white matter skeleton, red-yellow: areas of significant correlation between radial diffusivity and bladder score. Below: scatterplot, showing RD value and demeaned bladder score for PD group

Within the non-PD group, there were no significant white matter correlations with bladder score.

Combining the two groups, there was a significant correlation between FA and bladder score and radial diffusivity and bladder score across the two groups (figure 4.11-12) but also a significant interaction, for FA, MD and radial diffusivity, demonstrating white matter regions where a significant difference in correlation with bladder score existed between the two groups (figure 4.13-16).



X = 90, Y = 108, Z = 90

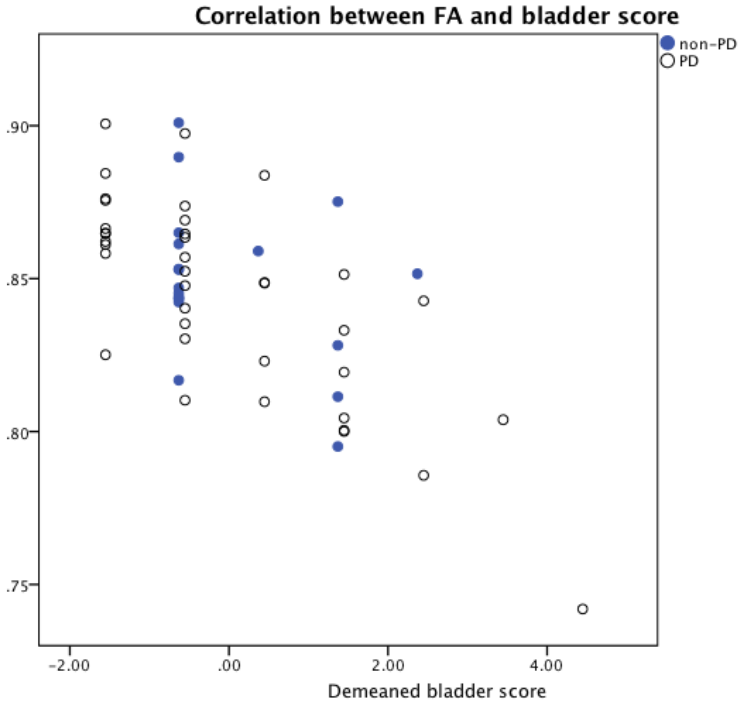


Figure 4.11. Above: White matter regions where FA significantly correlated with bladder score in both PD and non-PD groups. Green: white matter skeleton, blue-light blue: areas of significant correlation between FA and bladder score. Below: scatterplot showing relationship between FA and bladder score in significant regions of the white matter skeleton for PD and non-PD subjects

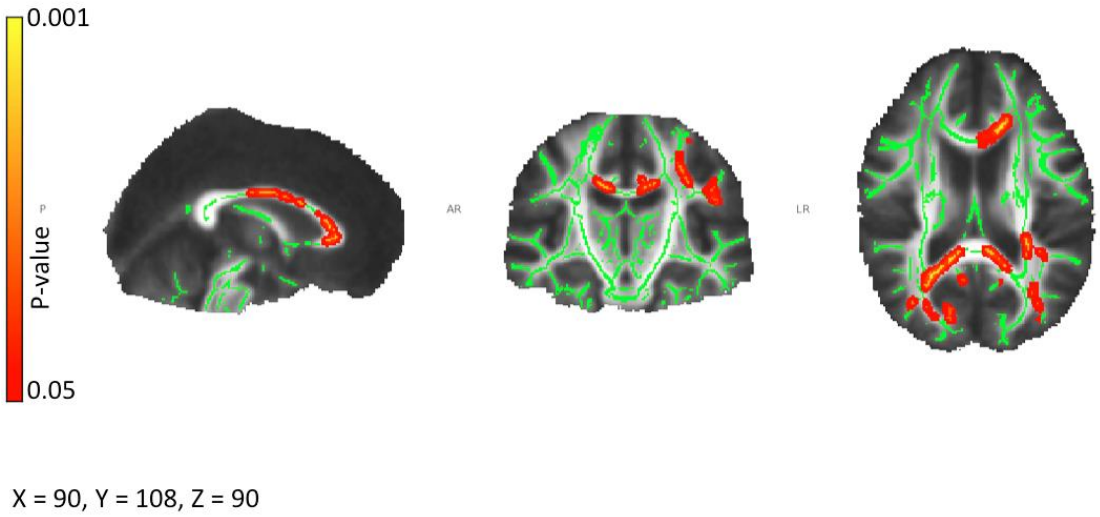
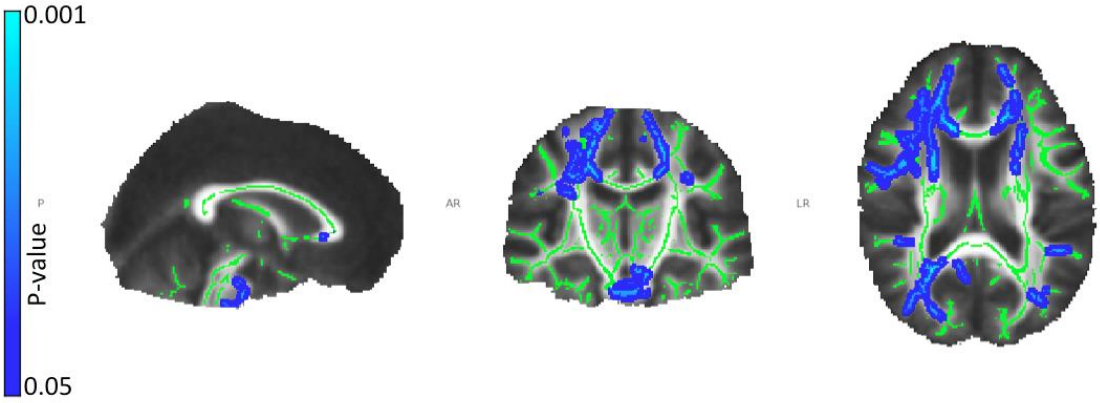


Figure 4.12. White matter regions in which radial diffusivity significantly correlated with bladder score in both PD and non-PD groups. Green: white matter skeleton, red-yellow: areas of significant correlation between RD and bladder score. Below: scatterplot showing relationship between radial diffusivity and bladder score in significant regions of the white matter skeleton for PD and non-PD subjects



X = 90, Y = 108, Z = 90

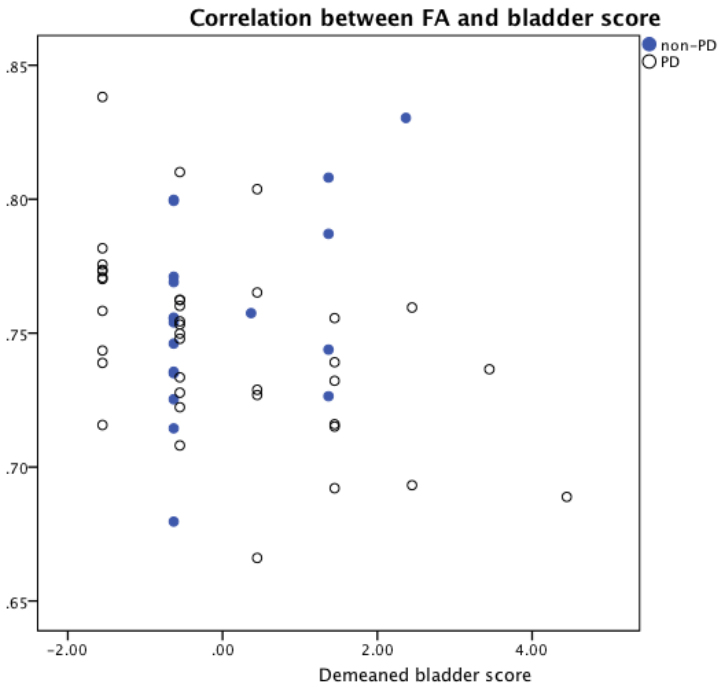
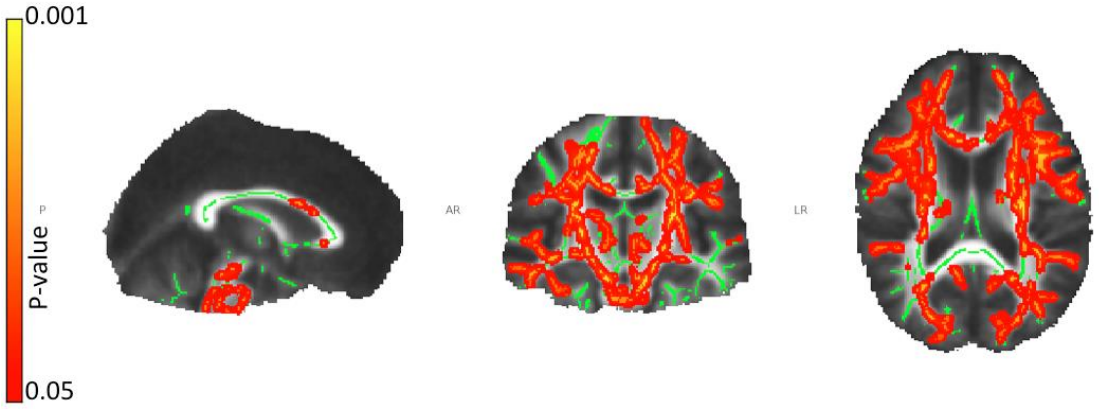


Figure 4.13. White matter regions, which correlated differently with bladder score in the PD vs non-PD group producing a significant interaction. Green: white matter skeleton, blue-light blue: areas of significant interaction between PD and non-PD groups in terms of relationship between FA and bladder score. Below: scatterplot showing FA values and bladder score for the PD (closed circles) and non-PD group (filled blue circles) in the regions where there was a significant interaction.



X = 90, Y = 108, Z = 90

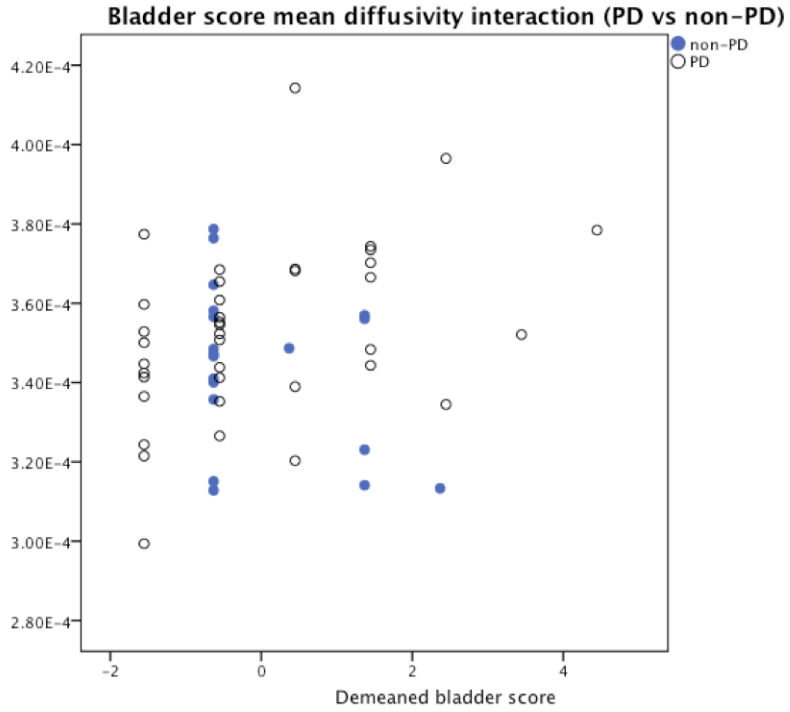
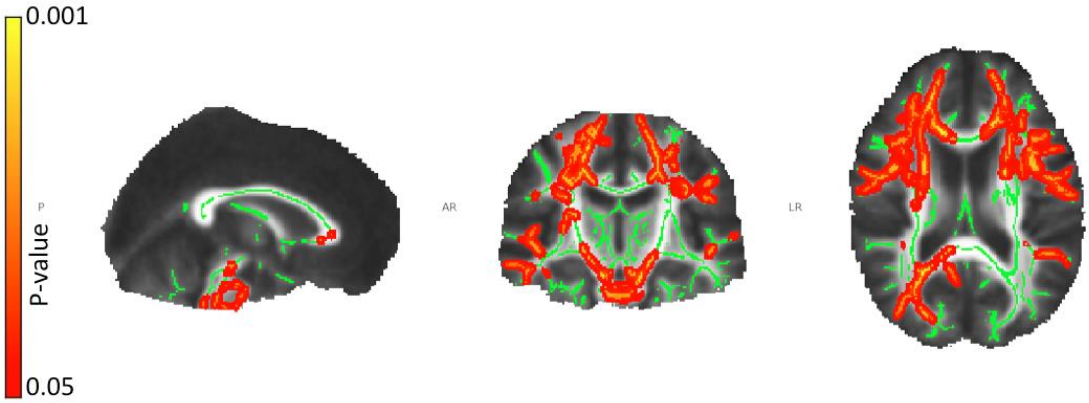


Figure 4.14. White matter regions in which MD correlated differently with bladder score in the PD vs non-PD group producing a significant interaction. Green: white matter skeleton, red-yellow: areas of significant interaction between PD and non-PD groups in terms of relationship between MD and bladder score. Below: scatterplot showing MD values and bladder score for the PD and non-PD group in the regions where there was a significant interaction



X = 90, Y = 108, Z = 90

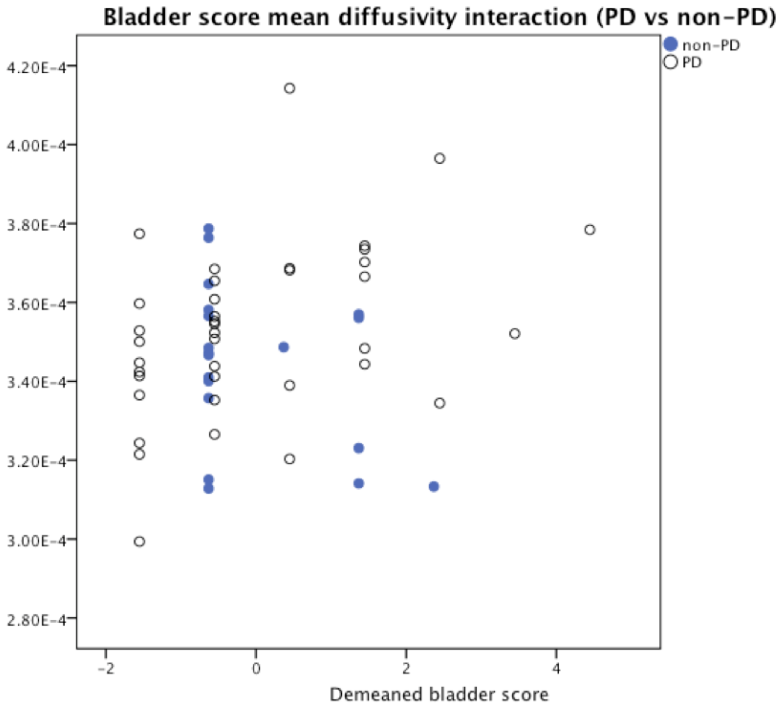


Figure 4.15. White matter regions in which radial diffusivity correlated differently with bladder score in the PD vs non-PD group. Green: white matter skeleton, red-yellow: areas of significant interaction between PD and non-PD groups in terms of relationship between RD and bladder score. Below: scatterplot showing radial diffusivity values and bladder score for the PD and non-PD group in the regions where there was a significant interaction

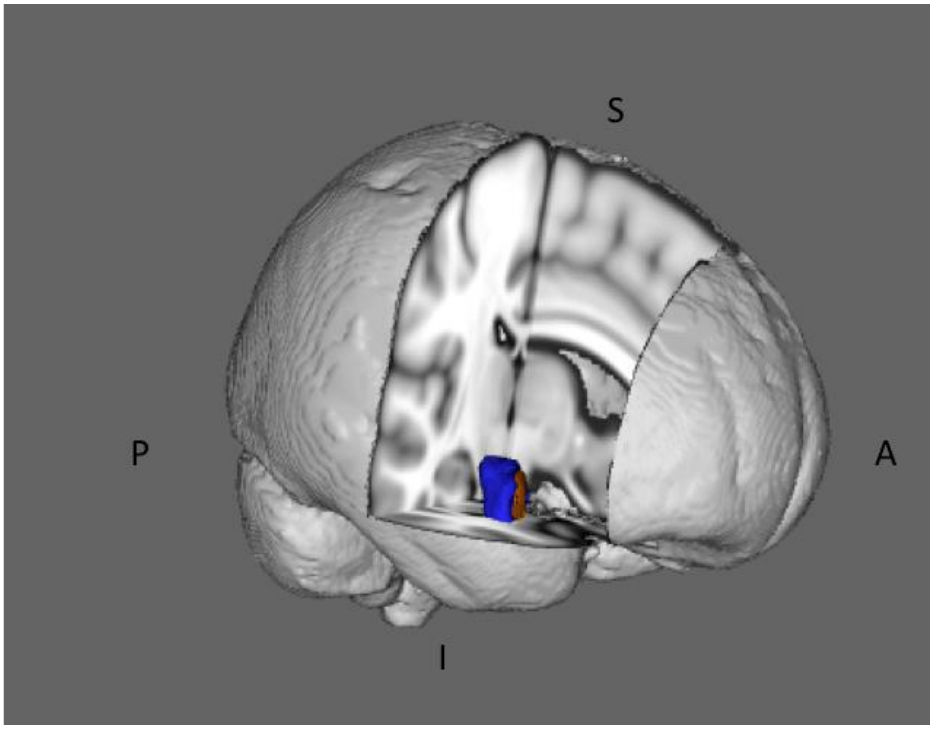
Group	FA	MD	L1	Radial diffusivity
PD	Sig	Sig	NS	Sig
Non-PD	NS	NS	NS	NS
Combined	Significant correlation	NS	NS	Significant correlation
Interaction	NS	Significant correlation	NS	NS

Table 2: Correlations between bladder score and DTI metrics

Grey matter correlations with bladder score

Voxel-based morphometry did not reveal any significant correlations between grey matter volume and bladder score. Subcortical segmentation of grey matter regions did not reveal significant correlations between the volume of grey matter structures and bladder score. Furthermore, vertex-wise comparisons did not reveal any individual group correlations with bladder score for the PD group or the non-PD group.

Shape analysis was also run for the amygdala and hippocampus for both groups combined. This analysis revealed a significant interaction between the PD and non-PD group for the correlation between bladder score and right amygdala shape (Figure 4.16).



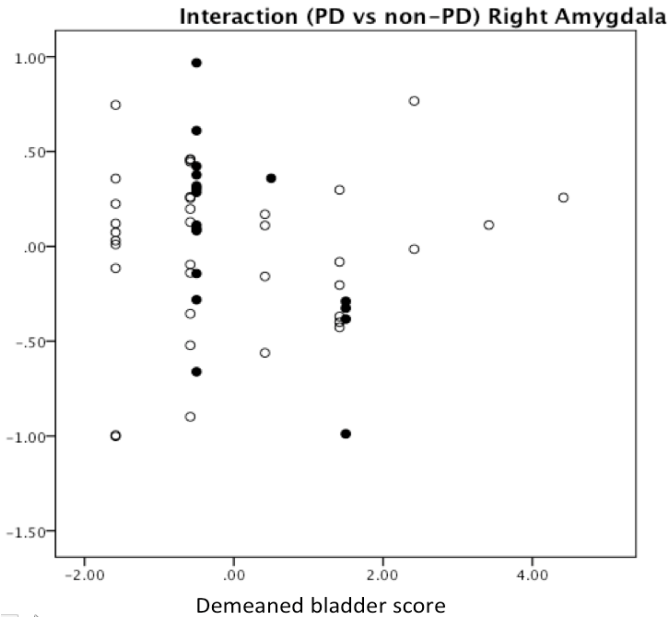


Figure 4.16. Above: 3-dimensional rendering of right amygdala. Red regions represent areas of significant interaction between the PD and non-PD group in terms of their relationship between vertex dimension and bladder score. Below: scatter plot to demonstrate interaction between PD (open circles) and non-PD (closed circles) groups with regard to right amygdala shape and bladder score.

Relationship between bladder score and concentration

Previous studies suggest that bladder control problems are associated with the function of brain regions responsible for attentional systems, such as the dorsal anterior cingulate cortex and prefrontal cortex (Tadic *et al*. (2010), Griffiths *et al*. (2015)). Given the widespread pattern of white matter change associated with bladder symptoms, which implicated frontal and pericingulate white matter pathways, we wanted to assess whether subjects who reported bladder symptoms were also more likely to report attentional deficits. All non-PD subjects and 36 out of 38 PD subjects had answered a "yes/no" question about whether they experienced difficulties keeping their mind on their task during their adult life. Combining PD and non-PD patients, those who had difficulty keeping their mind on their task had a significantly higher bladder score than those who did not report this difficulty ($p = 0.026$, Mann-Whitney U Test).

Discussion

In this analysis, my primary aim was to investigate the white and grey matter structural changes associated with autonomic symptoms in Parkinson's disease. Diffusion-weighted image analysis using tract-based spatial statistics revealed no significant correlation between whole-brain white matter microstructural values and autonomic score as assessed by the COMPASS-31 scale in the PD group alone. However, there was a significant relationship between grey matter (left hippocampal) structural volume and shape and autonomic score in the PD group. This suggests that

autonomic changes in the PD population may be driven by grey rather than white matter changes. It is known that Lewy neurites develop in the hippocampal region in PD (Braak *et al*. (2003)), and a recent study has described reduced left hippocampal grey matter volume in a PD population with autonomic impairment (Chen *et al*. (2016)) while baseline perfusion of the hippocampus has been linked to cardiovagal baroreflex sensitivity (Lasosirpisan *et al*. (2015)). Furthermore, the involvement of the hippocampus in autonomic control is undeniable based on numerous findings in non-PD populations (Harper *et al*. (2000), Critchley *et al*. (2000), Henderson *et al*. (2002), Harper *et al*. (1998)). When the PD subjects were combined with the non-PD subjects, white matter correlations with autonomic symptom severity emerged that were common to both groups. This suggests that there may be mild and non-specific white matter changes associated with autonomic dysfunction, perhaps secondary to impaired cerebral perfusion or other changes occurring in subjects with poor autonomic control (Udow *et al.* (2016)). There were also some areas of white matter interaction, where the relationship between autonomic score mean diffusivity was different in the PD and non-PD group. Unlike the shared areas of correlation, the interaction included brainstem regions, suggesting that microstructural changes in the brainstem may be more common in PD than non-PD subjects, which fits with the progressive ascending pattern of pathological changes attributed to PD (Braak *et al*. (2003)).

Considering the relationship between grey and white matter structural variables and bladder symptoms revealed a different pattern. In the case of bladder symptoms, strong correlations with white matter metrics were present for the PD group but not the non-PD group, when each group was considered separately. This finding is very

consistent with theories that bladder dysfunction in general is closely related to white-matter disease (Sakakibara *et al*. (2014), Smith *et al*. (2015)), although this theory is not undisputed (Wehrberger *et al*. (2014)). Moreover, when the two groups were combined, significant correlations with white matter and bladder score were present across the groups, further supporting this view.

An interaction in the relationship between right amygdala shape and bladder symptoms for the PD and non-PD group was observed, although the low dynamic range of the bladder score makes the result less convincing. Nevertheless the amygdala is closely connected with the insula and cingulate cortex which are frequently activated in brain imaging studies of the bladder, so its involvement in bladder control in these patient groups is not difficult to believe.

Attention and bladder control

A post-hoc analysis of questionnaire responses showed that subjects with concentration difficulties across both groups were significantly more likely to have problems with bladder control. This link between concentration/attentional control and bladder control should be further investigated in future studies to understand the neural basis of bladder control more fully. Brain regions commonly activated in fMRI studies of bladder filling include the dorsal anterior cingulate cortex and prefrontal cortex (Griffiths *et al*. (2007)) which are important in attentional allocation (Bishop 2009) and white matter changes that affect these prefrontal networks could be involved in continence problems in the elderly and those with neurodegenerative conditions.

Implications for deep brain stimulation

Accolla *et al*. (2015) found that more ventral subthalamic areas showed increased connectivity to medial temporal regions including amygdala and hippocampus. Thus ventral STN DBS might well be expected to improve autonomic symptoms more than dorsal STN DBS.

Limitations

The major limitation of this study was the reliance on questionnaire data rather than true physiological measurements of autonomic and bladder function. A future study taking a similar approach to white and grey matter analysis but employing sensitive physiological measures in addition to questionnaires would be more powerful and enable stronger conclusions to be drawn.

CHAPTER 5: LOCAL FIELD POTENTIALS AND THE CONTROL OF THE PELVIC FLOOR AND URINARY VOIDING

Introduction

Different techniques have been used to study the supraspinal control of voiding in human subjects, predominantly utilising neuroimaging, as discussed in the introduction. There has been very limited use of MEG or EEG to study central mechanisms of bladder control, probably due to the better spatial resolution of fMRI (Hutchison *et al*. (2014)), and to our knowledge, at the time of writing, there have been no reports of the use of local field potential recordings to study the role of deep brain nuclei in bladder control. Indeed, the whole area of visceral control has not been addressed in the LFP literature. In the general introduction (chapter 1), I discussed some of the inherent limitations of the BOLD signal for understanding the central bladder network. These included general limitations of BOLD in that it is a surrogate marker of neuronal activity with many additional contributors to the signal that need to be controlled for; and more specific challenges including the difficulty of using fMRI to image small subcortical structures which appear to be significant in the central bladder network, and the fact that participants have to adopt a posture which is not physiologically normal for voiding, thus brain activity may not reflect naturalistic brain responses to bladder filling or voiding. In this chapter, I investigate the possibility of using local field potentials (LFPs) to derive further understanding about the brain-bladder control network and the pathophysiology of urinary symptoms in Parkinson's disease. LFPs represent a complementary and in some ways superior approach to fMRI because the LFP is thought to be a more direct measure of neuronal activity though nevertheless still reflecting similar processes to those represented by the fMRI signal (Magri *et al*. (2012)) and also because whereas there are potential

technical limitations concerning the use of fMRI to resolve small subcortical structures, (particularly in the brainstem where there are artefacts due to pulsatile CSF flow), LFP recordings have excellent spatial resolution and can be informative about the activity within these small structures. Finally, identifying signals that correspond to particular physiological events or which are bladder-relevant pathophysiological signatures can represent potential input signals for intervention using closed loop stimulation in the future (Little *et al*. (2013), Tan *et al.* (2016)). This advantage is unique to the LFP, because it could be continuously recorded via an implanted DBS electrode in a future hypothetical scenario. In this introduction, I will outline the current state of knowledge about LFP signal from the four regions of interest described in the experiment and will also justify my choice of experimental paradigm. For a more general discussion about the nature of the local field potential, I refer the reader to the introduction.

LFP recordings from human subcortical structures

In this chapter, I describe LFP signals recorded from the STN and GPi, and in the following chapter, from the PAG and VPL. The choice is primarily based on loci available in Oxford for recording, nevertheless, all of these structures have been implicated in bladder control (see introduction). The main aim of these studies was to investigate whether any meaningful LFP signals could be found to relate to urinary tasks or symptoms, thus demonstrating that LFP recording could be a useful technique to further the field of neuro-urology.

Although no bladder-related LFPs have been studied from any intracranial loci in humans, LFP signals in general recorded from the human STN and GPi for PD have been relatively better investigated than the PAG and VPL (implanted for pain), mostly due to greater caseloads of patients for STN and GPi surgery. In particular, beta band LFP activity in PD has been well-described. Beta oscillations are thought to be a pathophysiological signature in PD (Neumann *et al*. (2016)), and recordings from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treated monkeys and PD patients indicate that elevated oscillatory synchrony in the beta band in the STN and GPi are one of the hallmarks of reduced dopaminergic input to the basal ganglia (Levy *et al*. (2000), Levy *et al*. (2002), Marceglia *et al*. (2006), Nini *et al*. (1995), Brown *et al*. (2001), Priori *et al*. (2002). Both DBS and dopaminergic medications reduce beta power (Brittain *et al*. (2014), Kuhn *et al*. (2016)), and the presence of beta oscillatory neurons in the STN correlates with patient benefit from dopaminergic medications (Weinberger *et al*. (2006)). Beta band signals are increased during tonic movement and reduced during the preparation for movement (Williams *et al*. (2005)), mental imagery (Kuhn *et al*. (2006)) and active movement itself (Engel & Fries (2010)). Given the closely intertwined relationship between the beta rhythm and the dopamine-related pathophysiological mechanism of PD, it might be expected that any bladder symptoms specifically related to this same pathophysiological process would also show correlations with beta band activity. We would expect such symptoms to improve with DBS, and perhaps even to correlate with the improvement of beta-related symptoms.

The role of other frequency band oscillations in the STN and GPi have also been investigated. Gamma band activity in particular has been demonstrated to be

informative about movement, including force of movement (Bruke *et al*. (2012)) and speed of movement (Joundi *et al*. (2012)) and can be complementary to beta in this regard, in the sense that when both gamma and beta are taken into account, they may provide an accurate prediction of motor parameters as demonstrated in a study of grip strength (Tan *et al*. (2016)). In the STN, activity within theta LFP band has been linked to the inhibition of impulsive actions during conflict (Zavala *et al*. (2015)).

Paradigm choice

Three main fMRI paradigm-types have been used to investigate the neural basis of urinary voiding. Pelvic floor contraction and relaxation during functional MRI has been used as a surrogate marker of urinary voiding (Seseke *et al*. (2006), (2008)), because the relaxation of the pelvic floor musculature is an integral part of voiding (Blok *et al*. (1997b)). A distributed network of brain areas, including the pons, PAG, cerebellum, thalamus, putamen, inferior frontal gyrus, occipital cortex, inferior parietal lobule, pre-SMA, SMA and sensorimotor cortex, and in males also the anterior cingulate cortex, have all been described as activated during pelvic floor activity in subjects with a full bladder (Seseke *et al*. (2006), (2008)), presumably representing the various sensory, motor and continence-maintaining elements all active during this process. This type of approach to understanding the neural basis of voiding essentially replicates as many muscle movements as possible that are associated with voiding, without actually asking a subject to fully void. Therefore, it would be expected that some commonalities exist between neural activations evoked by this task and urinary voiding, but that voiding would also have some associated differences, since the contraction of the detrusor muscle and relaxation of the external urethral sphincter does not occur in the pelvic floor task. A second approach has been

to investigate the psychophysiological aspects of bladder fullness and urinary voiding by asking subjects to "concentrate on sensations arising from the bladder and to call forth the urge to void as if initiating micturition, however, without actually passing urine"- essentially requiring subjects to imagine the sensory and cognitive experience of desiring and initiating voiding (Kuhtz-Bushbeck *et al*. (2009)). This task was associated with activation of the SMA, insular cortex, frontal operculum bilaterally, as well as the right middle frontal gyrus, right inferior parietal lobe and right angular gyrus. The PAG showed a tendency to activate ($p = 0.072$). It was also found that the PAG and right insula were more active in the full bladder condition than the empty bladder condition. The approach should be expected to reveal brain networks associated with paying attention to a full bladder and perhaps also preparing for, or anticipating urinary voiding, since the full bladder is a natural cue for voiding and focussing on these cues might evoke some anticipatory effects. Finally, other studies have examined the effect of micturition itself on patterns of brain activity during PET or fMRI scanning. Blok *et al*. (1997b) described increased blood flow in the right dorsomedial pontine tegmentum, the periaqueductal grey area, the hypothalamus and the right inferior frontal gyrus during voiding in male volunteers (Blok *et al*. 1997b), and increased blood flow in the right inferior frontal gyrus and right ventral pontine tegmentum in subjects who were unable to void despite attempting to do so. During fMRI scanning, micturition activates an extensive network of brain areas including the cerebellum, thalamus, caudate, lentiform nucleus, red nucleus, SMA, postcentral gyrus, anterior and posterior cingulate cortex, insula, left superior frontal gyrus, parahippocampal gyrus, inferior temporal gyrus, inferior frontal gyrus, orbital surface of frontal lobe precuneus, cuneus, occipital cortex and pons (Krhut *et al*. (2012), Shy *et al*. (2014)).

In choosing a paradigm type, it was important to include tasks that had some comparator in the LFP literature in order to be able to interpret the results. Motor activation tasks are commonly reported in LFP studies (Tan *et al*. (2013), Joundi *et al.* (2012), Green *et al.* (2007)), thus a paradigm which included pelvic floor relaxation as a condition was a good choice. Surprisingly, there are also published studies with a motor imagery or anticipation component in the LFP literature (Kuhn *et al*. (2006); Green *et al.* (2007)). Kinaesthetic imagery has been shown to involve a similar network of motor areas as the motor act itself and has been used in experimental paradigms to dissociate neural activity associated with sensory feedback from neural activity associated with motor planning (Kuhn *et al*. (2006)). Thus, given the fMRI literature concerning both pelvic floor relaxation/contraction and kinaesthetic imagery of voiding; and the LFP literature including motor control and imagined and anticipated motor events it seemed sensible to use similar designs for this study of lower urinary tract control. It would then be possible to include a "void" condition despite the absence of literature on the LFP response to visceral motor tasks because it could be compared to the motor and motor imagery results.

A bladder-filling paradigm was not included in this experiment, even though it is the dominant modality in the fMRI bladder literature. This is because, for a preliminary study, there were a number of disadvantages associated with this approach. In particular, many patients in their intra-operative week were reluctant to participate in an invasive procedure involving catheterisation, and furthermore, the experiment would have been challenging to carry out with a single experimenter due to the need to monitor the bladder and the LFP signals simultaneously. In addition, there are no

published LFP studies describing neural responses bladder filling. It seemed sensible to begin with a simple paradigm that many patients would find acceptable and that had a good foundation in previous research, rather than a more ambitious protocol that only one or two would consent to. Nevertheless, chapter 5 of this thesis does describe the results from a single subject who consented to have LFPs recorded during bladder filling.

Furthermore, and perhaps more importantly, there is no precedent for LFP recordings during bladder filling but there is an extensive literature describing LFP responses in PD during various motor tasks. For a completely new area of LFP experiments, it made sense to design a paradigm that would be in some ways comparable to existing LFP data, as well as neuroimaging data. Thus I decided to focus on a pelvic floor task and to use LFP signals during pelvic floor activity to correlate with reported urinary symptoms in PD patients. Finally, imagined void has been used in the fMRI literature and the LFP signal response to imagined motor actions has been described (Kuhn *et al*. (2006)), which may be a very significant point as regards brain-machine interfaces. Thus, an imagined void condition was also included to identify whether there was a specific fingerprint associated with imagined void that could be used to trigger a system to contract the bladder.

Subjects and Methods

Subjects

Subjects were recruited for the voiding study during the week between stage 1 and stage 2 DBS surgery, when the intracranial lead was still externalised and therefore available to record from. Participation was normally on day 3-6 after stage 1 DBS. Subjects with electrodes placed in the STN, GPi or PAG/VPL were recruited.

The task was explained to subjects at least 24 hours prior to the experiment in a written information sheet and later discussed face to face with the experimenter, either at the time of recruitment or prior to the experimental session. Subjects were asked to drink plenty of fluid and refrain from voiding prior to the time scheduled for testing, in order to have a full bladder. All subjects continued their normal anti-Parkinsonian or pain medications in order to capture LFPs in a physiologically "normalised" state (Joundi et al 2012; Tan et al 2016).

Subjects completed an ICIQ questionnaire at the time of testing (see appendix 1).

Task

The experiment consisted of 3 separate paradigms, the first two of which were conducted with eyes closed, and the third with eyes closed if possible for the subject.

The first paradigm required the subject to "imagine voiding", i.e. bring to mind the experience and sensation of voiding. This was done for 10 seconds, followed by a 10 second rest period. The beginning of each "imagine void" trial was signalled by an auditory "start" cue, and the beginning of each "rest" trial was signalled by an auditory "stop" cue. At least five trials were completed for each subject.

The second paradigm required the subject to “relax” their pelvic floor muscles over a ten second period. Each “relax” trial was preceded by a 10 second “contract” trial, whereby the subject was required to maintain a contracted pelvic floor. The contract trial was included because it was perceived that it would be difficult for subjects to voluntarily relax their pelvic floor muscles from a neutral position. However the trial of interest was the “relax” pelvic floor trial and this alone was used in the subsequent analysis.

The third paradigm was the voiding trial. Subjects voided as they would normally do, and uroflowmetry was performed wherever possible. Subjects were asked to initiate voiding with an auditory “start” signal. Subjects then attempted to initiate voiding, and indicated to the experimenter when flow had started. As many 10-second voiding trials as possible were recorded until the bladder was empty: i.e. after a 10 second void, the subject was asked to “hold” the void and re-start when signalled by the experimenter, after a pause of at least 10 seconds.

For the PAG/VPL subject (see chapter 7), the “imagine void” and “relax pelvic floor” tasks were repeated 9 times, in order to achieve a sufficient number of trials to perform meaningful statistics in a single subject. Also, in this subject, a “void” trial was counted as a 10 second trial in which the subject was attempting to void, either successfully (n = 4) or unsuccessfully (n = 5), again in order to generate enough trials for analysis.

RESULTS

Patients, surgery and electrode localisation

6 subjects with GPi DBS and 4 subjects with STN DBS were recruited and provided informed consent for participating. Within the GPi group there were 6 patients, mean age 53 (range 40-61); within the STN group there were 4 subjects, mean age 61 (range 56-71). Neither UPDRS nor ICIQ scores were available for subject 40 and this subject was therefore not included within the correlational analyses described here. For the other subjects, the mean UPDRS motor score was 54 (off medications) and 24.8 (on medications) in the GPi group, and was 51 (off medications) and 26.25 (on medications) in the STN group. The mean total ICIQ score was 13.4 in the GPi group and 10 in the STN group.

Demographic table

Subject ID	Electrode location	Electrode type	Age	Sex	Diagnosis	UPDRS off state	UPDRS part III off/on	ICIQ score
24	GPi	3387	54	M	PD	127	57/29	25
29	GPi	3387	61	F	PD	113	48/17	24
34	GPi	3387	58	M	PD	48	50/29	10
31	GPi	3387	48	M	PD	82	49/18	4
36	GPi	3387	58	M	PD	98	66/31	4
40	GPi	3387	40	M	PD	-	-	-
25	STN	3389	71	F	PD	92	53/24	16

26	STN	3389	56	F	PD	94	61/35	20
30	STN	3389	62	M	PD	90	63/38	2
39	STN	BS	55	M	PD	63	27/8	2

Table 1: Patient demographic details and information about electrode location and electrode type, as well as clinical scores. ICIQ refers to the International Consultation on Incontinence Questionnaire (see Appendix I) and UPDRS refers to the Unified Parkinson's Disease Rating Scale.

Statistical analysis

For the statistical analysis, n refers to the number of observations per trial for each nucleus examined (See Green *et al*. (2007), Magri *et al*. (2012)). For the STN subjects, there were 4 subjects and 7 nuclei (3 subjects had bilateral STN electrodes and one had a unilateral electrode), giving a total of 35 observations per condition. The contact pair with the highest signal in the beta band was selected for analysis in this group. For the GPi subjects, there were 6 subjects and 11 nuclei (5 bilateral and 1 unilateral recording). For the statistical analysis, correlations between LFP power in a certain condition and clinical score were performed using Pearson's correlation and considered significant at $p < 0.05$ (two-tailed).

Data exclusion

One GPi subject and one STN subject's data were excluded from the voiding analysis due to very short time duration of voiding periods (pt 24) and poor signal quality probably due to displacement of the ground electrode whilst the subject was changing position ready to void (pt 25).

Local field potential results

Beta and voiding symptoms

For the PD patients with a completed ICIQ questionnaire, the LFP signals were explored to identify correlations between LFP value and clinical measures. There were a total of 9 subjects with completed ICIQ questionnaires (5 GPi and 4 STN). There was a significant correlation between beta power during the "relax pelvic floor" condition and the severity of voiding symptoms as measured by the voiding score on the ICIQ ($r = 0.696$, $p = 0.037$, Pearson Correlation two-tailed), (figure 5.1). There was no correlation between the beta signal and the incontinence score, frequency score or overall ICIQ score. As the beta signal is recognised as a pathophysiological signal associated with the disease process in Parkinson's disease, we tested the correlation between voiding score and total pre-operative UPDRS. There was a highly significant correlation between severity of voiding and the pre-operative UPDRS score ($r = 0.818$, $p = 0.007$, Pearson Correlation two-sided), but not with the incontinence score, frequency score or overall ICIQ score (figure 5.2). As would be expected given these findings, the beta signal in the "relax pelvic floor" trial also significantly correlated with UPDRS ($r = 0.704$, $p = 0.034$, Pearson Correlation, two-tailed). This suggests that the voiding impairment described by the ICIQ questionnaire is closely related to the core pathophysiological processes in PD that are connected with the presence of the beta signal and represent a bladder or sphincter bradykinesia (Christmas *et al*. (1988)). Interestingly, there were no significant correlations between the motor component of the UPDRS and voiding score or the theta band during relax pelvic floor.

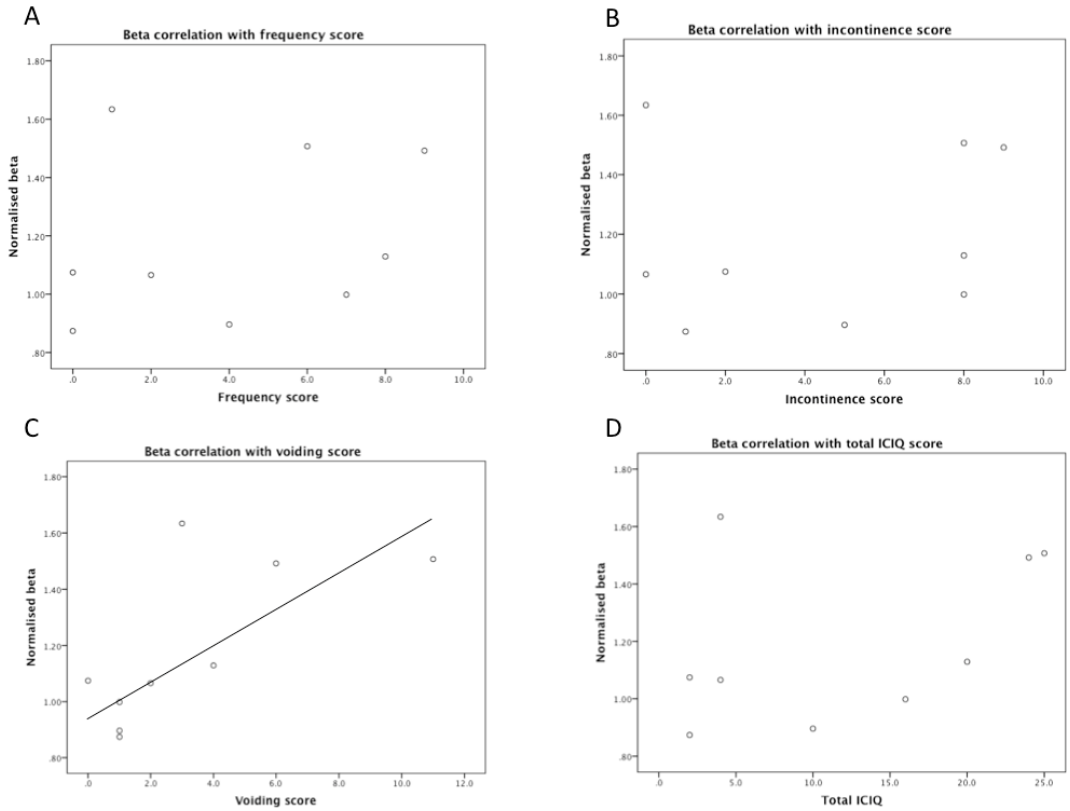


Figure 5.1: Relationship between beta power during pelvic floor relaxation and ICIQ scores. Significant correlation between beta power and voiding score (C); no significant correlation for frequency score (A), incontinence score (B) or total score (D)

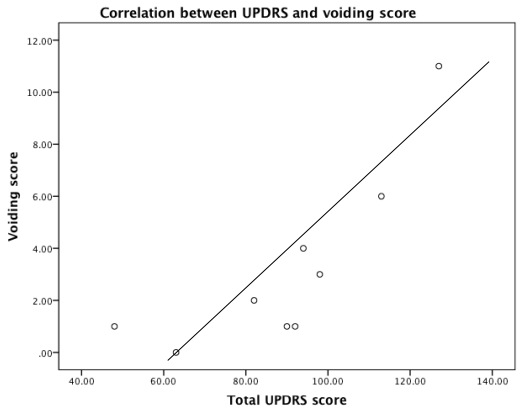


Figure 5.2: Correlation between total UPDRS score and voiding score for each subject

As noted in the introduction to this chapter, we would expect urinary symptoms that are related to the beta LFP band to be responsive both to DBS and also to dopaminergic medication, since these tend to reduce the degree of pathological beta. In the majority of patients, the voiding score did improve following DBS (improvement in 4/6, no change in 1/6, worsening in 1/6).

Theta and storage symptoms

Theta power during pelvic floor relaxation correlated significantly with the total ICIQ score ((r = 0.758, p = 0.018, Pearson Correlation, two-tailed) (figure 5.3), the frequency component of the ICIQ score (r = 0.709, p = 0.033, Pearson Correlation, two-tailed) and the incontinence component of the ICIQ score (r = 0.791, p = 0.029, Pearson Correlation, two-tailed), but not the voiding component of the score.

The UPDRS score did not correlate with the total ICIQ, the frequency or the incontinence scores, and nor did it correlate significantly with the theta signal during relax pelvic floor, although there was a trend towards correlation.

The pairing of theta and storage symptoms, independent of beta and beta related symptoms as indexed by the UPDRS score (including voiding), suggests a separate dimension of urinary impairment in Parkinson's disease that is less closely related to the conventionally recognised markers of PD including the UPDRS and beta and more closely related to the theta signal.

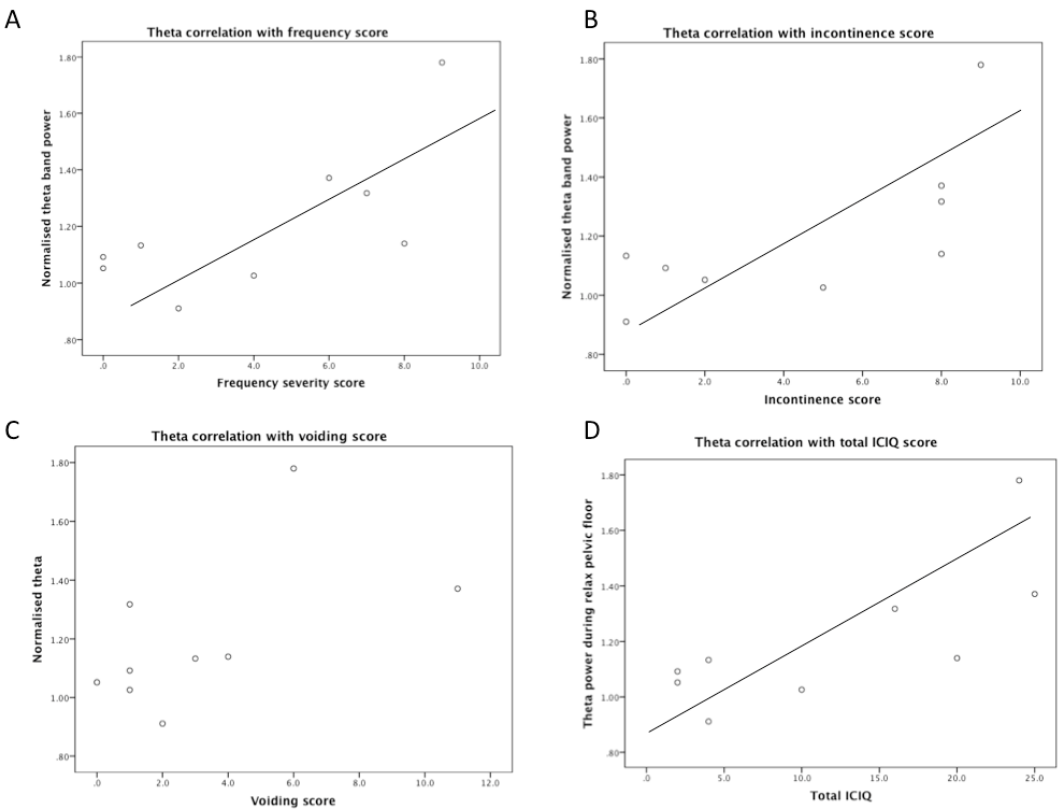


Figure 5.3: Relationship between theta power during pelvic floor relaxation and ICIQ scores. Significant correlation between theta power and frequency score (A),

incontinence score (B) or total score (D); no significant correlation for voiding score (C).

Gamma power and voiding

Gamma-band power during voiding correlated significantly with the UPDRS score ($r = 0.773$, $p = 0.042$, Pearson Correlation, two-tailed) (figure 5.4). This further supports the view that voiding impairments measured by the ICIQ are related to classical Parkinsonian impairments quantified by the UPDRS.

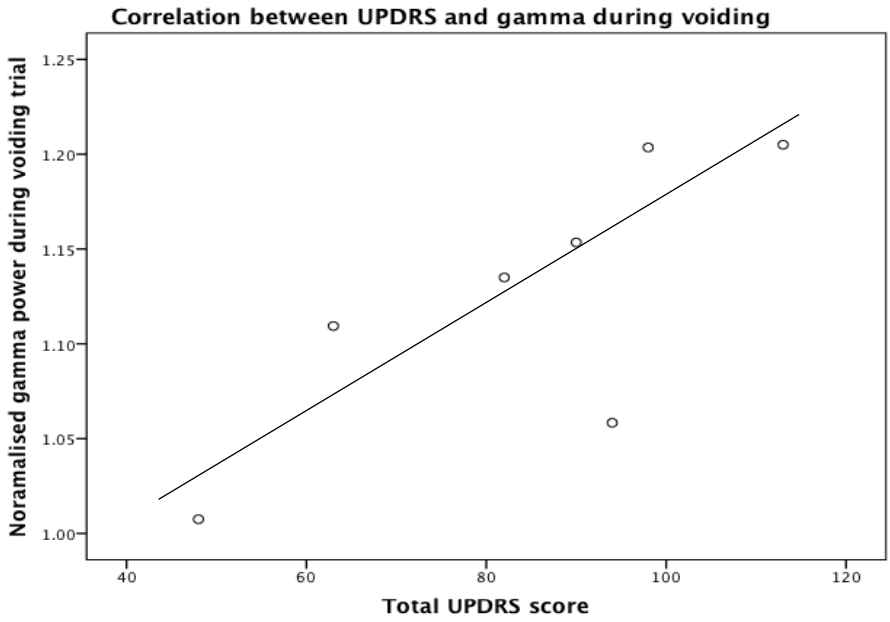


Figure 5.4: Correlation between gamma power during voiding and total UPDRS score, demonstrating a significant positive correlation ($p < 0.05$) between gamma power during voiding and the total UPDRS score for each patient.

Discussion

In this study we have described for the first time in humans, local field potential changes associated with urinary-control tasks. Recordings were made in the subthalamic nucleus and globus pallidus interna. The pelvic floor relaxation and imagined void tasks designed to elicit activity associated with motor and cognitive aspects of pre-voiding or the early stages of voiding.

In the published literature, effects of STN stimulation have been described at different stages of the micturition cycle. The majority of studies have demonstrated that STN stimulation in human subjects with PD, at clinically therapeutic stimulation parameters, increases sensory thresholds during bladder filling (Finazzi-Agro *et al*. (2003); Seif *et al*. (2004); Herzog *et al*. (2008)), facilitating a larger bladder capacity and enabling greater volume to be held in the bladder before the onset of detrusor overactivity. The only exception is a report by Winge *et al*. (2007), where no urodynamic changes were reported with STN DBS ON compared with STN DBS OFF, and a secondary measure of functional bladder control (interval between first desire to void and maximal bladder capacity) actually decreased following surgery (Winge *et al*. (2007)). There were no major differences between this study and the others, although only one ON DBS and OFF DBS trial was done, and details about the duration of time between ON and OFF fills are not given. Activity within the subthalamic nucleus has also been described during urinary voiding. In the animal

literature, a triphasic pattern of single unit activity that corresponds to stages of detrusor contraction during urinary voiding has been described in the cat (Porter *et al*. (1971)). It is surprising that improved urinary voiding function has not been described in human subjects following STN DBS, despite improvements in storage symptoms (Finazzi-Agro *et al*. (2003), Seif *et al*. (2004), Winge *et al*. (2007), Halim *et al*. (2011)). It may be that STN DBS improves the speed and ease of voiding initiation (i.e. reduced time to void start), rather than the motor quality of the void itself which is quantified by measures such as the maximal detrusor pressure, maximal flow rate and residual urine. Time taken to initate voiding has not, to our knowledge, been described in any published studies of STN DBS and urinary voiding.

Here, we found that there was a correlation between voiding symptoms on the ICIQ and the beta signal during the "relax pelvic floor" condition, whereas none of the other ICIQ symptoms correlated with the beta signal in any trial. This suggests that the voiding symptoms, but not storage symptoms in our cohort are associated with the beta band signal in PD, which is known to be pathological. Pathological beta is associated with symptoms of bradykinesia and rigidity, but not tremor (Kuhn *et al*. (2006), Ray *et al*. (2008)), and are reduced by movement, DBS and dopaminergic medications. Symptoms associated with the beta band signal would therefore be expected to improve with DBS, or dopaminergic medications, and to correlate with other symptoms related to the beta oscillation. The current findings fit well with reported observations that voiding symptoms were universally alleviated following the intake of L-DOPA/dopa-decarboxylase in a group of 12 patients, whereas the storage symptoms were not improved (in fact urgency and and urge incontinence were exacerbated by the L-DOPA/dopa-decarboxylase) (Uchiyama *et al*. (2003)). In

our cohort, the severity of voiding symptoms correlated with the UPDRS score, and the majority of subjects reported improved voiding symptoms with DBS.

Urinary storage symptoms (frequency and urgency) correlated with the power in the theta band during pelvic floor relaxation. The significance of the theta band is less well-established than that of the beta band, although it has been linked with impulse control disorders (Cavanagh *et al*. (2011)) and encoding conflict (Zavala *et al*. (2015)). Nevertheless, the fact that storage symptoms were not connected with beta but with theta clearly indicates not only that their pathophysiology may be different to that of voiding symptoms, but also that LFP signals may be significant in understanding their aetiology.

Study limitations

As this was the first time that LFP data has been collected in the context of micturition studies, we had used only published material from other experimental modalities to guide our experimental design. Furthermore, as this was the first time LFPs had been recorded with respect to the bladder, we aimed to be as non-invasive as possible to maximise recruitment, as patients in their intra-operative week are often reluctant to undergo uncomfortable or invasive procedures that they might otherwise be prepared to undertake. I was therefore unable in this study to obtain direct physiological measures with which to correlate the LFP signals. This would have provided a much more informative dataset with which to work and it may have been possible to link the LFP signal with specific physiological events, such as voiding initiation, relaxation of the external urethral sphincter or the rate of relaxation of the

pelvic floor musculature. It is my hope that this data could act as pilot material to allow these future investigations to be carried out.

Another limitation is that I was restricted to recording from sites that were dictated by clinical need (i.e. existing clinical DBS targets for PD). As a result, I was not able to preselect recording sites based on their relevance to neuro-urology. Furthermore, all participants had a pre-existing clinical condition, which makes questionable the generalizability of the results to the healthy population.

Finally, recordings were made in the week following DBS implantation, when a stun effect may influence oscillatory recordings. Future models of DBS systems, which allow LFPs to be downloaded remotely, will facilitate recordings to be made after the stun effect has settled.

CHAPTER 6: PAG LOCAL FIELD POTENTIALS DURING MICTURITION-RELATED TASKS

Introduction

The periaqueductal gray area (PAG) is a midbrain nucleus, which is used as a therapeutic target for deep brain stimulation (DBS) to treat chronic pain (Reynolds *et al* (1969), Richardson *et al.* (1977), Hosobuchi *et al*. (1977), Boccard *et al*. (2013)). In addition to its involvement in ascending/descending pain pathways, PAG DBS has been implicated in a variety of physiological functions, including respiration (Hyam *et al.* (2012)), blood pressure control (Green *et al*. (2006b), Green *et al*. (2010)) and micturition (Green *et al*. (2012)). It is thought that the PAG plays a critical role in the regulation of micturition by facilitating the switch from urine storage to voiding (Tai *et al*. (2009)), a role that is made possible by connections between the PAG and higher cortical and subcortical centres that regulate bladder function, as well as ascending sensory afferent information from the bladder. In a recent study, we showed that deep brain stimulation of the human PAG increased maximal bladder filling capacity during clinical urodynamic testing, a finding that mirrored the effect of stimulating the rat PAG (Green *et al*. (2012)). In order to understand the mechanism underlying the effect of PAG stimulation on bladder function, we used implanted depth electrodes to record local field potentials (LFPs) from the PAG and sensory thalamus (VPL) of a human subject during bladder filling. To investigate the effect of PAG DBS on information transfer along sensory pathways, we also recorded from the VPL during bladder filling with simultaneous PAG DBS.

Local field potentials are extracellular potentials that reflect the summed excitatory and inhibitory post-synaptic potentials of a local population of neurons. They are thought to reflect alterations in the efficiency of synaptic interactions, which in turn affect short- and long- range communications between brain areas (Friston *et al*. (2015)). In particular, LFPs have been implicated in mediating attentional processes (Feldman *et al*. (2010)). Oscillations in the alpha frequency band (8-12 Hz) are thought to play a role in predicting upcoming stimuli, while gamma oscillations may signal prediction errors- sensory inputs that differ from those predicted. These effects have been demonstrated in the visual system (Bauer *et al*. (2014)) but may also apply in other domains of exteroceptive and also interoceptive attention. Green *et al*. (2009) demonstrated that alpha frequency spindles in the PAG and VPL occurred concomitant with the subjective awareness of pain, along with generalised pain-related increases in activity in the 8-12 Hz frequency band within the PAG and in the 17-30 Hz frequency band of the VPL. Beta band (12-25 Hz) and gamma band (25-60Hz, 60-90Hz) power in the PAG increased during both anticipation of exercise and exercise itself (Green *et al*. (2007)). Elevated gamma band power in the dorsal PAG occurred following naloxone infusion and may be linked with pain sensation (Pereira *et al*. (2013)). PAG and VPL activity are thought to influence one another via short-range connections, as demonstrated by Wu *et al*. (2014) who showed that PAG stimulation inhibited activity within the sensory thalamus and sensory thalamus stimulation excited the PAG by increasing activity in the delta and theta frequency bands (Wu *et al*. (2014)).

Studying the relationship between the BOLD signal and LFPs in the visual cortex of monkeys, Magri *et al.* (2012) found that the BOLD signal correlated with the relative power in the alpha and gamma bands and that essentially, although the BOLD signal was more closely related to gamma, the alpha band carried important information that was not carried in the gamma frequency band. Thus, in this study, we were particularly interested in the individual alpha and gamma responses to bladder filling, and related tasks such as pelvic floor muscle contraction and imagined void. We were also interested in analysing the combined alpha and gamma signal changes during the filling process (also see Kilner *et al.* (2005)).

Patient information

The results presented in this chapter refer to recordings from 2 separate patients who underwent two different experimental protocols. Descriptions of the patients and protocols are detailed below.

Patient 1

A 47 year old female underwent DBS of the left PAG and left VPL for chronic pain. Her total ICIQ score was 12, indicating moderate to severe lower urinary tract symptoms. She gave informed consent for her participation in the study. The study protocol conformed with the declaration of Helsinki and received ethical approval from the Oxfordshire Local Research Ethics Committee (Oxfordshire REC B 09/H605/62).

Patient 2

A 51-year-old man underwent DBS of the left PAG and left VPL for the treatment of medication-refractory right arm phantom limb pain following a motorcycle accident 2 years previously. He had no co-morbid cardiovascular, respiratory, gastrointestinal or urogenital conditions and was taking no medications at the time of testing. Prior to testing he completed an autonomic questionnaire, which showed no evidence of autonomic disturbance, and a bladder diary, which showed normal bladder function with no urinary frequency, urgency, incontinence or difficulty passing urine. He was not taking any regular medications at the time of testing. The patient gave informed consent for his participation in the study. The study protocol conformed with the declaration of Helsinki and received ethical approval from the Oxfordshire Local Research Ethics Committee (Oxfordshire REC B 09/H605/62). The urodynamic data collected from this subject have been included in a previous publication (Green *et al*. (2012)).

Methods

DBS surgery

See general methods

Patient 1

Following stage I DBS, while the electrodes were externalized, the patient completed the "imagine void", "pelvic floor contract/relax" and "void" tasks as described in chapter 5. There were 9 observations for each condition per trial, apart from the "void" trial, where there were 9 attempted voids/voids in total. Please see chapter 6 for methods. Recordings were made from the left PAG and left VPL during each of these tasks and both electrodes remained OFF throughout the testing.

Patient 2

On day 3 after stage I DBS, while the electrodes were externalized and therefore available to record from, the patient underwent 4 cycles of urodynamic testing, prior to which he received a single dose of prophylactic antibiotics (nitrofurantoin 100mg orally). For the urodynamics, bladder catheterisation with a dual lumen catheter (8 Fr) was performed. A rectal catheter (4.5 Fr) was inserted to measure intra-abdominal pressure. The bladder was infused with a sterile solution of isotonic saline at a rate of 24 ml min^{-1} and the patient was asked to indicate when he experienced the following bladder sensations representing graded intensity of desire to void: (1) a first sensation of bladder filling (2) an initial desire to void [2] a strong desire to void and (4) a very strong ("desperate") desire to void. After this, filling continued until the patient asked the experimenter to stop (5) and this point was taken to be the 'maximal cystometric capacity'. The saline infusion was stopped immediately and after a short pause, the patient was either asked to void naturally or fluid was removed from the bladder via the catheter. The time of attempted void or fluid removal was recorded. Subjective pain scores were recorded at the beginning and the end of bladder filling.

The VPL electrode was turned off throughout testing and connected to a portable physiological measuring system (Porti 7, TMSI International) for LFP recording. Two bladder filling cycles were performed with PAG stimulation turned ON, and two filling cycles were carried out with PAG stimulation turned OFF, in the order OFF/ON/OFF/ON. Each cycle was separated from the next by a 10 minute 'rest' period, and a 5 minute interval was maintained between changing a stimulation setting and the subsequent filling cycle. While the PAG stimulation was turned OFF, the PAG lead was connected to the portable amplifier system and LFPs were recorded. Stimulation parameters were as follows: PAG (1.0V, 210μs, 50Hz) and VPL (2.9V, 210μs, 50Hz).

Power Spectral Analysis

Signals were analysed off line using Spike II software (version 7, Cambridge Electronic Design, Cambridge, UK) and the NSPL toolbox in Matlab (Mathworks Inc, MA, USA). First, bipolar channels were created by subtracting the signal in adjacent channels, to create two bipolar channels for the PAG electrode and one bipolar channel for the VPL electrode (due to poor signal quality in one of the four VPL electrodes). For patient 1, the LFP signals were analysed as described in chapter 6. For patient 2, the time of each new bladder sensation (on a scale of 1-5 as described above in the urodynamic methods) was located on the data trace by identifying a marker made at the time of the reported sensation, and double-checking this against the recorded time that the patient had reported a new sensation. A bipolar data segment of 10-second duration immediately preceding the new sensation was identified, downsampled to 1000 Hz and exported to NSPL. Bipolar signals were low pass filtered at 200 Hz, high pass filtered at 2 Hz, and bandstop filtered at 50 Hz using a Butterworth filter to exclude mains artefact. Power spectral density analysis was performed on the 10 second data

segments using a 1.5 second Hanning window and the power in the following frequency bands was recorded: 4-8 Hz, 8-12 Hz, 12-25 Hz, 25-60 Hz, 60-90 Hz, 90-150 Hz for each of the four filling cycles. Signals were analysed for each fill separately, and also averaged within the "ON" and "OFF" DBS states. LFP power in each band was normalised relative to "rest" for each fill, which was defined as power during a 10 second segment of initial filling prior to any reported bladder sensation.

Statistical Analysis

Statistical analyses were carried out in SPSS (version 20, IBM). Comparisons between LFP power during "imagine void" and "stop imagining void" trials, "contract pelvic floor" and "relax pelvic floor" trials were carried out using Wilcoxon sign rank test as the data were not normally distributed. The normalized LFP power in the "imagined void" and "void" and "relax pelvic floor" and "void" trials were compared using t-tests and corrected for multiple comparisons using a Bonferroni test. For patient 2, correlational analyses were performed using Pearson's correlation (two-tailed) and significance at $p < 0.05$ is reported.

Results

Patient 1

Electrode pair L4-L3 was selected for analysis in the PAG and the VPL.

Start vs. stop imagining void

In the PAG, there was no significant modulation in LFP power in the theta, beta or gamma bands between “imagine void” and “stop imagining void” conditions. However there was a significant change in the alpha band power during this task, with the alpha power increasing during the “imagine void” trial. This was statistically significant ($p < 0.01$, Wilcoxon sign rank test) (figure 5.1 and 5.3).

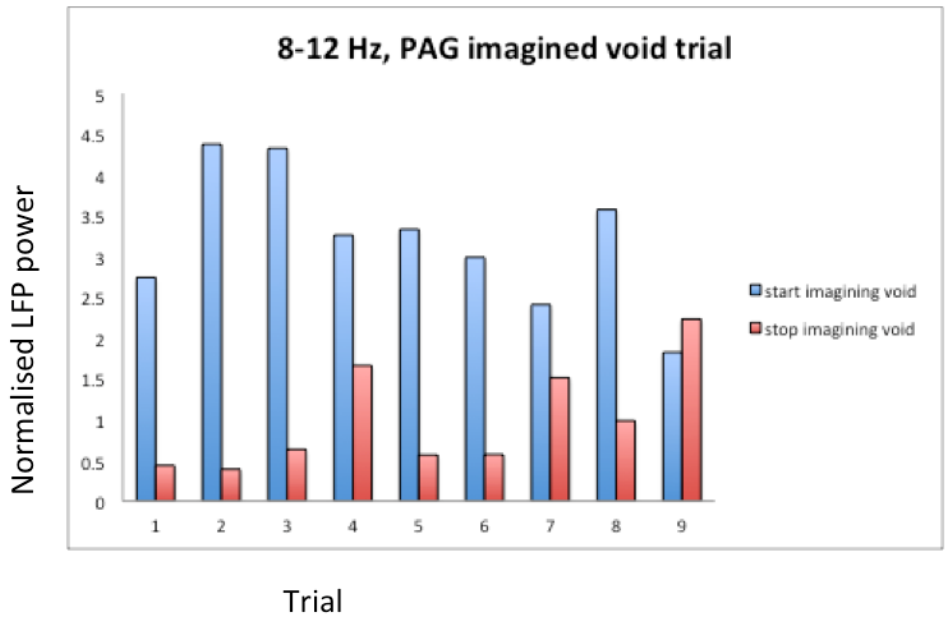


Figure 6.1 Normalised LFP power in the alpha frequency band within the PAG during the imagined void trial, across trials 1-9. Blue bars represent power during “imagine void” trials and red bars represent power during “stop imagining void” trials. It can be seen that, apart from the final trial, the alpha power during the “imagine void” trials is higher than during the “stop imagine void” trials.

In the VPL there was no significant modulation in LFP power in the theta, beta or gamma bands between imagine void and stop imagining void. However there was a significant change in the alpha band power during this task, with the alpha power increasing during the imagine void trial. This was statistically significant ($p < 0.05$, Wilcoxon sign rank test).

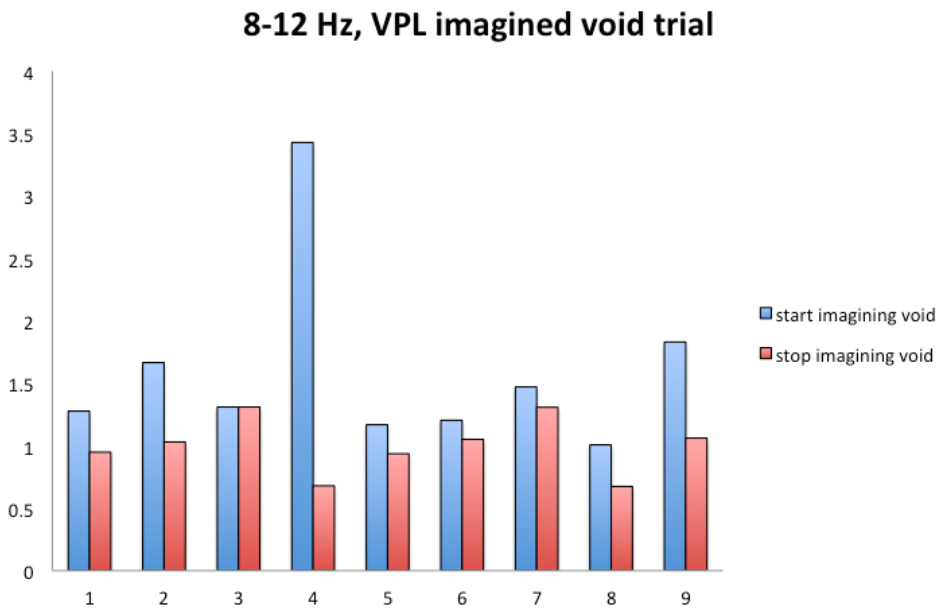


Figure 6.2 Normalised LFP power in the alpha frequency band within the VPL during the imagined void trial, across trials 1-9. Blue bars represent power during "imagine void" trials and red bars represent power during "stop imagining void" trials.

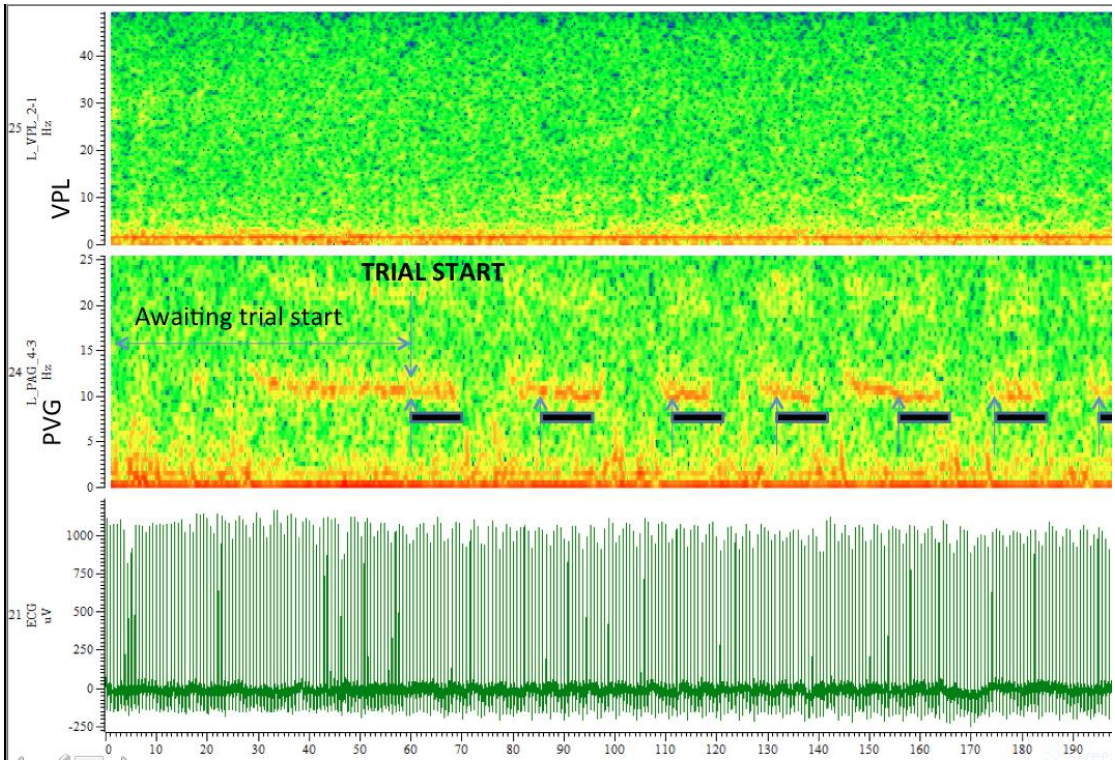


Figure 6.3: Time frequency plot showing VPL power (top line) and PAG power (middle line) during the "imagine void" trial. Black lines correspond to the duration of the trial and intervening time is rest.

Contract vs. relax pelvic floor

There was no significant modulation in LFP power in the theta, beta or gamma bands between contract and relax pelvic floor. However there was a significant change in the alpha band power during this task, with the alpha power increasing during the "relax pelvic floor" trials compared with the "contract pelvic floor" trials (figure 6.4). This was statistically significant ($p < 0.05$, Wilcoxon sign rank test).

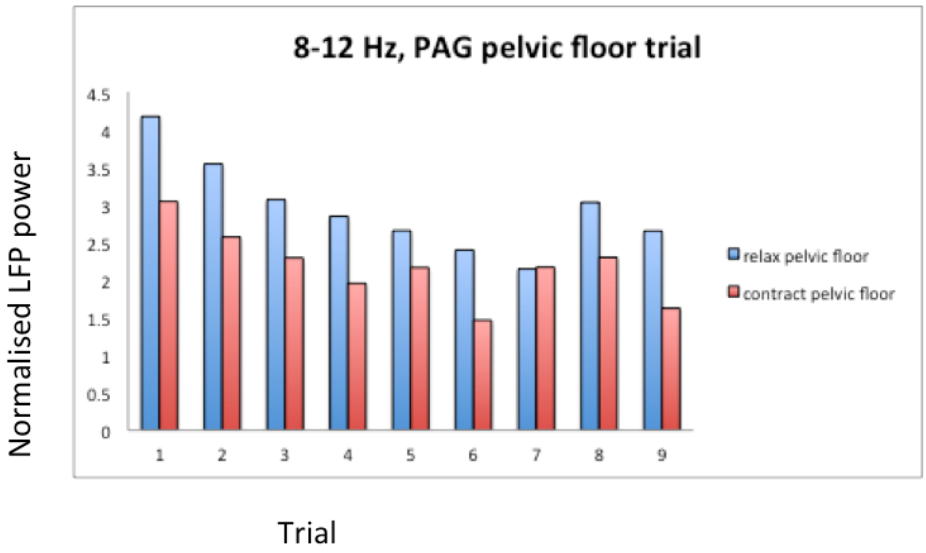


Figure 6.4 Normalised LFP power in the alpha frequency band within the PAG during the pelvic floor trial, across trials 1-9. Blue bars represent power during "relax pelvic floor" trials and red bars represent power during "contract pelvic floor" trials.

The VPL did not show any significant modulation of LFP power between pelvic floor contraction and pelvic floor relaxation (figure 6.5).

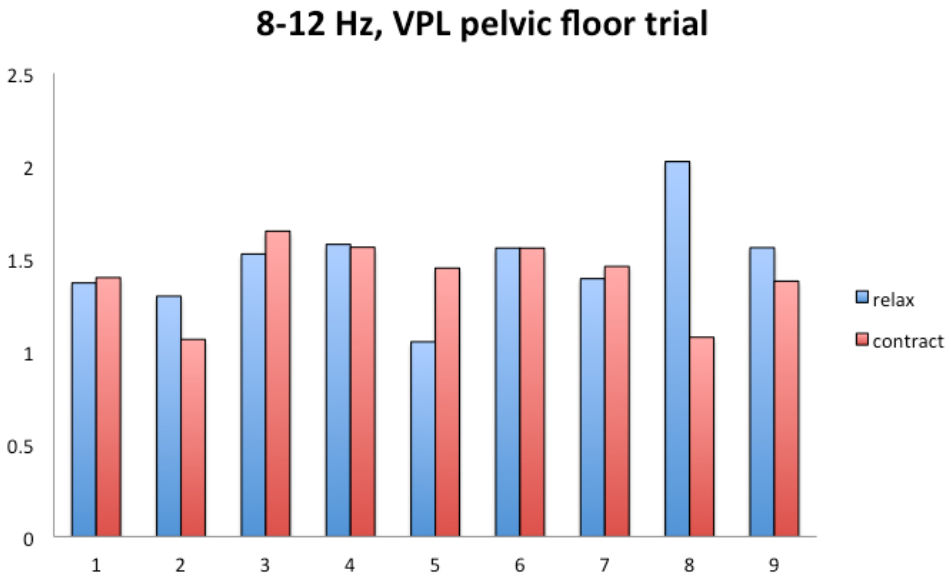


Figure 6.5 Normalised LFP power in the alpha frequency band within the VPL during the pelvic floor trial, across trials 1-9. Blue bars represent power during “relax pelvic floor” trials and red bars represent power during “contract pelvic floor” trials.

Voiding trials compared with imagined void and pelvic floor relaxation

Normalised scores for the voiding trials were compared with those for the imagined void and relax pelvic floor trial. T-tests were used with a Bonferroni correction for multiple comparisons as these results were normally distributed. Alpha power during the void trials (which included attempted void and actual void) was significantly less than that during the “relax pelvic floor” trial ($p < 0.05$) and the “imagined void” trial ($p < 0.05$) (figure 6.6).

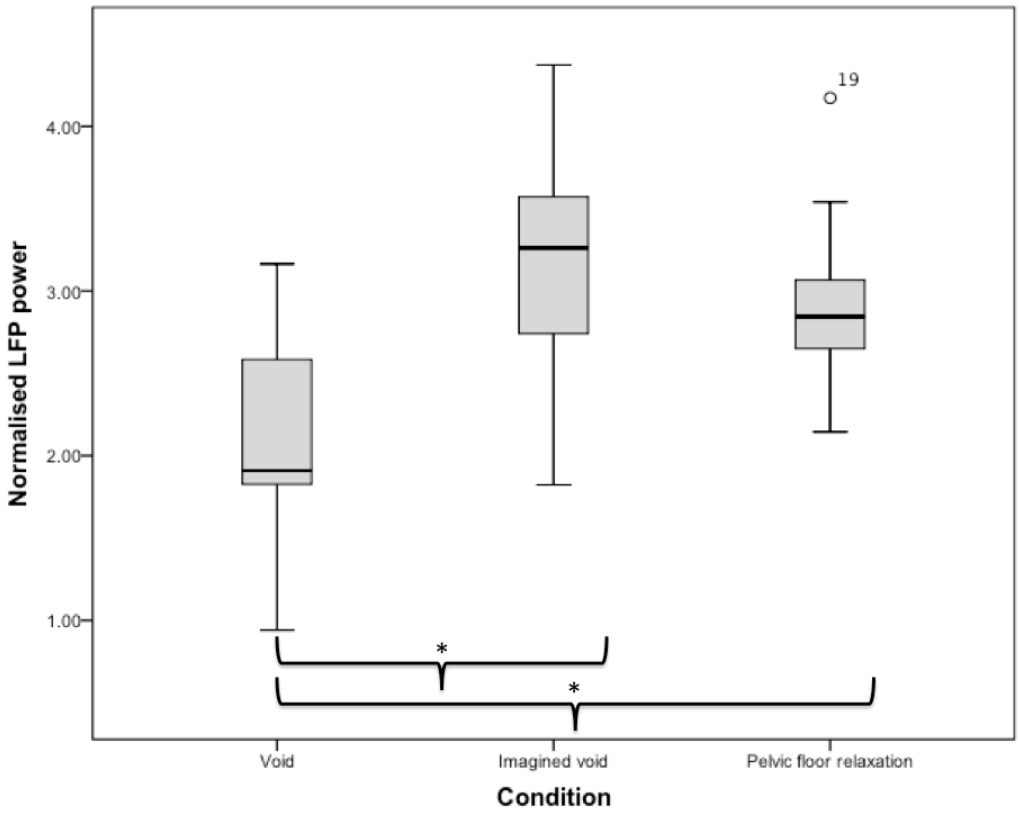


Figure 6.6: Box and whisker plot to show distribution of LFP power between the void, imagined void and pelvic floor relaxation trials. The ends of the box are the upper and lower quartiles for each distribution, the whiskers extend to the highest and lowest values, and the median is indicated by a horizontal line within the box. * indicates a significant difference between 2 conditions, with a significance level of $p < 0.05$.

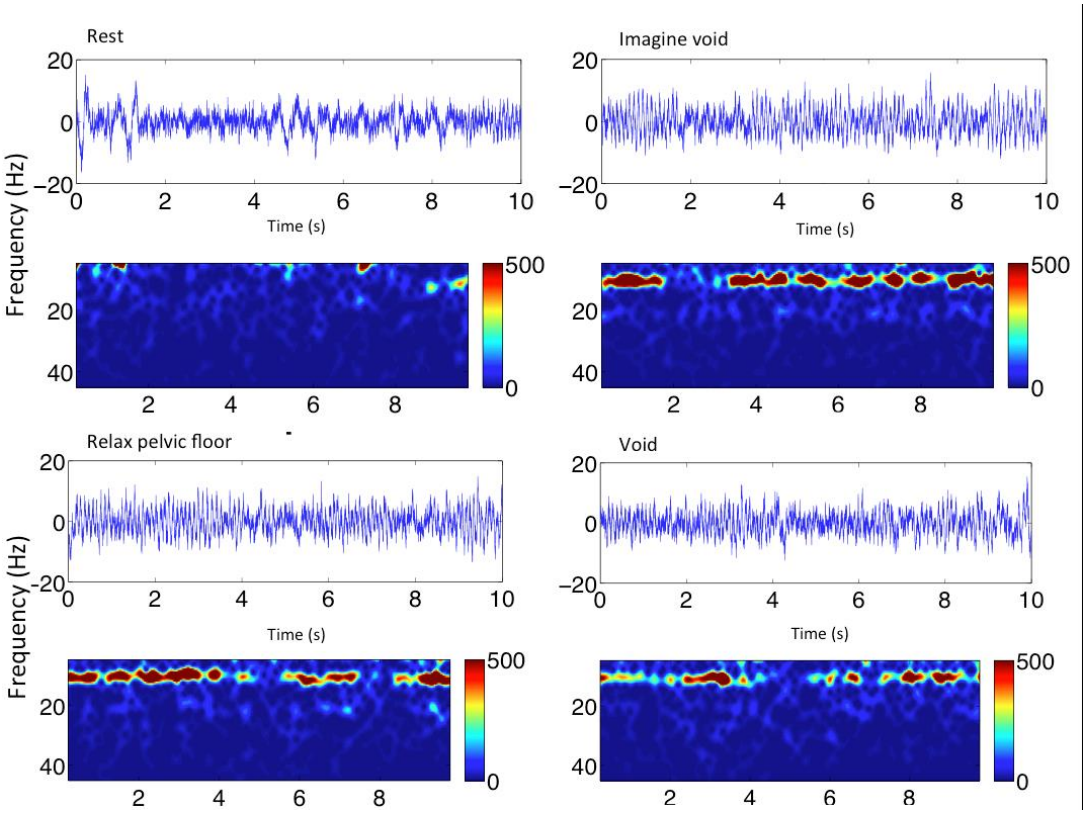


Figure 6.7: PAG signal in all four conditions of the voiding study. Raw signal above and time-frequency plot below for rest (top left), imagine void (top right), relax pelvic floor (bottom left) and void (bottom right).

Patient 2

Urodynamics

The mean maximal bladder capacity with PAG DBS ON was 201mls and PAG DBS OFF was 168mls. The mean pain score out of 10 (10 representing severe pain and 1 representing no pain) was 4 with PAG DBS ON and 5.5 with PAG DBS OFF.

Local field potentials

Correlations between PAG and VPL signals

There was a significant correlation between LFP signal in the PAG and VPL in the alpha (8-12 Hz) frequency band (p = 0.003) (figure 6.1).

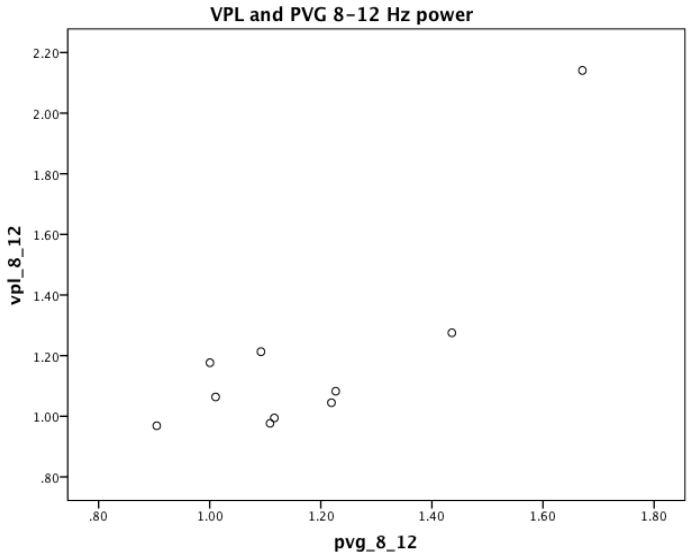


Figure 6.1: Correlation between alpha power in the PAG and the VPL during bladder filling (DBS OFF)

Signal change during bladder filling (DBS OFF)

In the VPL, there was a significant inverse correlation between bladder volume and LFP power in the high gamma frequency band (90-150 Hz) (p = 0.039) (figure 6.2).

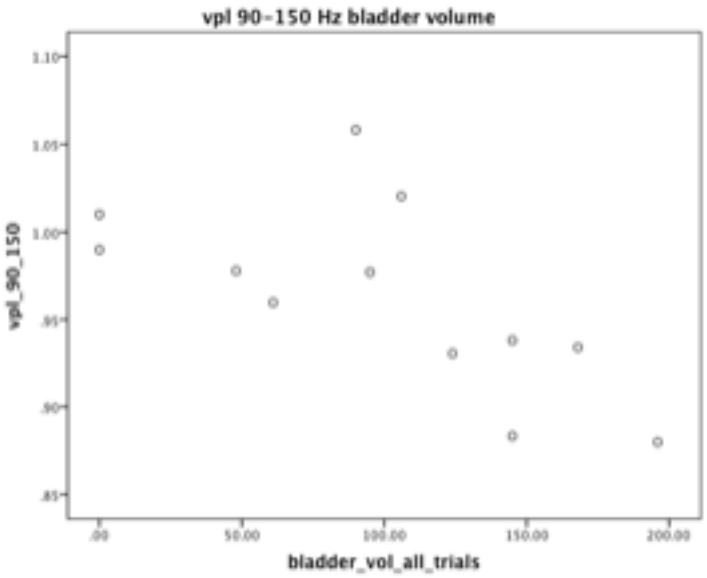


Figure 6.2: Correlation between 90-150 Hz power in the VPL with increasing bladder volume

In the PAG, there was no significant correlation between bladder volume and LFP power in any single frequency band, or between reported intensity of desire to void in any frequency band.

However, examining each of the two filling trials separately, there appeared to be a plausible response to filling in the gamma frequency band in the first trial in which gamma power correlated significantly with bladder volume ($p = 0.033$) (figure 6.3a) and a clear non-linear response in the alpha frequency band to bladder filling during the second filling trial (figure 6.3b).

Finally, we examined the partial correlation between gamma band power and bladder volume, controlling for alpha, based on previous literature suggesting that the combined alpha and gamma spectral profile might be more informative than the

separate profiles. Across both filling trials, there was a significant correlation between gamma power and bladder volume controlling for alpha ($p = 0.046$).

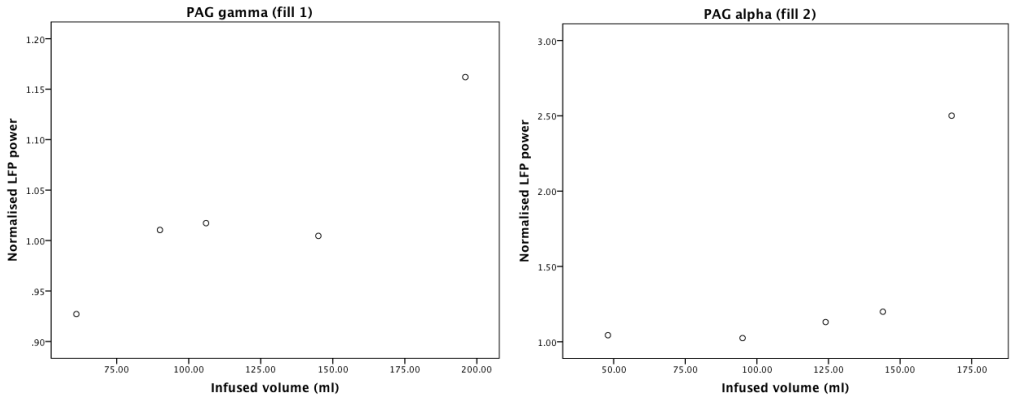


Figure 6.3: (a) Response to bladder filling in the 60-90 Hz frequency band in the PAG during the first fill (b) Response to bladder filling in the 8-12 Hz frequency band during the second DBS "OFF" fill.

Difference between DBS OFF and DBS ON

In the VPL, the response to bladder filling between the DBS OFF and DBS ON trials was compared.

Firstly, we examined the signal in the 90-150 Hz frequency band, and found that there was no longer a significant inverse correlation between gamma power and bladder volume in the VPL.

We then assessed the signal in the 8-12 Hz frequency band. During the first, but not the second fill with DBS ON, there was a negative correlation between alpha power and bladder volume ($p = 0.01$) (figure 6.5). Also, there was significant suppression of normalized alpha power overall during bladder filling with DBS ON relative to DBS OFF (paired samples t-test, $p < 0.001$).

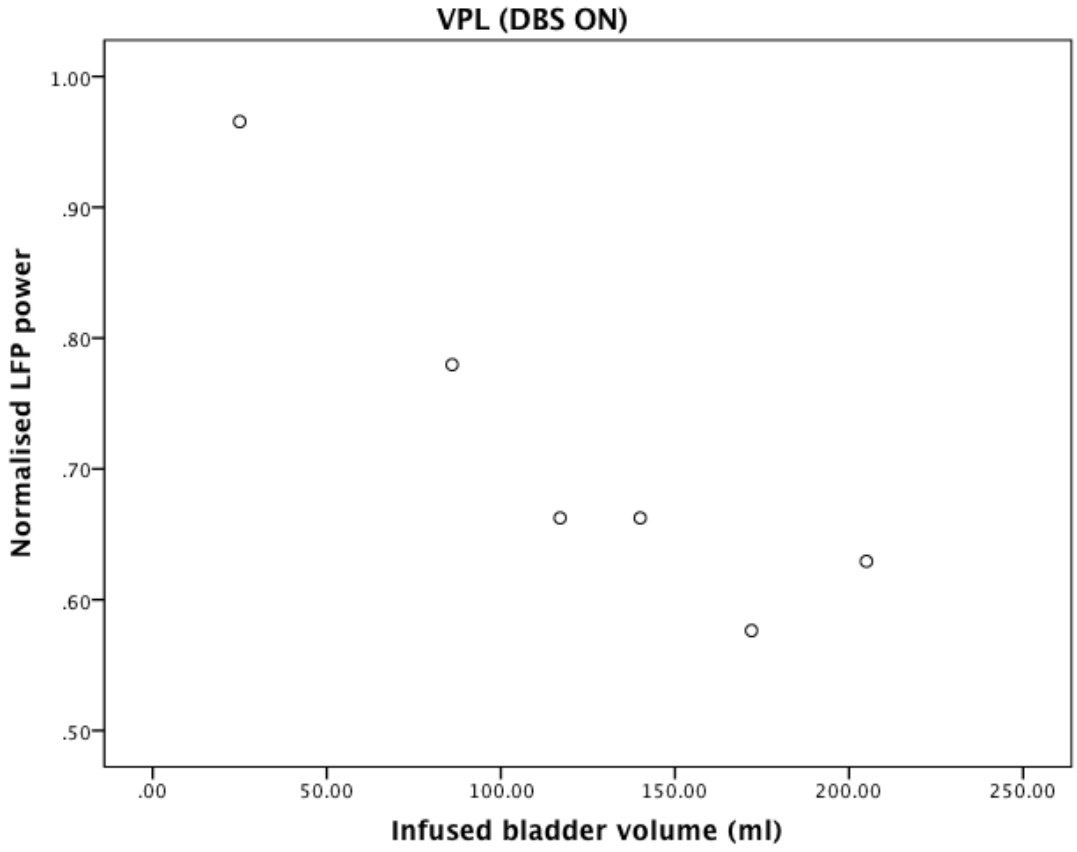


Figure 6.4: Normalised alpha power in the VPL with increasing bladder volume for DBS ON, trial 1.

Discussion

The present study is the first to describe the results of intracranial LFP recordings made from the PAG and VPL during bladder related tasks. The first set of results refer to changes in the PAG and VPL during imagined vs. stop imagined void, contract vs. relax pelvic floor, and actual voiding.

There was a striking increase in alpha synchrony in the PAG during imagined void, as well as significant but smaller increases associated with pelvic floor relaxation, with no significant changes in other frequency bands. Furthermore, the alpha modulation during imagined void and pelvic floor relaxation were significantly greater than the alpha power oscillations during the void/attempted void trial.

The PAG has long been believed to be an integral part of bladder control pathways, based on anatomical tracer studies demonstrating projections from bladder afferents to the PAG (Blok & Holstege (1994), Blok *et al.* (1995)) and also numerous experiments using functional imaging demonstrating consistent PAG activation during bladder filling (see Linnman *et al*. (2012) for a summary). Furthermore, deep brain stimulation of the PAG results in increased bladder capacity (Green *et al*. (2012)). It is known to integrate emotional, autonomic and somatic inputs (An *et al*.

(1998)), and for this reason its functions may incorporate multiple aspects of the emotional and sensory and motor control of the bladder.

Alpha band synchrony has been linked with anticipation and attention. Alpha oscillations are implicated in stimulus prediction during spatial attention tasks (Bauer *et al*. (2014)), visual and auditory attention (Mazaheri *et al*. (2014)) and, within the PAG specifically, with pain perception (Green *et al*. (2009)). PAG activation has also been described following exposure to other cues predicting the onset of a sensory or emotionally valent event, including predictors of fear stimuli (Wager *et al*. (2004)) and breathing resistance (Faull *et al*. (2016)). The increased 8-12 Hz signal during the "imagine void" and "relax pelvic floor" condition may represent a combination of increased attention to "painful" or "uncomfortable" full bladder sensations and an anticipatory effect of these bladder sensations, which are endogenous voiding cues, perhaps combined with an attempt to inhibit the switch to voiding or a response to the bladder-related motor cues relating to pelvic floor control. Whether the large increase in alpha synchrony was primarily dependent on the perceptual, anticipatory or inhibitory aspects of the task is not clear and further experiments are required to dissociate the different elements involved. Interestingly, PAG activation narrowly missed the threshold of statistical significance in an fMRI study, which required patients to focus on bladder sensations and internally generate the urge to void without actually voiding (Kuhtz-Bushbeck *et al*. (2009)). PAG activation has been described during pelvic floor relaxation and contraction in subjects with a full bladder (Seseke *et al*. (2006), (2008), (2013)) and also during micturition (Shy *et al*. (2014)). These studies did not draw conclusions about whether the PAG was specifically involved in the sensory, motor or cognitive aspects of these events. The fact that alpha

oscillatory power was lower during the void trial than the pelvic floor relaxation or imagined void trial supports the view that alpha is involved in the sensory or anticipatory aspects of bladder circuitry.

Ventroposterolateral thalamic nucleus

There was a significant increase in 8-12 Hz power in the VPL during the "imagine void" trial (contrasting "imagined void" with "stop imagined void") but no significant modulation of alpha power during the pelvic floor trial.

The VPL is thought to be an output nucleus of the PAG in the bladder sensory pathways (Herzog *et al*. (2008)), and is known to respond to signals from the distended bladder (Bruggeman *et al*. (1993)). Thus, corresponding patterns of activity seen here between the PAG and VPL during imagined void are unsurprising and probably represent bladder related sensations experienced in each of the trials- during voiding, pelvic floor relaxation and imagining voiding. The sensory component of the "imagine void" trial presumably relates to the subject focussing more attention on the afferent signals from the bladder. Neural activity in the PAG and VPL have been shown to correlate in a previous study of pain-related local field potential activity (Wu *et al*. (2014)), further supporting this observation. It is not clear why the alpha power in the VPL during the imagined void trial does not reach significance. It may be that the VPL is more specifically engaged by sensory signals, whereas the PAG is activated by a broader range of bladder related stimuli, both motor and sensory.

Bladder filling study

In patient 2, we describe changes in the LFP within the PAG during bladder filling. Our first finding was a significant correlation between activity in the PAG and VPL in the alpha (8-12 Hz) frequency band during bladder filling. This correlated activity strongly supports the view that afferent information about bladder filling reaching the PAG from spinal pathways (Holstege (2005)) then passes via the sensory thalamus (VPL) to the insula (Herzog *et al*. (2008)). Further support for a role of the VPL in receiving sensory afferent information from the bladder is the fact that we were able to demonstrate significant correlations between VPL activity and bladder filling stages. Activity within the high gamma band (90-150 Hz) was inversely correlated with increasing bladder volume while activity within the alpha band (8-12 Hz) correlated with bladder volume during the second filling trial, DBS OFF. Interestingly, previous studies of single-unit extracellular potential activity in the monkey VPL in response to urinary bladder distension and other upper and lower body stimuli found that a population of neurons responsive to other lower body stimuli were excited by urinary bladder distension, whereas a second population of neurons typically excited by upper body somatic receptive fields were inhibited by bladder distension (Chandler *et al*. (1992)). Thus, it is unsurprising to discover that within the human VPL, certain neuronal populations that are inhibited and others that are excited by bladder distension, as we appear to have demonstrated here.

In this subject, the PAG appeared to respond differently to bladder filling over the two filling trials. On the first filling trial, there was no response in the alpha band to increasing bladder volume, but an increase in activity within the gamma frequency band. On the second filling trial, there was no longer a response in the gamma band, but a clear response in the alpha frequency band. Both gamma and alpha band activity

are known to be implicated in attentional processes. In one study of visual attention, gamma band oscillations encoded "surprise"; i.e. differences between internal predictions and sensory events, whereas the alpha band was important in tracking predictable stimuli. Since the first recorded fill here represented the first time the patient had undergone urodynamics, it is likely that the stimuli were "unpredictable" and therefore perhaps triggered gamma band activity, whereas by the second "DBS OFF" fill, the relationship between the urodynamic task and sensation had been learned, reflected by the alpha oscillation (Bauer *et al*. (2014)). In monkeys, the combined alpha and gamma spectral profile was shown to be more informative about the BOLD signal than either profile in isolation (Magri *et al*. (2012)). The possibility that the combined output of the PAG in the alpha and gamma band carries information about bladder filling is supported by our observation that gamma power correlates significantly with bladder volume after controlling for alpha.

In addition to examining PAG and VPL LFP signals during bladder filling, we also wanted to understand whether PAG DBS influenced sensory processing of signals in the VPL, as this could provide an explanation for the effect of PAG DBS on bladder capacity in humans. In the alpha and gamma frequency bands, in which a relationship between bladder volume and LFP power in the "DBS OFF" state had been established, we found that there was no longer a correlation when DBS was turned ON. Furthermore, comparing normalized VPL power in the alpha band between DBS OFF and ON states revealed a significant suppression of power with DBS ON. This is consistent with previous studies, which have also demonstrated that PAG DBS causes suppression of alpha activity within the VPL (Wu *et al*. (2014)) and has been previously discussed as a possible mechanism by which PAG DBS alleviates pain,

since alpha is thought to be important in encoding painful signals (Green *et al*. (2009)).

Interestingly, although the PAG is well established as an important region in the afferent and efferent limbs of the micturition pathway, its precise activity during different stages of the cycle has not been possible to fully predict based on existing imaging data due to complex variations according to experiment type, stage of the cycle and patient group. For example, in a study by Athwal *et al*. (2001), activity within the PAG correlated with bladder filling but not sensations of urgency during PET examination, while in fMRI studies of subjects with poor bladder control, Griffiths *et al*. (2007) found relatively *weak* PAG activation, particularly at large bladder volumes. In an fMRI study of rats during bladder filling and voiding, Tai et al reported activation of the PAG during bladder filling with an enhancement of PAG activity at micturition threshold (the micturition switch) (Tai *et al.* (2009)). In this study also, we found that activity within the PAG varied between filling trials, and suggest that its role in sensory processing of bladder information is complex, with information carried in multiple frequency bands, requiring further investigation before a full understanding is reached.

CHAPTER 7: EFFECTS OF PEDUNCULOPONTINE NUCLEUS STIMULATION ON HUMAN BLADDER FUNCTION

Introduction

Deep brain stimulation is an evolving technology with well-established applications including the treatment of certain movement disorders such as Parkinson's disease (Deuschl *et al*. (2006)) and dystonia (Volkmann *et al*. (2012)). Furthermore there is growing interest in its applications in many other spheres including neuropsychiatry (Alonso *et al*. (2015), Richardson *et al*. (2015)), autonomic regulation (Hyam *et al*. 2012) and bladder control (Seif *et al*. (2004), Green *et al*. (2012)). Research on the use of DBS for urinary indications in humans has been a result of clinical studies in which subjects with DBS electrodes implanted are willing to undergo urodynamic testing for the benefit of research. Through this research, it has been demonstrated that deep brain stimulation of the subthalamic nucleus (STN) typically increases bladder capacity and reduces detrusor overactivity (Finazzi-Agro *et al.* (2003); Seif *et al.* (2004); Herzog *et al*. (2006), (2008)), while DBS of the thalamus significantly decreases bladder volume at first desire to void, strong desire to void and maximum bladder capacity (Kessler *et al.* (2008)). DBS of the globus pallidus interna in patients with dystonia appears to reduce urine flow rate, increase post-void residual and reduce detrusor overactivity (Mordasini *et al*. (2014)), and DBS of the PAG was shown in a series of patients with chronic pain to increase maximal bladder capacity (Green *et al.* (2012)). This growing literature provides valuable information about the potential targets for functional manipulation of bladder function through brain stimulation and also outlines possible side effects that could arise following DBS at different targets. DBS-bladder studies are also an excellent supplement to research on

the neural control of the bladder derived from other investigative modalities including functional MRI.

According to a recent case report, a patient with Parkinson's disease (PD) experienced new onset lower urinary tract symptoms immediately after unilateral, right-sided deep brain stimulation of the pedunculopontine nucleus (PPN), initially associated with stimulation of the most caudal contact, not having experienced urinary symptoms pre-operatively. It was suggested that the new onset urodynamic detrusor overactivity observed, along with symptoms of urinary frequency, urgency and urge incontinence, occurred due to an effect of the stimulation on the pontine micturition centre in the dorsolateral pons (Aviles-Olmos *et al*. (2011); Blanco *et al*. (2013)). The pedunculopontine nucleus (PPN) is a diffuse cell group located in the rostral pontine/caudal mesencephalic tegmentum (Zrinzo *et al*. (2008); Alam *et al*. (2011)). It is part of the mesencephalic locomotor region and is known to undergo degeneration in PD (Zweig *et al*. (1989); Hirsch *et al*. (1987)). In humans, the PPN has been stimulated bilaterally, with varying results, for the treatment of severe gait disturbance in PD (Mazzone *et al*., 2005; Plaha and Gill, 2005; Stefani et al 2007), but as far as we are aware, there is only a single case-report describing the effect of unilateral electrical stimulation in this region on bladder function.

In this study we were interested to discover whether a detrimental effect on bladder function occurs with bilateral stimulation of the PPN, which is the usual clinical mode of implantation (Mazzone *et al*. (2005), Plaha *et al*. (2005); Stefani *et al*. (2007)). Thus, the primary motivation for this investigation was to use urodynamics to assess whether worsening of urinary symptoms occurs consistently after PPN DBS, which

would highlight an important side-effect to inform patients about prior to DBS surgery. The second aim was to understand the mechanism of any demonstrated actions of PPN DBS on urological function. In particular, the aim was to evaluate whether any effects of DBS on bladder symptoms was more likely to be mediated via local effects on the pontine micturition and pontine continence centres (PMC and PCC); via effects on the PAG; or via distant effects on nodes within the "central bladder network", the complex network of sensory, motor and autonomic regions involved in bladder sensation, continence and voiding. To this end, we carried out urodynamics ON and OFF PPN DBS in a small series of subjects, and used diffusion tensor imaging in two of these subjects to map the probabilistic white matter pathways connecting the DBS "volume of activated tissue" with the rest of the brain.

Subjects and methods

6 subjects with PD who had bilateral PPN DBS electrodes inserted for the control of movement disorder symptoms participated in the study. All patients gave consent to participate and for their data to be used for research purposes. All parts of this study were carried out in accordance with the Declaration of Helsinki and received approval from the Oxfordshire Research Ethics Committee B (study 09/H0605/62).

Surgical procedure

Surgical implantation techniques are described in the methods chapter (chapter 2).

Imaging

Subjects underwent a diffusion-weighted MRI and a high resolution T1-weighted MRI as described in the methods (chapter 2).

Urodynamics

All patients had their Parkinson's disease medications withheld for 12 hours prior to testing, with the exception of one patient (patient 5) who took a quarter of the normal dose of medication.

Urodynamics were carried out in accordance with International Continence Society guidelines.

Participants were asked to void urine in order to fully empty their bladder prior to testing and urinalysis was performed to exclude a urinary tract infection. A dual lumen urethral catheter (8 Fr) was used to fill the bladder and measure intravesical pressure. A rectal catheter (4.5 Fr) was inserted to measure abdominal pressure and thus derive detrusor pressure by subtracting the abdominal pressure from the intravesical pressure.

The bladder was infused with a sterile solution of isotonic saline at an average rate of 30 ml min^{-1}. Urodynamic parameters were evaluated, including detrusor contraction amplitude and bladder volume at initial sensation of bladder filling, initial desire to void, normal desire to void, strong desire to void, and maximal capacity. When the

patient reported being unable to tolerate further filling (i.e. reaching maximal capacity), the saline infusion was stopped immediately. For each patient, bladder filling was carried out twice with PPN DBS turned 'ON' and twice with PPN DBS turned 'OFF'.

We did not collect information about voiding parameters due to difficulties in mobilising the patients to a voiding position for each of the 'ON' and 'OFF' trials, given that they were in an "off medication" state, which exacerbated their akinesia and tremor. We were also concerned that catheter displacement would occur while mobilising the subjects to a voiding position, and felt that this would potentially make it difficult to complete the filling part of the study if patients were not able to tolerate multiple catheterisations.

Diffusion tensor image analysis

Diffusion tensor image analysis was carried out in two of the subjects who participated in this study. Images were analysed using the FMRIB Software Library (FSL), Release 5.0 © 2012. A detailed description of the analysis methods can be found in Fernandes *et al*. (2015). In summary, the post-operative CT was skull-stripped and used to locate the DBS electrode contacts. The post-operative CT and the DTI b0 volume were co-registered to T1 space using the FLIRT tool (FMRIB, Oxford) (Jenkinson *et al*. 2002) and the resulting transformation matrix from DTI to T1 space was used to transform the DBS contact masks from T1 space to DTI space with a trilinear interpolation method. With the transformed electrode contact mask in

DTI space, a mask of the voxel of maximum intensity was made. This mask was taken to represent the voxel within DTI space with the maximum probability of containing the electrode contact. The electric field distribution around the active electrode contacts (volume of activated tissue, (VAT)) was then modelled according to a previously described method (Fernandes *et al*. (2015)). In order to quantify and compare connectivity profiles and their relative strengths, the brain was parcellated into 116 cortical, subcortical and cerebellar regions based on the automated anatomical labelling (AAL) atlas (Tzourio-Mazoyer *et al*. (2002)); each of the 116 regions then represented a network node to which connectivity could be described.

The AAL template (originally in MNI space) was transformed into the DTI space of each individual patient using the existing T1 to DTI transformation matrix obtained in a previous step (see above), concatenated with the transformation matrix derived from linearly co-registering the ICBM152 in MNI space into the T1 space of the structural image with an affine registration (12 degrees of freedom) using a nearest neighbour interpolation. A separate PAG atlas (Ezra *et al*. (2015)) was obtained and also transformed into the DTI space of each individual patient using the same method.

Tractography

The FDT toolbox (version 5.0, www.fmrib.ox.ac.uk.fsl) in FSL was used to process the diffusion data. Each subject's diffusion-weighted image was individually inspected for gross distortions, corrected for head motion and eddy currents and brain extracted. A Markov Chain Monte Carlo sampling algorithm was used to estimate the

local probability of fibre direction in each voxel, using an automated estimation of two fibre directions within each voxel. Using the estimated VAT as a seed region, probabilistic tractography was applied at a single voxel level, sampling 5000 streamlines per voxel.

Results

Subjects

One subject was tested within a week of surgery and did not undergo the full cycle of 4 fills due to an operational failure with the urodynamics system. His data was therefore excluded from subsequent analysis. The remaining subjects (n =5) were all male and the mean age was 62 years (range 54-71). The mean disease duration was 22.4 years (range 16-26 years) and the mean time since DBS implantation was 27 months (range 4 months- 48 months). Stimulation parameters were either bipolar or monopolar in configuration, with amplitude range 1.5 to 3.5V; pulse width 60 to 90μs; and frequency range 20 to 40Hz (see table 1 for details). Electrode co-ordinates in MNI space are given in table 7.1.

Urodynamics results

There was a slight increase in bladder volume at first desire to void with DBS 'ON' compared with DBS 'OFF', however this effect was non-significant (91.4 mls 'ON', (range 24-142); 53.8 mls 'OFF' (range 22-87.5)). The mean bladder volume at maximal capacity was 199 mls (range 103-440) ON stimulation compared with 131

mls (range 39-230) OFF stimulation. This effect was statistically significant ($p < 0.05$, Wilcoxon test).

Patient	Sex	Age	Disease duration (years)	Handedness/side of disease onset	Medications (mg/24 hrs)	Bladder symptoms at time of testing	Change in gait score following PPN DBS[a]	Change in freezing score following PPN DBS[b]	Stimulation parameters (V; μs; Hz)	Electrode location (MNI space, x,y,z)	Mean maximal bladder capacity OFF and ON DBS (ml)
1	M	54	25	R/R	Benserazide 150; levodopa 600	Urgency	Improvement	Improvement	R: 3.5; 90; 35 L: 3.5; 90; 35 Bipolar	R: 10, -28, -23 L: -10, -28, -23	PPN OFF: 154 PPN ON: 168
2	M	71	25	R/R	Entacapone 600; selegiline 10; amantadine 200; carbidopa 212.5; levodopa 850	Frequency, nocturia, urgency	Improvement	Improvement	R: 3; 60; 35 L: 2; 60; 35 Monopolar	R: 9, -28, -24 L: -7, -31, -30	PPN OFF: 89 PPN ON: 113
3	M	58	16	R/not known	Amantadine 200; pramipexole 2.1; benserazide 325; levodopa 1300	-	Worsening	No change	R: 3; 60; 30 L: 3; 60; 30 Bipolar	R: 6, -32, -23 L: -7, -20, -23	PPN OFF: 39 PPN ON: 103
4	M	56	26	R/not known	Trihexyphenidyl 8; benserazide 150; levodopa 600	Urgency	No change	No change	R: 3; 60; 35 L: 3; 60; 35 Bipolar	R: 12, -30, -27 L: -10, -28, -19	PPN OFF: 229.5 PPN ON: 440.5
5	M	71	20	R/R	Rasagiline 1; entacapone 200; carbidopa 850; levodopa 1000	Frequency, nocturia, urgency	Improvement	Improvement	R: 1.7; 60; 20 L: 1.5; 60; 20 Monopolar	R: 7, -29, -16 L: -5, -29, -18	PPN OFF: 143.75 PPN ON: 175

Table 7.1 Demographic and clinical information for each of the 5 patients who underwent urodynamics testing. Information includes age, disease duration, medications, urinary symptoms, change in gait and freezing score after PPN DBS and stimulation parameters. Information about change in maximal bladder capacity on bladder filling is also included.

There was no evidence that PPN DBS altered the amplitude or incidence of detrusor activity; only one subject displayed detrusor overactivity (patient 2) and this was present in all 4 filling trials, regardless of the DBS status. None of the other subjects had detrusor overactivity during any of the trials. (Figure 7.2, table 7.2).

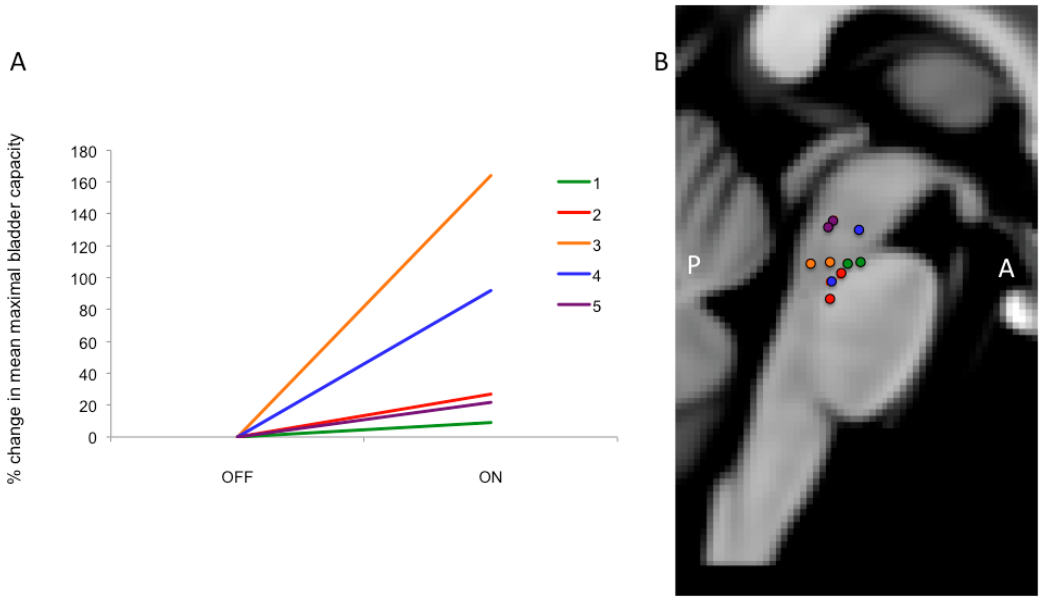


Figure 7.1A. Line graph to show change in mean maximal bladder capacity with DBS OFF and DBS ON. Figure 7.1B. Schematic depicting location of electrodes. Electrode colours correspond with the key in figure 7.2A.

Tractography results

Tractography demonstrated extensive connectivity between the volume of activated tissue and cortical, subcortical and cerebellar regions for patients 2 and 4 (figure 7.3A,B). Areas of connectivity common to both subjects included right sensorimotor cortex, bilateral thalamus, bilateral pallidum, bilateral cerebellum, cerebellar vermis and bilateral precuneus (table 7.1). However, examining the position of the tractography paths in relation to the PAG, there was no overlap between the paths and the outlined region of the PAG in either patient, although patient 2's tracts passed

very close to the outer margins of the PAG (figure 7.3A,B). There is no validated atlas of the pontine bladder regions (PMC and PCC) available, however, both patients had significant projections through the pons. In particular, subject 4's tracts were positioned in the dorsomedial tegmentum close to where the PMC might be expected to be found, whereas projections from subject 2 were more extensive, covering ventral and dorsal regions (figure 7.3C).

Subject	**Projections**
Areas common to both subjects	Right supplementary motor area Right postcentral gyrus Right precentral gyrus Right superior frontal gyrus Right superior parietal lobule Right precuneus Left precuneus Left paracentral lobule Right thalamus Left thalamus Right pallidum Left pallidum Left putamen Right cerebellum Left cerebellum Cerebellar vermis
Areas unique to subject 2	Right superior frontal gyrus Left supplementary motor area Left paracentral lobule Left cuneus Left superior parietal lobule Left post-central gyrus Left anterior cingulate gyrus Right mid cingulate gyrus Left mid cingulate gyrus

	Right posterior cingulate gyrus Left posterior cingulate gyrus Right hippocampus Right cuneus Right caudate
Areas unique to subject 4	Right paracentral lobule

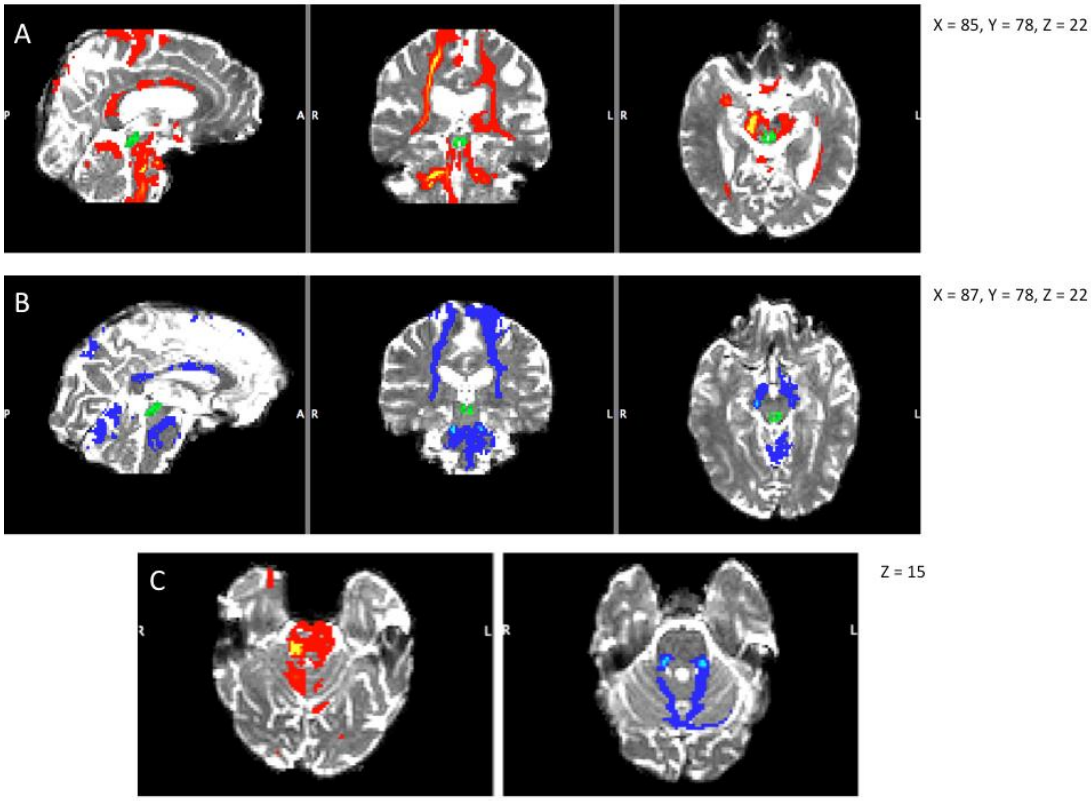


Figure 7.3: Probabilistic projections from volume of activated tissue for subject 2 (A), and subject 4 (B). The anatomical location of the PAG is indicated in green. C: Passage of tracts through the pons for subject 2 (left, red) and subject 4 (right, blue). Tracts are thresholded between 10 streamlines and 5000 streamlines for each subject.

Table 7.2: AAL regions traversed by tracts common to both subjects, or unique to one subject

Discussion

Our main conclusion is that in 5 PD subjects with chronically implanted bilateral PPN DBS electrodes, PPN stimulation produced no significant worsening of urodynamic parameters. In particular there was no change in the incidence or amplitude of detrusor overactivity with DBS ON compared with DBS OFF, and furthermore, there was no significant reduction in bladder capacity or any other urodynamic parameter during the urodynamic studies.

We also observed a small increase in maximal bladder capacity with the PPN DBS turned ON compared with DBS OFF. This is an important observation, because it demonstrates that, rather than provoking urinary frequency, urgency and detrusor overactivity, the effect of bilateral DBS in this small cohort of patients with chronically implanted stimulators was to increase the storage capacity of the bladder.

Pre-operative diffusion tensor imaging scans were available for two participants (subject 2 and subject 4) and probabilistic tractography was used to map the white matter connectivity associated with the volume of activated tissue for each DBS electrode. Tractography demonstrated long range connections to a number of regions that have been described in functional MRI studies of bladder function as being linked with urinary storage or voiding, including the supplementary motor area (Fukuyama *et al*. (1996), Athwal *et al*. (2001), Zhang *et al*. (2005), Khutz-Bushbeck *et al*. (2009), Shy *et al*. (2014)), the post-central gyrus (Shy *et al*. (2014)) the superior frontal gyrus (Khutz-Bushbeck et al), the thalamus (Griffiths *et al*. (2009), Shy *et al*. (2014)), the left putamen (Griffiths *et al*. (2007), Zhang *et al*. (2005)), the pallidum (Shy *et al*. (2014)), the cerebellum (Athwal *et al*. (2001), Zhang *et al*. (2005), Shy *et al*. (2014))

and the precuneus, which has been described particularly in studies using a connectivity-based analytic approach (Khutz-Bushbeck *et al.* (2009), Jarrahi *et al.* (2015)). It is possible that stimulation of long-range connections with some or all of these regions could bring about changes in the physiological response to bladder filling, particularly the very subtle effects that we describe in this study.

We also used a PAG atlas (Ezra *et al*. (2015)) to indicate the likely position of the PAG for each of the two subjects. We found that the white matter pathways in these individuals did not pass through the PAG. In particular, projections from subject 4, for whom DBS had a larger proportional effect on bladder capacity did not pass close to the region of the PAG. The PAG has been hypothesised to act as a "micturition switch" (Griffiths & Fowler (2013)) and modulation of activity within the PAG by PPN DBS could be hypothesised to alter micturition control. However, based on these tractography appearances, it appears unlikely that projections to the PAG are the main factor driving the observed effects of PPN DBS on bladder function.

Finally, we examined the white matter projections through the pons. Although we had no validated atlas of the pontine bladder regions available (L-region and M-region), it was clear that both subjects had projections through the pons, including the dorsomedial tegmentum close to where the pontine micturition centre might be expected to be found. Based on these appearances, it is certainly possible that PPN DBS could influence activity within the pontine micturition centre which in turn might affect the micturition threshold based on feedback to higher centres or intrinsic changes in the excitability of PMC neurons.

There are a number of limitations that prevent us from drawing firm conclusions from this study. The first major limitation is the small number of subjects. PPN DBS is performed in relatively low volumes for PD compared with STN or GPi DBS, and the pool for recruitment is therefore very small. The low subject numbers necessitate cautious interpretation of the experimental findings, particularly as with such small numbers, experimental factors such as the ON/OFF order could not be perfectly balanced across the group. However, given the lack of other published studies on this subject apart from a single case report (Aviles-Olmos *et al*. (2011)), we believe that these findings are nonetheless important, particularly for neurosurgical teams who want to be more informed about possible side effects of PPN DBS.

We also note that these results describe the effect of bilateral PPN DBS in patients with chronically implanted stimulators. By contrast, the previous case-report described the effect of unilateral right-sided DBS in the acute post-operative period (Aviles-Olmos *et al*. (2011)). There is some evidence that the pontine control of micturition may be right-lateralised (Blok *et al*. (1997)). The effect of laterality is an important subject for future investigation, as deterioration in bladder symptoms may occur more commonly with unilateral (or specifically right-sided) stimulation. However, it was our aim in this study to test the effect of bilateral chronic PPN DBS on bladder filling parameters, as this is the standard pattern of implantation for patients in our unit and has been reported by many other centres (Mazzone *et al*. (2005) Plaha *et al*. (2005); Stefani *et al*. (2007)) and we hope that the question of laterality will be addressed in future work. Finally, we were not able to test voiding parameters in this study. This is an important area, particularly given the possible influence of DBS on the PMC, and should be investigated in a separate study.

To summarise: we have studied the effect of PPN DBS on bladder filling parameters in 5 male subjects with PD. PPN DBS did not provoke detrusor overactivity or reduce sensory bladder filling thresholds. There was a small increase in maximal bladder capacity with PPN DBS ON, but larger studies are essential to validate this finding. Probabilistic tractography demonstrated a possible mechanism for the observed effects either via long-range connections to important cortical, subcortical and cerebellar regions for bladder control or via direct effects on the PMC.

CONCLUDING REMARKS

In this thesis I addressed two separate but overlapping subject areas. The first concerned the central control of autonomic function and the second was the question of the neural control of the bladder. My approach was not bottom-up or particularly systematic; rather I attempted to add new insights to already studied fields, using the research techniques and clinical populations available to me. The main insights derived from the work in this thesis come from the application of different combinations of techniques and clinical populations than have been used in the past.

Regarding autonomic neuroscience and the central organisation of autonomic control, I first considered the effect of STN DBS on autonomic function and used tractography to associate a connectivity profile with different autonomic outcomes; and then I used whole brain diffusion MRI techniques to investigate grey and white matter structural changes linked with autonomic symptoms. The connecting features between both studies were the use of PD subjects and the same autonomic symptom questionnaire for both studies. The main conclusion from this section was that white matter changes in autonomic dysfunction tend to be somewhat non-specific whereas grey matter changes in certain key regions such as the hippocampus may well drive autonomic deterioration in PD. As we did not adjust for pre-existing degenerative changes in these key grey matter autonomic centres, the results of the STN fingerprint study may not tell the full story about important connectivity patterns associated with autonomic improvement following DBS, and this study could be repeated in the future with larger patient numbers, physiological testing for autonomic assessment as well as

questionnaires and controls built into the algorithms to account for pre-existing degenerative change in the white and grey matter.

The second part of the thesis dealt more specifically with the neural control of the bladder. I did not directly address the overlap between autonomic control and bladder control, since urinary bladder control enlists both autonomic and somatic mechanisms. However, the results of the TBSS study in chapter 4 confirm that there is not a complete overlap between central autonomic networks and bladder control networks in the brain. I first investigated the possibility of using local field potential signals to study in detail the role of the STN, GPi, VPL and PAG in urinary storage and voiding. Although these findings are in essence pilot data, as such experiments have not been performed before, the LFP data, despite the limitations of the experimental design, were highly informative. In particular, it was interesting to see that the spectral profile of the STN and GPi associated with voiding differed to that during pelvic floor relaxation, strongly implying that these basal ganglia regions have a unique role in voiding that is not fully accounted for by their their role in pelvic floor relaxation. The combined correlation (across GPi and STN) between "relax pelvic floor" beta power and voiding symptoms also suggests that the beta signal may be relevant in the pathogenesis of voiding dysfunction in PD, shedding new light on pathophysiological mechansims. The development of this experimental paradigm, with EMG recordings, ON and OFF dopamine testing and increased patient numbers would be likely to hold useful and important insights for the field of neuro-urology. PAG and VPL testing, although only in one subject for voiding and filling respectively, also provided some fascinating pilot data. The dramatic response in the alpha band of the PAG during imagined void and pelvic floor relaxation confirm its

role in the cognitive motor aspects of voiding preparation, while the significant signal change in higher frequencies during voiding itself suggests that it is also involved in the voiding process. As the gamma signal, which corresponds best to the BOLD signal, was significantly raised during voiding and pelvic floor relaxation, these might be the responses that are most easily detected by fMRI. The lower frequency responses however confirm additional activity that might be more difficult to assess using conventional imaging techniques. Data from the filling LFP study importantly provided information to support the proposal that the PAG and VPL are connected in the sensory bladder information transfer pathway.

The final study of the thesis was a clinical approach to assess the effect of PPN DBS on bladder filling parameters. Although I only report here on a small, homogeneous subject group (n = 5, all bilateral and chronic PPN DBS), there was a significant improvement in bladder capacity within this cohort. This suggests that the effect of PPN DBS on bladder function is not always an adverse one, but may possibly have beneficial effects in some patients, probably depending on exact placement, stimulation parameters and individual subject anatomy. A tractography study in two of the five subject suggested that the mechanism of action was unlikely to be primarily via the PAG but could be a result of current spread to the pontine micturition centres.

The combined results of the LFP and PPN DBS chapters represent a distant goal in neuro-urology, which would be to develop an implantable closed-loop system for bladder control, able to respond to the spectral profile associated with either the cognitive will or desire to void, or with a particular bladder volume, and then able to

initiate voiding via stimulation of the appropriate structures. This would be relevant in patients with voiding dysfunction or spinal cord injury, preventing them from initiating voiding. The converse design would be one that detected signals associated with urinary urgency in patients with overactive bladder, and either suppressed these signals, or inhibited the pontine micturition centre and deferring voiding, at least for a sufficient length of time to prevent incontinence. Although such devices may seem unlikely clinical applications at the present time, it is hopeful that as surgical techniques become safer and technology more sophisticated, implantable devices for urinary control may be possible in the future.

FUTURE DIRECTIONS

Understanding the neural control of the autonomic nervous system and bladder in the human is still in the relatively early stages of its development compared with some other branches of neurophysiology and cognitive science. As a result it has been possible for me to write a thesis using several different techniques, all of which had rarely been applied to the question of autonomic or bladder control. However, the consequence of this is that almost every aspect of the studies described here are in need of refinement or development; each chapter has major limitations or steps that were not performed due to contstraints of time, finances or other resources (including willing subjects!)

For example, in the autonomic fingerprint chapter, the analysis should be repeated prospectively. Here, the significant areas for autonomic control that we identified would be utilised as part of a reverse-tractography analysis pre-operatively to locate the optimal placement of the electrode within the subthalamic nucleus. Patients would then be evaluated post-operatively to determine whether this produces a superior autonomic result to existing targeting methods. The fingerprint study could also be carried out in far greater depth, incorporating a multivariate analysis style to look at relevant connectivity profile for each subset of autonomic symptoms, and also incorporating physiological measures of autonomic function in addition to validated questionnaires to define autonomic symptom severity.

In the TBSS study (chapter 4) use of higher resolution scanning techniques with cardiac gating to control for CSF pulsatility may enable a similar study to be repeated but to yield more specific insights about brainstem structural changes in PD that are associated with autonomic symptoms. Also, recruiting a larger cohort of patients and carrying out serial scans over time, would allow us to correlate changes in autonomic profile with changes in the TBSS profile for individual patients.

In the LFP study, the main immediate clinical application is in the potential for the stratification of urinary symptoms in Parkinson's disease and the suggestion of a link between beta oscillations and voiding symptom severity, and theta oscillations and the severity of frequency, incontinence and urgency. It suggests that these two groups of symptoms must be considered, and treated, separately in the clinical context. There is also a possibility that by targeting or suppressing theta oscillations in the basal ganglia (or at another anatomical point within a linked oscillatory network), improvements in urinary storage symptoms might be achieved. However, both the basal ganglia study and the PAG/VPL study need to be repeated or extended with better recruitment and monitoring during the trials. In particular, a means of recording the strength of pelvic floor muscle activity is essential for fully interpreting the meaning of the LFP signal during pelvic floor contraction and relaxation, and for the PAG/VPL work to be of any real significance, more subjects need to be recruited. Regarding the PAG and VPL, there is clearly much work to be done in understanding the significance of the alpha signal, both in terms of the neuronal population that drives its synchrony, the functional significance of alpha, and its clinical utility.

Finally, in terms of the pedunculopontine nucleus (PPN) a prospective trial on the effects of PPN DBS for bladder function, including pre-operative urodynamics and questionnaires, followed by post-operative urodynamics, may help to confirm the results presented in this chapter. High resolution structural and functional imaging studies to look for altered structure or function within the PPN in patients with bladder symptoms, would help confirm the relevance of the PPN in the bladder control network.

As a truly final comment, two important approaches that were absent from this thesis are histology and brain tissue electrophysiology. Correlating patient symptoms with histological evidence of degenerative changes in putative autonomic and bladder-related brain structures (both grey and white matter) would add weight to the PPN chapter as well as the early chapters on structural MRI. Understanding more about the significance of neuronal oscillations within and between the PAG and VPL would take the LFP study to a deeper level beyond simple observation and correlation with behavioural features.

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APPENDIX 1

DBS Autonomic questionnaire

COMPASS-31 questionnaire and scoring algorithm

ICIQ male and female

DBS 'side-effects' Questionnaire

The aim of this questionnaire is to assess whether you have any conditions that may be affected by Deep Brain Stimulation (DBS). By assessing before and after stimulation, we aim to find out if DBS either improves those symptoms or makes them worse. We will use this data to adjust your care if necessary. With your permission, we would like to use the data (anonymously) in a study to test whether DBS affects body systems other than the one you are undergoing DBS to treat.
If you would not like us to use this data, please tick here: □

Your Name:.....
Date of Birth:.....
Today's Date:.....Pre-op □ Post op □
Your Diagnosis:.....
Target Nuclei:.....(we will fill this in)
Hospital number:.....

Pre-op medications	Post-op medications

Medical History
Do you or have you ever suffered from any of the following diseases?

Yes No

1a. High Blood Pressure (whether treated or not)	☐	☐
1b. Postural or Orthostatic Hypotension	☐	☐
2. Heart Failure	☐	☐
3. Atrial Fibrillation	☐	☐
4. Other abnormal heart rhythm	☐	☐
5. Angina	☐	☐
6. Asthma	☐	☐
7. Chronic Obstructive Pulmonary Disease (e.g. chronic bronchitis)	☐	☐
8. Irritable Bowel Syndrome	☐	☐
9. Inflammatory Bowel Disease	☐	☐
10. Neurogenic or unstable bladder	☐	☐
11. Eczema	☐	☐
12. Hayfever	☐	☐
13. Rheumatoid Arthritis	☐	☐
14. SLE (Lupus)	☐	☐
15. Other Autoimmune disease (e.g. Sjogren's)	☐	☐
16. Immunodeficiency Syndrome	☐	☐
17. Hyperthyroidism	☐	☐

Autonomic Scores

18. In the past year, have you ever felt faint, dizzy, or "goofy" or had difficulty thinking soon after standing up from a sitting or lying down position?

□1. Yes
□2. No

If you marked YES go to question 19.
If you marked No go to question 37.

19. When standing up, how frequently do you get these feelings or symptoms?

□1. Rarely
□2. Occasionally
□3. Frequently
□4. Almost always

20. How would you rate the severity of these feelings or symptoms?

□1. Mild
□2. Moderate
□3. Severe

21. For how long have you been experiencing these feelings or symptoms?

□1. Less than 3 months
□2. 3 to 6 months
□3. 7 to 12 months
□4. 13 months to 5 years
□5. More than 5 years
□6. As long as I can remember

22. In the past year, how often have you ended up fainting soon after standing up from a sitting or lying down position?

□0. Never
□1. Once
□2. Twice
□3. Three times
□4. Four times
□5. Five or more times

23. How cautious are you about standing up from a sitting or lying down position?

□1. Not cautious at all
□2. Somewhat cautious
□3. Extremely cautious

24. What part of the day are these feelings worst? (*Mark only one*)

□1. Early morning
□2. Rest of morning
□3. Afternoon
□4. Evening
□5. At night, when I get up after I've been asleep
□6. No particular time is worst
□7. Other time, please specify_____

25. In the past year, have these feelings or symptoms that you have experienced:

□1. Got much worse
□2. Got somewhat worse
□3. Stayed about the same
□4. Got somewhat better
□5. Got much better
□6. Completely gone

Please rate the average severity you have experienced in the past year for each of
the following symptoms;

	1. Not experienced	2. Mild	3. Moderate	4. Severe
26. Rapid or increased heart rate? (palpitations)	□	□	□	□
27. Sickness to your stomach (nausea) or vomiting	□	□	□	□
28. A spinning or swimming sensation?	□	□	□	□
29. Dizziness?	□	□	□	□
30. Blurred vision?	□	□	□	□
31. Feeling of weakness?	□	□	□	□
32. Feeling shaky or shaking (sensation)	□	□	□	□
33. Feeling anxious or nervous?	□	□	□	□
34. Turning pale?	□	□	□	□
35. Clammy feeling to your skin?	□	□	□	□

36. Do you have any biological (blood, natural) relatives among your parents, grandparents, brothers, sisters, or children who have frequent dizziness after standing from a sitting or lying down position?

□1. Yes
□2. No

If Yes, please list their names and relationship to you.

Name	Relationship
_____	_____
_____	_____
_____	_____
_____	_____

In the past year, have you ever felt faint, dizzy, or "goofy" or had difficulty thinking:

37. soon after a meal? □1. Yes □2. No

38. after standing for a long time? □1. Yes □2. No

39. during or soon after physical activity or exercise? □1. Yes □2. No

40. during or soon after being in a hot bath, shower, tub, or sauna? □1. Yes □2. No

41. Have you ever felt dizzy or faint or actually fainted when you saw blood or had a blood sample taken?

□1. Yes □2. No

In the past year, have you fainted:

42. whilst passing urine? □1. Yes □2. No

43. whilst coughing? □1. Yes □2. No

44. whilst pressing on side of neck? □1. Yes □2. No

45. before a public speech? □1. Yes □2. No

46. any other time? □1. Yes □2. No

If you marked "Yes" to any of these questions on fainting, please describe the circumstances.

_____

_____

47. In the past year, have you ever completely lost consciousness after a spell of dizziness?

□1. Yes □2. No

48. In the past year, have you had any seizures or convulsions?

□1. Yes □2. No

please describe the circumstances

_____

_____

In the past 5 years how would you rate the amount of trouble, if any, you have had:

	1.None	2.Some	3.A Lot	4.Constant
49. with paralysis in parts of your face?	□	□	□	□
50. with feelings of complete weakness all over your body?	□	□	□	□
51. with attacks of uncontrollable movements of your arms or legs?	□	□	□	□
52. with attacks in which you couldn't control your speech?	□	□	□	□

53. Have you ever in your adult life had a spell of dizziness?

□1. Yes □2. No

54. In the past year, have you ever noticed color changes in your skin, such as red, white, or purple?

□1. Yes □2. No

If Yes, complete the following box.
If No, go to question 65.

What color changes have occured?
(*Mark all that apply*.)
□55.My skin turns red
□56.My skin turns white
□57.My skin turns purple
□58.Other

If other, please
specify_____

What parts of your body are affected by these color changes?
(*Mark all that apply*.)
□59.My hands
□60.My feet
□61.Other parts

If other please specify _____

□62.Entire body

□63.For how long have you been experiencing these changes in skin colour?

□1. Less than 3 months

□2. 3 to 6 months
□3. 7 to 12 months
□4. 13 months to 5 years
□5. More than 5 years
□6. As long as I can remember

64. Are these changes in your skin color:

□1.Getting much worse
□2. Getting somewhat worse
□3. Staying about the same
□4. Getting somewhat better
□5. Getting much better
□6.Completely gone

65. In the past year, after a long hot bath or shower, have you ever noticed the pads on the ends of your fingers wrinkle up?

□1. Yes □2. No

66. In the past 5 years, what changes, if any, have occurred in your general body sweating?

□1. I sweat much more than I used to
□2. I sweat somewhat more than I used to
□3. I haven't noticed any changes in my sweating
□4. I sweat somewhat less than I used to
□5. I sweat much less than I used to

67. In the past 5 years, what changes, if any, have occurred in the amount your feet sweat?

□1. They sweat much more than they used to
□2. They sweat somewhat more than they used to
□3. I haven't noticed any changes
□4. They sweat somewhat less than they used to
□5. They sweat much less than they used to

68. In the past 5 years, what changes, if any, have occurred in facial sweating after eating spicy foods?

□1. I sweat much more than I used to
□2. I sweat somewhat more than I used to
□3.. I haven't noticed any changes in my sweating
□4. I sweat somewhat less than I used to
□5. I sweat much less than I used to
□6. I avoid eating spicy foods because I sweat so much
□7. I avoid eating spicy foods for other reasons

In the past 5 years, what changes, if any, have occurred in your ability to tolerate heat during a hot day, strenuous work or exercise, hot bath or shower, hot tub, or sauna?
(*Mark all that apply*.)

69. I now get more overheated □1. Yes □2. No
70. I now get dizzy □1. Yes □2. No
71. I now get short of breath □1. Yes □2. No
72. Other changes, please specify

_____

73. No change □1. Yes
□2. No

74. Do your eyes feel excessively dry? □1. Yes
□2. No

75. Does your mouth feel excessively dry? □1. Yes
□2. No

76. Do you have excessive amounts of saliva formation? □1. Yes
□2. No

77. What is the longest period of time that you have had any one of these symptoms: dry eyes, dry mouth, or increased saliva production?

□0. I have not had any of these symptoms
□1. Less than 3 months
□2. 3 to 6 months
□3. 7 to 12 months
□4. 13 months to 5 yesrs
□5. More than 5 years
□6. As long as I can remember

78. For the symptom of dry eyes, dry mouth, or increased saliva production that you have had for the longest period of time, is this symptom:

□0. I have not had any of these symptoms
□1. Getting much worse
□2. Getting somewhat worse
□3. Staying about the same
□4. Getting somewhat better
□5. Getting much better
□6. Completely gone

79. What weight changes, if any, have you had over the past year?

□1. I have lost about_____kg
□2. My weight has not changed

□3. I have gained about_____kg

80. In the past year, have you noticed any changes in how quickly you get full when eating a meal?

□1. I get full a lot more quickly now than I used to
□2. I get full more quickly now than I used to
□3. I haven't noticed any change
□4. I get full less quickly now than I used to
□5. I get full a lot less quickly now than I used to

81. In the past year, have you felt excessively full or persistently full (bloated feeling) after a meal?

□1. Never
□2. Sometimes
□3. A lot of the time

82. In the past year, have you felt like you had a persistent upset stomach (nausea)?

□1. Never
□2. Sometimes
□3. A lot of the time

83. In the past year, have you vomited after a meal?

□1. Never
□2. Sometimes
□3. A lot of the time

84. In the past year, have you had a cramping or colicky abdominal pain? If your answer is ‘never’ go to question 87.

□1. Never
□2. Sometimes
□3. A lot of the time

85. Are these pains usually after a meal? □1. Yes □2. No

86. How long have you had these cramping or colicky abdominal pains?

□1. Less than 3 months
□2. 3 to 6 months

□3. 7 to 12 months
□4. 13 months to 5 years
□5. More than 5 years
□6. As long as I can remember

87. In the past year, have you had any bouts of diarrhoea?

□1. Yes □2. No
（If Yes, please complete questions 88 to 93. If No, go to 94)

88. How frequently does this occur?

□1. Rarely
□2. Occasionally
□3. Frequently, _____times per month
□4. Constantly

89. How severe are these bouts of diarrhoea?

□1. Mild
□2. Moderate
□3. Severe

90. What part of the day do they seem to be worse?

□1. First thing in the morning
□2. Rest of the morning
□3. Afternoon
□4. Evening
□5. During the night
□6. No particular time

91. Do these bouts of diarrhoea usually occur after meal? □1. Yes
□2. No

92. Are these bouts of diarrhoea accompanied by a lot of rectal gas (flatus)?

□1. Never
□2. Occasionally
□3. Frequently
□4. Always

93. Are your bouts of diarrhoea:

□1. Much worse
□2. Somewhat worse
□3. Staying the same

□4. Somewhat better
□5. Much better
□6. Completely gone

94. In the past year, have you been constipated? □1. Yes □2. No

(If Yes, please complete Q95 to 97. If No, please go to Q98.)

95. How frequently are you constipated?

□1. Rarely
□2. Occasionally
□3. Frequently, _____times per month
□4. Constantly

96. How severe are these episodes of constipation?

□1. Mild
□2. Moderate
□3. Severe

97. Is your constipation getting:

□1. Much worse
□2. Somewhat worse
□3. Staying the same
□4. Somewhat better
□5. Much better
□6. Completely gone

98. Overall, are your abdominal symptoms of vomiting, diarrhoea, constipation, or weight loss getting:

□0. I have not had these symptoms
□1. Much worse
□2. Somewhat worse
□3. Staying the same
□4. Somewhat better
□5. Much better
□6. Completely gone

99. Which one of the following symptoms have been most troublesome for you? (Mark only one.)

□0. None
□1. Vomiting
□2. Diarrhoea

□3. Constipation
□4. Weight loss

100. How long have you had this most troublesome symptom?

□0. I do not have any of these symptoms
□1. Less than 3 months
□2. 3 to 6 months
□3. 7 to 12 months
□4. 13 months to 5 years
□5. More than 5 years
□6. As long as I can remember

101. Is this most troublesome symptom getting:

□0. I do not have any of these symptoms
□1. Much worse
□2. Somewhat worse
□3. Staying the same
□4. Somewhat better
□5. Much better
□6. Completely gone

BH IBS 1.

During the past 4 weeks, how often have you:

	1.All the time	2.Most of the time	3.Some of the time	4.A little of the time	5.Never
B1. Had discomfort or pain in your abdomen?					
B2. Had loose, mushy or water bowel motions?					
B3. Been troubled with diarrhoea?					
B4. Been troubled by hard bowel motions?					
B5. Felt the need to strain to pass a stool?					
B6. Been troubled by constipation?					
B7. Experienced pain or discomfort after eating?					

102. In the past 5 years, how would you rate the amount of trouble, if any, you have had with difficulty in swallowing?

□1. No trouble
□2. Some trouble
□3. A lot of trouble
□4. Constant trouble

103. In the past 5 years, how would you rate the amount of trouble, if any, you have had with everything you eat tasting the same?

□1. No trouble
□2. Some trouble
□3. A lot of trouble
□4. Constant trouble

Have you ever in your adult life:
104. been nauseated or vomited? □1. Yes
□2. No
105. had a bout of diarrhoea? □1. Yes
□2. No
106. lost your appetite for at least part of a day? □1. Yes
□2. No
107. felt discomfort or pain in the pit of your stomach? □1. Yes
□2. No

108. In the past year, have you ever leaked urine or lost control of your bladder function?
□1. Never
□2. Occasionally
□3. Frequently, _____times per month
□4. Constantly
109. In the past year, have you had difficulty passing urine?

□1. Never
□2. Occasionally
□3. Frequently, _____times per month
□4. Constantly

110. In the past year, have you had trouble completely emptying your bladder?

□1. Never
□2. Occasionally
□3. Frequently, _____times per month
□4. Constantly

If you have a urinary/bladder problem, please answer the following questions. If not, please go to Question 111.

KH 1. How would you describe your health at the present?

□1.Very good □2.Good □3.Fair □4.Poor
□5.Very poor

KH2. How much do you think your bladder problem affects your life?

□1.Not at all □2.A little □3.Moderately □4.A lot

Below are some daily activities that can be affected by bladder problems.
KH3. How much does your bladder problem affect:
A.Household tasks?
□1.Not at all □2.A little □3.Moderately □4.A lot

B.Your job or your normal daily activities outside the home?
□1.Not at all □2.A little □3.Moderately □4.A lot
KH4. How much does your bladder problem affect:
A. Physical activities e.g. walking, running, going to the gym, playing sport?
□1.Not at all □2.A little □3.Moderately □4.A lot

B.Your ability to travel?
□1.Not at all □2.A little □3.Moderately □4.A lot

C.Your social life?
□1.Not at all □2.A little □3.Moderately □4.A lot

D.Your ability to see and visit friends?
□1.Not at all □2.A little □3.Moderately □4.A lot

KH5. How much does your bladder problem affect:
A. Your relationship with your partner?

□1.NA □2.Not at all □3.A little □4.Moderately □5.A lot

B. Your sex life?

□1.NA □2.Not at all □3.A little □4.Moderately □5.A lot
C. Your family life?

□1.NA □2.Not at all □3.A little □4.Moderately □5.A lot
KH6. Does your bladder problem make you feel:
A. Depressed?

□ 1.Not at all □2.Slightly □3.Moderately □4.Very much
B. Anxious or nervous?

□ 1.Not at all □2.Slightly □3.Moderately □4.Very much
C. Bad about yourself?

□ 1.Not at all □2.Slightly □3.Moderately □4.Very much

KH7. Does your bladder problem:
A. Affect your sleep?

□ 1.Never □2.Sometimes □3.Often □4.All the time

B. Make you feel worn out and tired?

□ 1.Never □2.Sometimes □3.Often □4.All the time

KH8. Do you do any of the following? If so, how much?

A. Wear pads to keep dry?

□ 1.Never □2.Sometimes □3.Often □4.All the time

B. Be careful about how much fluid you drink?

□ 1.Never □2.Sometimes □3.Often □4.All the time

C. Change your underclothes if they get wet?

□ 1.Never □2.Sometimes □3.Often □4.All the time

D. Worry incase you smell?

□ 1.Never □2.Sometimes □3.Often □4.All the time

KH9. From the list below, select how much each of the following problems affect you.

	1.Neve	2.A litt	3.Moderatel	4.A lo
A.Frequency: going to the toilet very often				
B.Nocturia: getting up at night to pass urine				
C.Urgency: a strong and difficult to control desire to urine				
D.Urge incontinence: urinary leakage accompanied strong desire to pass urine				
E.Stress incontinence: urinary leakage with physica activity e.g. coughing, running				
F.Nocturnal eneuresis: wetting the bed at night				
G.Intercourse incontinence: urinary leakage with se intercourse				
H.Waterworks infections				
I.Bladder pain				

111. How would you describe your current sexual desire?

□1. Completely absent
□2. Greatly reduced
□3. Somewhat reduced
□4. About the same or more than in the past

IF YOU ARE MALE, please complete Q 112 to 123. If not, please go to Q124

112. Are you able to have a full erection?

□1. Never, under any circumstances
□2. Much less frequently than in past
□3. Somewhat less frequently than in past
□4. The same, or more frequently, than in past

Which of the following statements apply to your situation?
(*Fill in all that apply*.) (Tick = 1)

113. □ My ability to have intercourse is the same
114. □I have erections but am unable to have intercourse
115. □I can have intercourse only some of the time
116. □My erections are definitely impaired
117. □ I am able to have intercourse, but am unable to ejaculate
118. □ I have "dry orgasms" and afterward my urine looks milky
119. □ I have been unable to have erections or they have been impaired since I started taking a medication called _____

120. □ Other situation, please describe_____

121. □ None of the above apply

122. How long have you had difficulty with erectile function?

□0. I do not have this difficulty
□1. Less than 3 months
□2. 3 to 6 months
□3. 7 to 12 months
□4. 13 months to 5 years
□5. More than 5 years
□6. As long as I can remember

123. Is this difficulty getting:

□0. I have not had difficulty
□1. Much worse
□2. Somewhat worse
□3. Staying the same
□4. Somewhat better
□5. Much better
□6. Completely gone

124. In the past year, without sunglasses or tinted glasses, has bright light bothered your eyes?
□1. Never □2. Occasionally □3. Frequently □4. Constantly

125. How severe is this sensitivity to bright light?

□1. Mild □2. Moderate □3. Severe

126. In the past year, have you had trouble focusing your eyes?

□1. Never □2. Occasionally □3. Frequently □4. Constantly

127. How severe is this focusing problem?

□1. Mild □2. Moderate □3. Severe

128. In the past year, have you had blurred vision?

□1. Never □2. Occasionally □3. Frequently □4. Constantly

129. How severe is this blurred vision?

□1. Mild □2. Moderate □3. Severe

130. In the past year, have you had difficulty seeing at night?

□1. Never □2. Occasionally □3. Frequently □4. Constantly

131. How severe is this night vision problem?

□1. Mild □2. Moderate □3. Severe

132. In the past year, has the same degree of light seemed:

□1. Excessively dimmer
□2. Much dimmer
□3. About the same
□4. Much brighter
□5. Excessively brighter

133. Which one of the following eye symptoms is the most troublesome for you? (Mark only one)

□0. None
□1. Trouble Focusing
□2. Blurred Vision
□3. Difficulty seeing at night

134. How long have you had this most troublesome eye symptom?

□0. I don't have any of these symptoms
□1. Less than 3 months
□2. 3 to 6 months
□3. 7 to 12 months
□4. 13 months to 5 years
□5. More than 5 years
□6. As long as I can remember

135. Is this most troublesome symptom with your eyes getting:

□0. I don't have any of these symptoms
□1. Much worse
□2. Somewhat worse
□3. Staying the same
□4. Somewhat better
□5. Much better
□6. Completely gone

136. In the past year, have you ever noticed or been told that while sleeping you stop breathing for several seconds?

□1. Yes □2. No

137. In the past year, have you ever noticed or been told that while sleeping you snore loudly?

□1. Yes □2. No

Have you ever been told you have or been diagnosed as having:

138. Narcolepsy? □1. Yes □2. No □3. Don't know

139. Obstructive sleep apnoea? □1. Yes □2. No □3. Don't know

140. Abnormal or disordered sleep patterns? □1. Yes □2. No □3. Don't know

141. Currently, how refreshing and restorative is your sleep?
□1. Not at all restorative - derive no benefit
□2. Some slight restorative value
□3. Restorative, but not adequate

□4. Relatively satisfactory
□5. Very satisfactory - feel completely refreshed

142. Compared with a year ago, how would you rate your own sleep over the last month?

□1. Last month was much worse than a year ago
□2. Last month was slightly worse than a year ago
□3. Last month was about the same as a year ago
□4. Last month was slightly better than a year ago
□5. Last month was much better than a year ago

143. Have you ever in your adult life had difficulty getting to sleep or staying asleep once you were asleep?

□1. Yes □2. No

144. In the past year, have you ever noticed or been told that during the day you sometimes breathe very loudly (e.g.,croup)?

□1. Yes □2. No

How would you describe your alcohol use over the past year?
(Mark all that apply.)

145. □I have not drunk any alcohol over the past year
146. □I drink socially only
147. □I have used alcohol excessively in the past year
148. □I have been intoxicated one or more times in the past year
149. □I have passed out from drinking too much alcohol one or more times in the past year

How would you describe your illicit drug use over the past year?
(Mark all that apply.)

150. □I have not used drugs over the past year
151. □ I have used drugs excessively in the past year
152. □I have been intoxicated one or more times in the past year
153. □ I have passed out from excessive drug use one or more times in the past year

154. Have you ever felt that you have used alcohol or drugs excessively?

155. Have you ever been told or have you been diagnosed as having alcohol or drug dependency?

156. Have you ever received treatment for alcohol or other drug dependency?

Please list the drugs involved, including alcohol
1._____
2._____
3._____
4._____

Which of the following describe your cigarette smoking?
(*Mark all that apply*)

157. I have never smoked cigarettes □1. Yes □2. No
158. I have smoked cigarettes in the past but have stopped: □1. Yes □2. No
159. Date quit:.....
163. □I am currently smoking □1. Yes □2. No

164. ..... cigarettes per day (if currently smoking)
166. In the past 5 years, how would you rate the amount of trouble, if any, you have had with over-sensitive hearing?

□1. None □2. Some □3. A lot □4. Constant

167. Have you ever in your adult life had difficulty keeping your mind on your job or task?

□1. Yes □2. No

Immune questions

Allergy/anaphylaxis

A1. Do you suffer from nasal itch, sneezing or a running/blocked nose at certain times of year or as a response to coming into contact with something you are allergic to?

□1. Yes □2. No

(B)If you answered 'yes', please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.

Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □

A2. Do you suffer from itchy, red eyes at certain times of year or as a response to coming into contact with something you are allergic to?

□1. Yes □2. No

(B)If you answered 'yes', please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.

Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □

A3. Do you ever suffer from wheeze, breathlessness, chest tightness or cough in response to certain triggers?

□1. Yes □2. No

(B)If you answered 'yes', please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.

Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □

A4. Do you have a known food allergy or intolerance?

□1. Yes □2. No

If 'yes' please give details:

Allergy to.....

What happens when you have this food.....

(B)If you answered 'yes', please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.

Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □

A5. Do you suffer from eczema?

□1. Yes □2. No

(B)If you answered 'yes', please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.

Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □

A6. Do you/have you ever suffered from contact dermatitis?

□1. Yes □2. No

(B)If you answered 'yes', please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.

Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □

A7. Have you ever had an adverse reaction in response to medical drugs or to blood products?

□1. Yes □2. No
If ‘yes’ please give details:
Adverse reaction to.....
What happened.....
(B)If you answered ‘yes’, please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.
Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □
A8. Have you ever had a systemic allergic reaction (i.e. anaphylaxis)?
□1. Yes □2. No
If ‘yes’, please give details:
Adverse reaction to.....
What happened.....

Organ specific/non organ specific immunological disease

A9. Do you or have you ever suffered from any of the following diseases? Please circle as appropriate:

1.(a)Glomerulonephritis (b)Other autoimmune kidney disease
2.(a)Rheumatoid arthritis (b)Ankylosing spondylitis (c)Reiter's syndrome (d)Psoriatic arthritis (e)Enteropathic arthritis (f)Behcet's syndrome (g)Other autoimmune arthropathy
3. (a)Systemic lupus erythematosis (SLE) (b)Sjogren's syndrome (c)polymyositis (d)scleroderma (e)other connective tissue disease
4.(a)Polyarteritis nodosa (b)giant cell arteritis (c)Churg Strauss disease (d)Takayasu's arteritis (e)Wegener's granulomatosis (f)cerebral vasculitis (e)other form of vasculitis
5.(a)Eczema (b)psoriasis (c)contact dermatitis (d)pemphigoid (e)vitiligo (f)other autoimmune skin disease
6.(a)Asthma (b)sarcoidosis (c)extrinsic allergic alveolitis (e.g. farmer's lung, bird fancier's lung) (d)other autoimmune lung disease
7.(a)Atrophic gastritis (b)pernicious anaemia (c)celiac disease (d)inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis) (e)autoimmune hepatitis (f)primary biliary cirrhosis (g)other autoimmune gastrointestinal or liver disease
8.(a)Type I diabetes (b)Addison's disease (c)Grave's disease (d)Hashimoto's thyroiditis (e)other autoimmune endocrine disease
9.(a)Autoimmune haemolytic anaemia (b)autoimmune thrombocytopenia (c)other autoimmune haematological disease
10.(a)Multiple sclerosis (b)myasthenia gravis (c)Guillan-Barre syndrome (d)chronic inflammatory demyelinating neuropathy (d)other neurological autoimmune disease

(B)If you circled one of the above conditions, please rate the severity of your symptoms on a scale of 1-10 where 1 is mild and 10 is severe.

Pre-op severity of symptoms (1-10) □ Post-op severity of symptoms (1-10) □

A10.

Please indicate how frequently you have suffered from the following problems over the past year

	1. Not at all	2. Rarely	3. Often	4. Very frequently
A.Common cold				
B.Gastroenteritis				
C.Chest infection				
D.Mouth ulcers				
E.Thrush				
F.Athlete's foot				
G. Sore throat				

A11.
Please indicate how severely you have suffered from the following problems over the past year

	1. Not at all	2. Mildly	3. Moderately	4. Severely
A.Common cold				
B.Gastroenteritis				
C.Chest infection				
D.Mouth ulcers				
E.Thrush				
F.Athlete's foot				
G. Sore throat				

A12.
Have you suffered from any of the following conditions for more than 6 months?

A.Depression □1. Yes □2. No
B.Anxiety □1. Yes □2. No
C.Psychosis/schizophrenia □1. Yes □2. No
D.Eating disorder □1. Yes □2. No

St George's Respiratory Questionnaire
Part 1
Describe how often your lung problems have affected you over the past 4 weeks. Please place a mark in the box for each question.

	1.Almost every day	2.Several days a week	3.A few days a month	4.Only with lung/respiratory infections	5.Not at all
1.Over the past 4 weeks I have coughed:					
2.Over the past 4 weeks I have brought up phlegm (sputum):					
3.Over the past 4 weeks I have had shortness of breath:					
4.Over the past 4 weeks I have had episodes of wheezing:					

5.During the last 4 weeks how many severe/very unpleasant attacks of respiratory problems have you had? Mark one answer only.
□1. More than 3 episodes
□2. 3 episodes
□3. 2 episodes
□4. 1 episode
□5. 0 episodes

6.How long did the worst episode of lung/respiratory problem last? Mark one answer only. Go to question 7 if you didn't have a severe episode.
□1. 1 week or more
□2. 3 days or more
□3. 1 or 2 days
□4. less than 1 day

7.Over the last 4 weeks, in the average week, how many good days (with few lung/respiratory problems) have you had? Mark one answer only.
□1. None
□2. 1 or 2
□3. 3 or 4
□4. Nearly every day
□5. Every day

8.If you wheeze is it worse in the morning? If you don't wheeze, go to part 2.
□1. No
□2. Yes

Part 2
Section 1:
A.How would you describe your lung/respiratory problem? Mark one answer only.
□1. The most important problem I have
□2. Causes me a lot of problems
□3. Causes me a few problems
□4. Causes me no problems

B.If you have ever held a job, mark one of these answers:
□1. My lung/respiratory problem made me stop my job
□2. My lung/respiratory problem interferes with my job/made me change my job
□3. My lung/respiratory problem does not affect my job

Section2:
These are questions about what activities usually make you short of breath. Mark the option that applies to you now.

	1.True	2.False
1.Sitting or lying still		
2.Washing yourself or dressing		
3.Walking in the house		
4.Walking outside on level ground		
5.Walking up a flight of stairs		
6.Walking up hills		
7.Playing sports or active games (baseball, tennis etc)		

Section 3:
These are questions about your shortness of breath. Mark either True or False as it applies to you now.

	1.True	2.False
1.Coughing hurts		
2.Coughing makes me tired		
3.I am short of breath when I talk		
4.I am short of breath when I bend over		
5.My coughing or breathing disturbs my sleep		
6.I become exhausted easily		

Section 4:
These are questions about other effects your lung/respiratory problem might have on you. Mark either true or false as it applies to you now.

	1.True	2.False
1.My coughing or breathing is embarrassing in public		
2.My lung/respiratory problem is a nuisance for my family, friends or neighbours		
3.I panic or get afraid when I cannot catch my breath		
4.I feel that I am not in control of my lung/respiratory problem		
5.I do not expect my lung/respiratory problem to get better		
6.I have become frail/an invalid because of my lung/respiratory problem		
7.Exercise is not safe for me		
8.Everything seems like too much of an effort		

Section 5:
These are questions about your lung/respiratory medication including oxygen, inhalers and pills. If you are not receiving medication go to question 6. Mark true or false according to what you are receiving now.

	1.True	2.False
1.My lung/respiratory medication does not help me very much		
2.I get embarrassed using my lung/respiratory medication in public		
3.I have unpleasant side effects from my lung/respiratory medication		
4.My lung/respiratory medication interferes with my life a lot.		

Section 6:
These are questions about how your activities might be affected by your breathing problem. For each question answer true if one part applies to you because of your breathing problem. Otherwise answer false.

	1.True	2.False
1.I take a long time to get washed or dressed		
2.I cannot take a bath or shower or I take a long time to do it		
3.I walk slower than other people my own age, or I have to stop to rest		
4.If I walk up one flight of stairs, I have to go slowly or stop		
5.If I hurry or walk fast I have to stop or slow down		
6.My breathing problem makes it difficult to do things like walking up hills, carrying things up stairs, light gardening such as weeding, dancing, playing golf or light sport such as horseshoes.		
7.My breathing problem makes it difficult to do things like carrying heavy loads, digging in the garden or shovelling snow, jogging or walking briskly, playing tennis or swimming		
8.My breathing problem makes it difficult to do things like very heavy manual labour, riding a bike, running, swimming fast or playing competitive sports.		

Section 7:
We would like to know how your breathing **usually** affects your daily life. Mark true or false if it applies to you because of your lung/respiratory problem.

	1.True	2.False
A.I cannot play sports or active games		
B.I cannot go out for entertainment or recreation		
C.I cannot go out of the house to do grocery shopping		
D.I cannot do household chores		

E.I cannot move far from my bed or chair		

Here is a list of other activities that your lung/respiratory problem may prevent you from doing. (You do not need to fill these in- they are just to remind you of ways in which shortness of breath may affect you).

- Going for walks or walking the dog
- Doing activities or chores at home or in the garden
- Having sexual intercourse
- Going to church or place of entertainment
- Going out into bad weather or smoky rooms
- Visiting family or friends or playing with children

Please write any other important activities that your lung/respiratory problem may stop you from doing.

Now, would you mark the one statement you feel best describes how your lung/respiratory problem affects you:

□It does not stop me from doing anything I would like to do
□It stops me from doing one or two things I would like to do
□It stops me from doing most of the things I would like to do
□It stops me from doing everything I would like to do

Thank you very much for taking the time to fill in this questionnaire

Supplemental Appendix 2. Instrument - COMPASS 31

1. In the past year, have you ever felt faint, dizzy, "goofy", or had difficulty thinking soon after standing up from a sitting or lying position?
   1 Yes
   2 No (if you marked No, please skip to question 5)

2. When standing up, how frequently do you get these feelings or symptoms?
   1 Rarely
   2 Occasionally
   3 Frequently
   4 Almost Always

3. How would you rate the severity of these feelings or symptoms?
   1 Mild
   2 Moderate
   3 Severe

4. In the past year, have these feelings or symptoms that you have experienced:
   1 Gotten much worse
   2 Gotten somewhat worse
   3 Stayed about the same
   4 Gotten somewhat better
   5 Gotten much better
   6 Completely gone

5. In the past year, have you ever noticed color changes in your skin, such as red, white, or purple?
   1 Yes
   2 No (if you marked No, please skip to question 8)

6. What parts of your body are affected by these color changes? (Check all that apply)
   1 Hands
   2 Feet

7. Are these changes in your skin color:
   1 Getting much worse
   2 Getting somewhat worse
   3 Staying about the same
   4 Getting somewhat better
   5 Getting much better
   6 Completely gone

8. In the past 5 years, what changes, if any, have occurred in your general body sweating?

1 I sweat much more than I used to
2 I sweat somewhat more than I used to
3 I haven't noticed any changes in my sweating
4 I sweat somewhat less than I used to
5 I sweat much less than I used to

9. Do your eyes feel excessively dry?

1 Yes
2 No

10. Does you mouth feel excessively dry?

1 Yes
2 No

11. For the symptom of dry eyes or dry mouth that you have had for the longest period of time, is this symptom:

1 I have not had any of these symptoms
2 Getting much worse
3 Getting somewhat worse
4 Staying about the same
5 Getting somewhat better
6 Getting much better
7 Completely gone

12. In the past year, have you noticed any changes in how quickly you get full when eating a meal?

1 I get full a lot more quickly now than I used to
2 I get full more quickly now than I used to
3 I haven't noticed any change
4 I get full less quickly now than I used to
5 I get full a lot less quickly now than I used to

13. In the past year, have you felt excessively full or persistently full (bloated feeling) after a meal?

1 Never
2 Sometimes
3 A lot of the time

14. In the past year, have you vomited after a meal?

1 Never
2 Sometimes
3 A lot of the time

15. In the past year, have you had a cramping or colicky abdominal pain?
    1 Never
    2 Sometimes
    3 A lot of the time

16. In the past year, have you had any bouts of diarrhea?
    1 Yes
    2 No (if you marked No, please skip to question 20)

17. How frequently does this occur?
    1 Rarely
    2 Occasionally
    3 Frequently _____ times per month
    4 Constantly

18. How severe are these bouts of diarrhea?
    1 Mild
    2 Moderate
    3 Severe

19. Are your bouts of diarrhea getting:
    1 Much worse
    2 Somewhat worse
    3 Staying the same
    4 Somewhat better
    5 Much better
    6 Completely gone

20. In the past year, have you been constipated?
    1 Yes
    2 No (if you marked No, please skip to question 24)

21. How frequently are you constipated?
    1 Rarely
    2 Occasionally
    3 Frequently _____ times per month
    4 Constantly

22. How severe are these episodes of constipation?
    1 Mild
    2 Moderate
    3 Severe

23. Is your constipation getting:
1 Much worse
2 Somewhat worse
3 Staying the same
4 Somewhat better
5 Much better
6 Completely gone

24. In the past year, have you ever lost control of your bladder function?
1 Never
2 Occasionally
3 Frequently _____ times per month
4 Constantly

25. In the past year, have you had difficulty passing urine?
1 Never
2 Occasionally
3 Frequently _____ times per month
4 Constantly

26. In the past year, have you had trouble completely emptying your bladder?
1 Never
2 Occasionally
3 Frequently _____ times per month
4 Constantly

27. In the past year, without sunglasses or tinted glasses, has bright light bothered your eyes?
1 Never (if you marked Never, please skip to question 29)
2 Occasionally
3 Frequently
4 Constantly

28. How severe is this sensitivity to bright light?
1 Mild
2 Moderate
3 Severe

29. In the past year, have you had trouble focusing your eyes?
1 Never (if you marked Never, please skip to question 31)
2 Occasionally
3 Frequently
4 Constantly

30. How severe is this focusing problem?
1 Mild
2 Moderate
3 Severe

31. Is the most troublesome symptom with your eyes (i.e. sensitivity to bright light or trouble focusing) getting:

1 I have not had any of these symptoms
2 Much worse
3 Somewhat worse
4 Staying about the same
5 Somewhat better
6 Much better
7 Completely gone

COMPASS-31 SCORING ALGORITHM

Domain	Item	Answer	Points
Orthostatic Intolerance			
	1	1	1
	2	2	1
		3	2
		4	3
	3	1	1
		2	2
		3	3
	4	1	3
		2	2
		3	1
Vasomotor			
	5	1	1
	6	1	1
		2	1
	7	1	3
		2	2
		3	1
Secretomotor			
	8	1	1
		4	1
		5	2
	9	1	1
	10	1	1
	11	2	3
		3	2
		4	1
Gastrointestinal			
	12	1	2
		2	1
	13	2	1
		3	2
	14	2	1
		3	2
	15	2	1
		3	2
	16	1	1
	17	2	1
		3	2
		4	3
	18	1	1
		2	2
		3	3

	19	1	3
		2	2
		3	1
	20	1	1
	21	2	1
		3	2
		4	3
	22	1	1
		2	2
		3	3
	23	1	3
		2	2
		3	1
Bladder			
	24	2	1
		3	2
		4	3
	25	2	1
		3	2
		4	3
	26	2	1
		3	2
		4	3
Pupillomotor			
	27	2	1
		3	2
		4	3
	28	1	1
		2	2
		3	3
	29	2	1
		3	2
		4	3
	30	1	1
		2	2
		3	3
	31	2	3
		3	2
		4	1

Initial number

ICIQ-MLUTS 01/06

CONFIDENTIAL

DAY MONTH YEAR

Today's date

Urinary symptoms

Many people experience urinary symptoms some of the time. We are trying to find out how many people experience urinary symptoms, and how much they bother them. We would be grateful if you could answer the following questions, thinking about how you have been, on average, over the PAST FOUR WEEKS.

1. Please write in your [redacted]

2a. Is there a delay before you can start to urinate?

never 0
occasionally 1
sometimes 2
most of the time 3
all of the time 4

2b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

3a. Do you have to strain to continue urinating?

never 0
occasionally 1
sometimes 2
most of the time 3
all of the time 4

3b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

4a. Would you say that the strength of your urinary stream is...

normal 0
occasionally reduced 1
sometimes reduced 2
reduced most of the time 3
reduced all of the time 4

4b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

ICIQ-MLUTS 01/06

5a. Do you stop and start more than once while you urinate?

never ☐ 0
occasionally ☐ 1
sometimes ☐ 2
most of the time ☐ 3
all of the time ☐ 4

5b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

6a. How often do you feel that your bladder has not emptied properly after you have urinated?

never ☐ 0
occasionally ☐ 1
sometimes ☐ 2
most of the time ☐ 3
all of the time ☐ 4

6b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

VS: sum scores 2-6 ☐ ☐

7a. Do you have a sudden need to rush to the toilet to urinate?

never ☐ 0
occasionally ☐ 1
sometimes ☐ 2
most of the time ☐ 3
all of the time ☐ 4

7b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

8a. **Does urine leak before you can get to the toilet?**

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

8b. **How much does this bother you?**
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

9a. **Does urine leak when you cough or sneeze?**

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

9b. **How much does this bother you?**
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

10a. **Do you ever leak for no obvious reason and without feeling that you want to go?**

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

10b. **How much does this bother you?**
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

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ICIQ-MLUTS 01/06

11a. Do you leak urine when you are asleep?

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

11b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

12a. How often have you had a slight wetting of your pants a few minutes after you had finished urinating and had dressed yourself?

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

12b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

IS: sum scores 7-12 ☐ ☐

13a. How often do you pass urine during the day?

1 to 6 times ☐	0
7 to 8 times ☐	1
9 to 10 times ☐	2
11 to 12 times ☐	3
13 or more times ☐	4

13b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

14a. During the night, how many times do you have to get up to urinate, on average?

none	0
one	1
two	2
three	3
four or more	4

14b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

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Thank you very much for answering these questions.

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Initial number

ICIQ-FLUTS 08/04

CONFIDENTIAL

DAY MONTH YEAR

Today's date

Urinary symptoms

Many people experience urinary symptoms some of the time. We are trying to find out how many people experience urinary symptoms, and how much they bother them. We would be grateful if you could answer the following questions, thinking about how you have been, on average, over the PAST FOUR WEEKS.

1. Please write in your [redacted]

2a. During the night, how many times do you have to get up to urinate, on average?

- none ☐ 0
- one ☐ 1
- two ☐ 2
- three ☐ 3
- four or more ☐ 4

2b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**

not at all — a great deal

3a. Do you have a sudden need to rush to the toilet to urinate?

- never ☐ 0
- occasionally ☐ 1
- sometimes ☐ 2
- most of the time ☐ 3
- all of the time ☐ 4

3b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**

not at all — a great deal

4a. Do you have pain in your bladder?

- never ☐ 0
- occasionally ☐ 1
- sometimes ☐ 2
- most of the time ☐ 3
- all of the time ☐ 4

4b. How much does this bother you?

Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**

not at all — a great deal

ICIQ-FLUTS 08/04

5a. How often do you pass urine during the day?

1 to 6 times	0
7 to 8 times	1
9 to 10 times	2
11 to 12 times	3
13 or more times	4

5b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

F score: sum scores 2a-5a ☐☐

6a. Is there a delay before you can start to urinate?

never	0
occasionally	1
sometimes	2
most of the time	3
all of the time	4

6b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

7a. Do you have to strain to urinate?

never	0
occasionally	1
sometimes	2
most of the time	3
all of the time	4

7b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

ICIQ-FLUTS 08/04

8a. Do you stop and start more than once while you urinate?

never	0
occasionally	1
sometimes	2
most of the time	3
all of the time	4

8b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

V score: sum scores 6a+7a+8a ☐☐

9a. Does urine leak before you can get to the toilet?

never	0
occasionally	1
sometimes	2
most of the time	3
all of the time	4

9b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

10a. How often do you leak urine?

never	0
once or less per week	1
two to three times per week	2
once per day	3
several times per day	4

10b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

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11a. Does urine leak when you are physically active, exert yourself, cough or sneeze?

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

11b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

12a. Do you ever leak urine for no obvious reason and without feeling that you want to go?

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

12b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

13a. Do you leak urine when you are asleep?

never ☐	0
occasionally ☐	1
sometimes ☐	2
most of the time ☐	3
all of the time ☐	4

13b. How much does this bother you?
Please ring a number between 0 (not at all) and 10 (a great deal)

0 1 2 3 4 5 6 7 8 9 **10**
not at all — a great deal

I score: sum scores9a-13a ☐ ☐

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Thank you very much for answering these questions.

APPENDIX 2

List of aal areas as they appear along the x axis of the structural connectivity plots:

1 Precentral_L 2001

2 Precentral_R 2002

3 Frontal_Sup_L 2101

4 Frontal_Sup_R 2102

5 Frontal_Sup_Orb_L 2111

6 Frontal_Sup_Orb_R 2112

7 Frontal_Mid_L 2201

8 Frontal_Mid_R 2202

9 Frontal_Mid_Orb_L 2211

10 Frontal_Mid_Orb_R 2212

11 Frontal_Inf_Oper_L 2301

12 Frontal_Inf_Oper_R 2302

13 Frontal_Inf_Tri_L 2311

14 Frontal_Inf_Tri_R 2312

15 Frontal_Inf_Orb_L 2321

16 Frontal_Inf_Orb_R 2322

17 Rolandic_Oper_L 2331

18 Rolandic_Oper_R 2332

19 Supp_Motor_Area_L 2401

20 Supp_Motor_Area_R 2402

21 Olfactory_L 2501

22 Olfactory_R 2502

23 Frontal_Sup_Medial_L 2601

24 Frontal_Sup_Medial_R 2602

25 Frontal_Mid_Orb_L 2611

26 Frontal_Mid_Orb_R 2612

27 Rectus_L 2701

28 Rectus_R 2702

29 Insula_L 3001

30 Insula_R 3002

31 Cingulum_Ant_L 4001

32 Cingulum_Ant_R 4002

33 Cingulum_Mid_L 4011

34 Cingulum_Mid_R 4012

35 Cingulum_Post_L 4021

36 Cingulum_Post_R 4022

37 Hippocampus_L 4101

38 Hippocampus_R 4102

39 ParaHippocampal_L 4111

40 ParaHippocampal_R 4112

41 Amygdala_L 4201

42 Amygdala_R 4202

43 Calcarine_L 5001

44 Calcarine_R 5002

45 Cuneus_L 5011

46 Cuneus_R 5012

47 Lingual_L 5021

48 Lingual_R 5022

49 Occipital_Sup_L 5101

50 Occipital_Sup_R 5102

51 Occipital_Mid_L 5201

52 Occipital_Mid_R 5202

53 Occipital_Inf_L 5301

54 Occipital_Inf_R 5302

55 Fusiform_L 5401

56 Fusiform_R 5402

57 Postcentral_L 6001

58 Postcentral_R 6002

59 Parietal_Sup_L 6101

60 Parietal_Sup_R 6102

61 Parietal_Inf_L 6201

62 Parietal_Inf_R 6202

63 SupraMarginal_L 6211

64 SupraMarginal_R 6212

65 Angular_L 6221

66 Angular_R 6222

67 Precuneus_L 6301

68 Precuneus_R 6302

69 Paracentral_Lobule_L 6401

70 Paracentral_Lobule_R 6402

71 Caudate_L 7001

72 Caudate_R 7002

73 Putamen_L 7011

74 Putamen_R 7012

75 Pallidum_L 7021

76 Pallidum_R 7022

77 Thalamus_L 7101

78 Thalamus_R 7102

79 Heschl_L 8101

80 Heschl_R 8102

81 Temporal_Sup_L 8111

82 Temporal_Sup_R 8112

83 Temporal_Pole_Sup_L 8121

84 Temporal_Pole_Sup_R 8122

85 Temporal_Mid_L 8201

86 Temporal_Mid_R 8202

87 Temporal_Pole_Mid_L 8211

88 Temporal_Pole_Mid_R 8212

89 Temporal_Inf_L 8301

90 Temporal_Inf_R 8302

91 Cerebelum_Crus1_L 9001

92 Cerebelum_Crus1_R 9002

93 Cerebelum_Crus2_L 9011

94 Cerebelum_Crus2_R 9012

95 Cerebelum_3_L 9021

96 Cerebelum_3_R 9022

97 Cerebelum_4_5_L 9031

98 Cerebelum_4_5_R 9032

99 Cerebelum_6_L 9041

100 Cerebelum_6_R 9042

101 Cerebelum_7b_L 9051

102 Cerebelum_7b_R 9052

103 Cerebelum_8_L 9061

104 Cerebelum_8_R 9062

105 Cerebelum_9_L 9071

106 Cerebelum_9_R 9072

107 Cerebelum_10_L 9081

108 Cerebelum_10_R 9082

109 Vermis_1_2 9100

110 Vermis_3 9110

111 Vermis_4_5 9120

112 Vermis_6 9130

113 Vermis_7 9140

114 Vermis_8 9150

115 Vermis_9 9160

116 Vermis_10 9170

Appendix 3

Single subject connectivity data from chapter 3 (STN fingerprint).

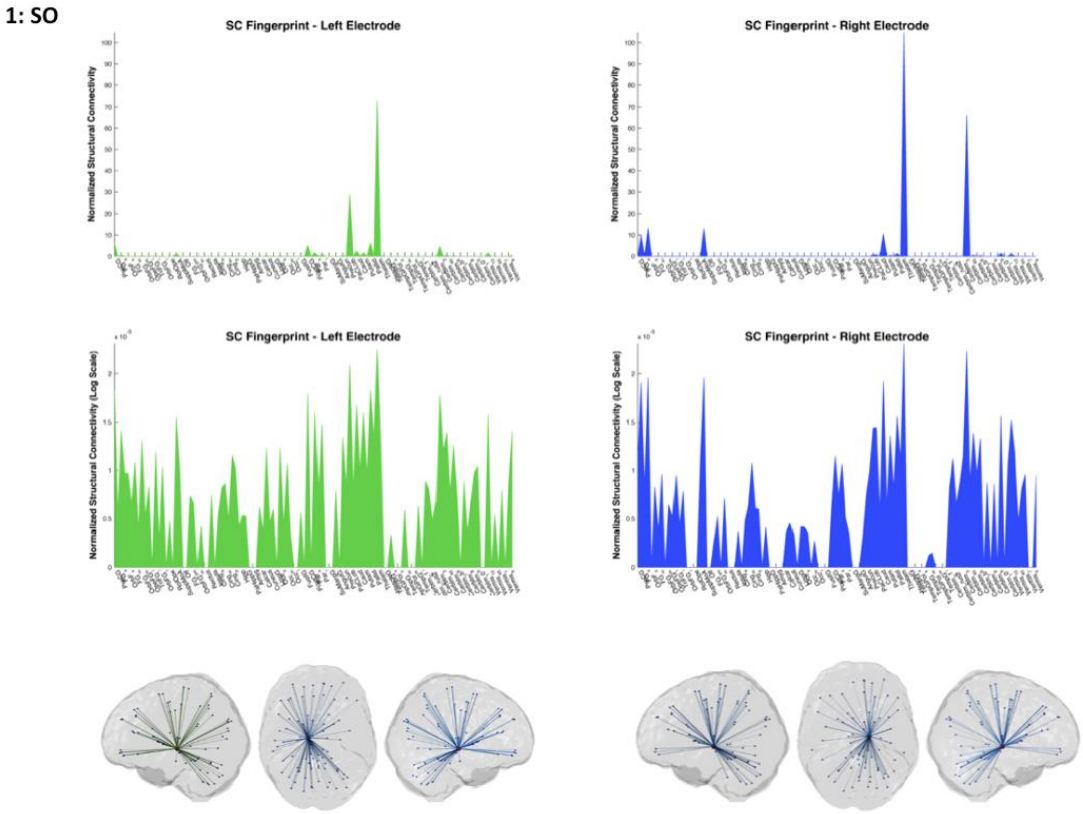


Figure 1: Subject 1. Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

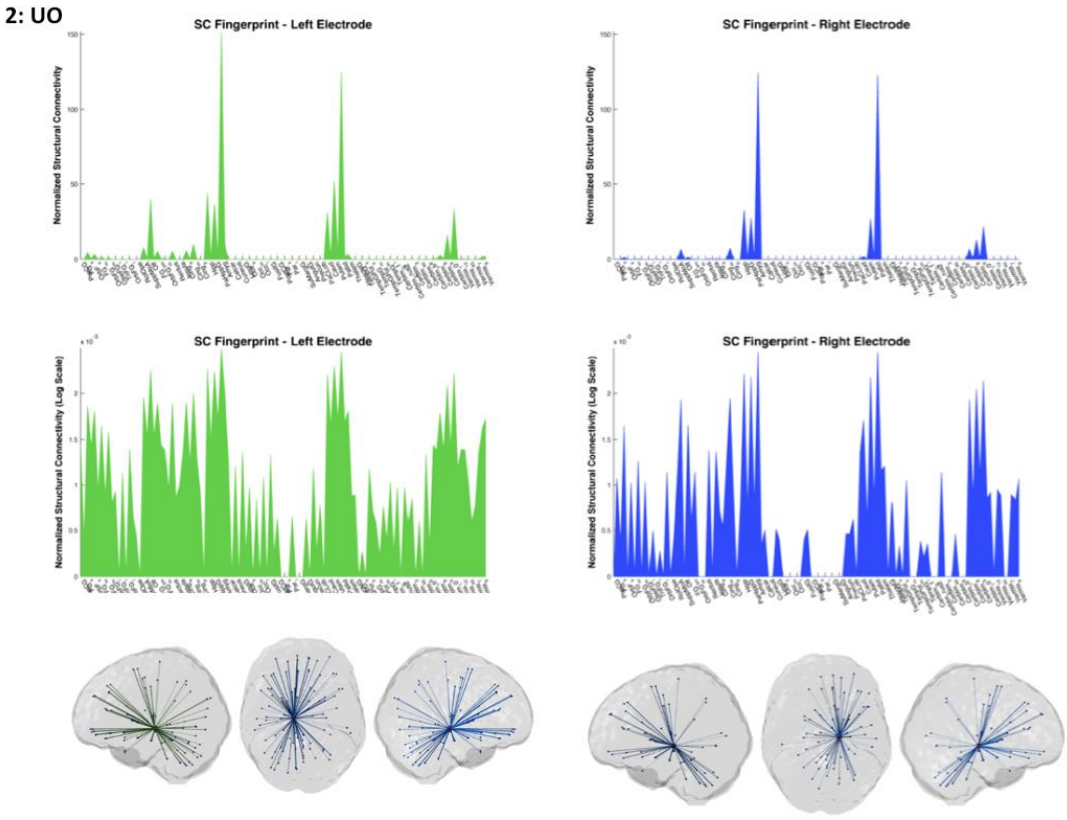


Figure 2: Subject 2 Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

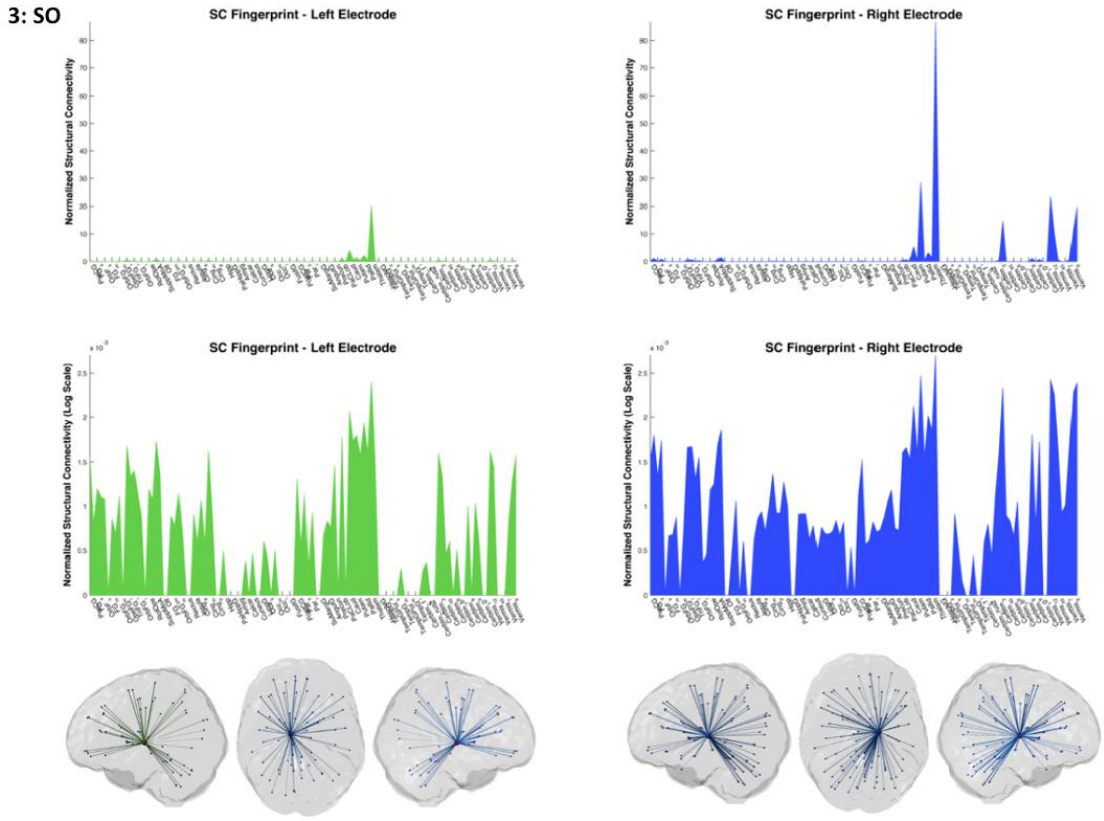


Figure 3: Subject 3. Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

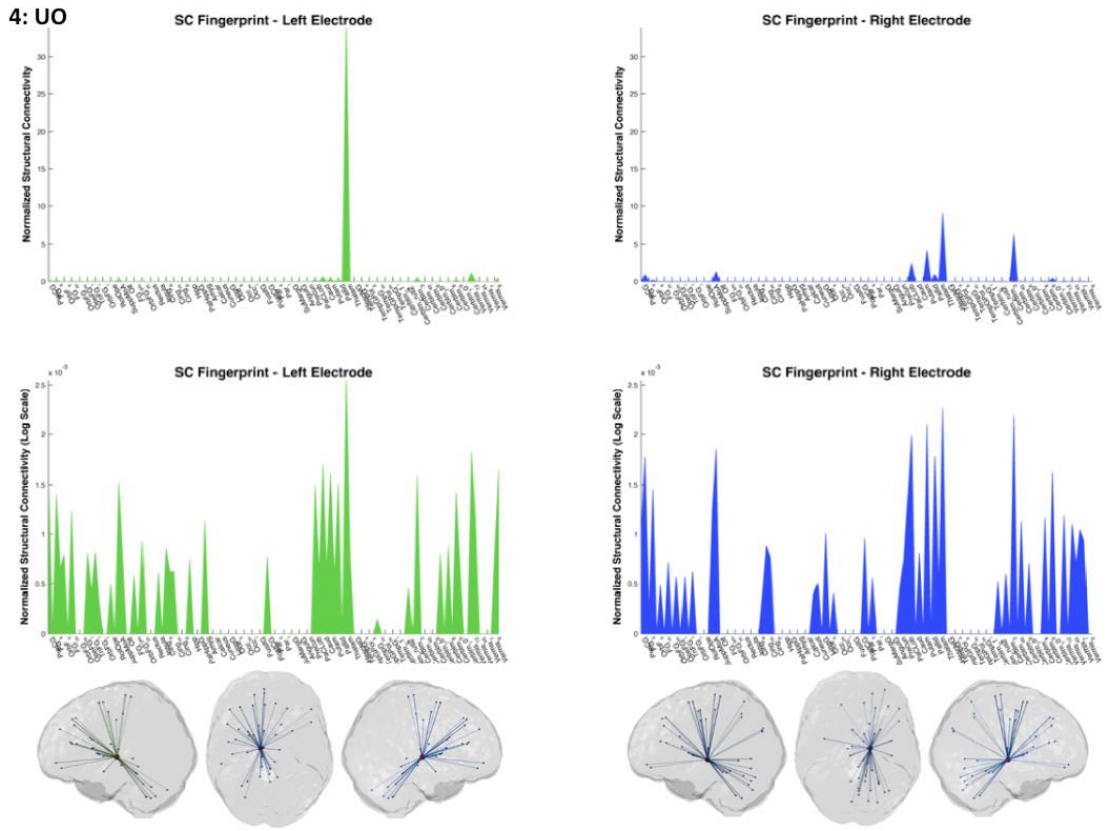


Figure 4: Subject 4. Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

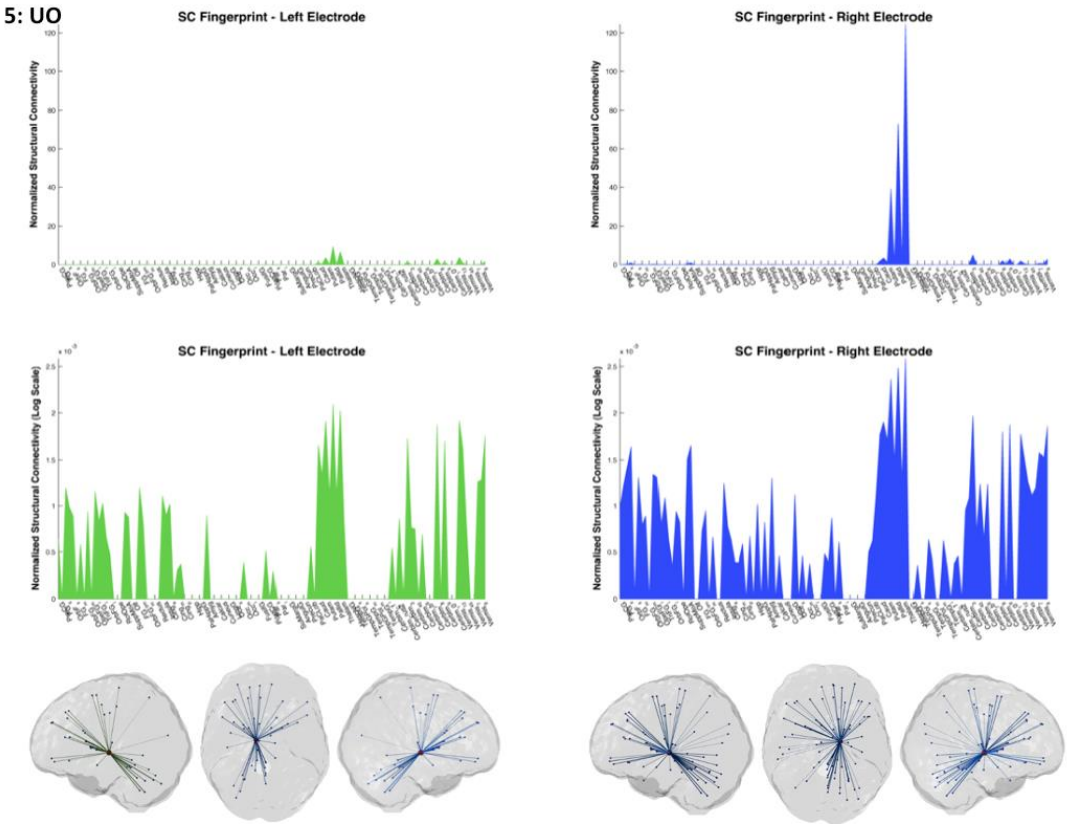


Figure 5: Subject 5. Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

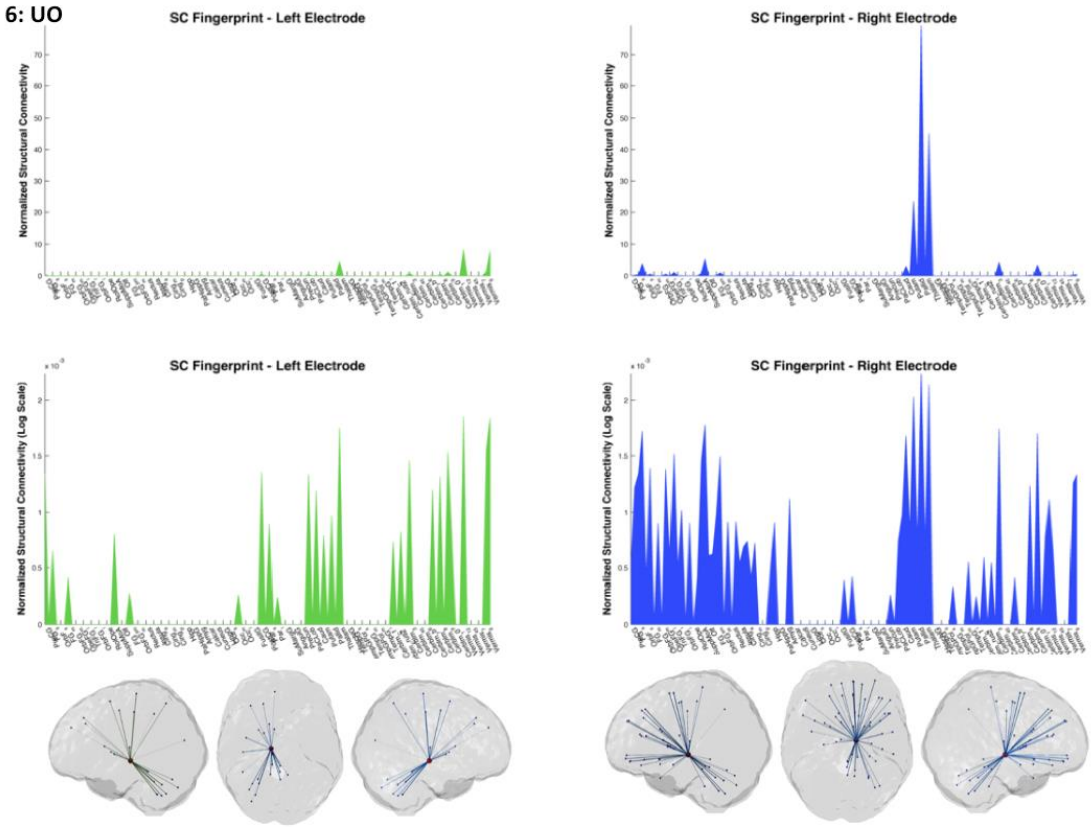


Figure 6: Subject 6. Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

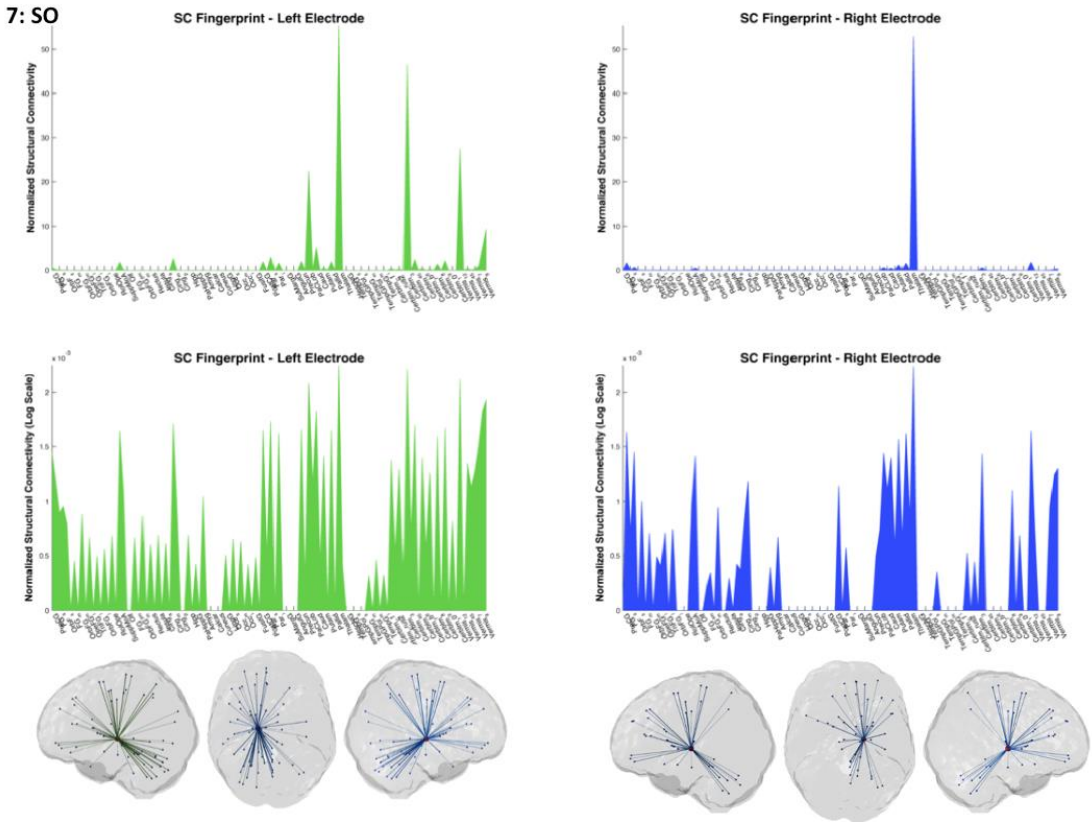


Figure 7: Subject 7. Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

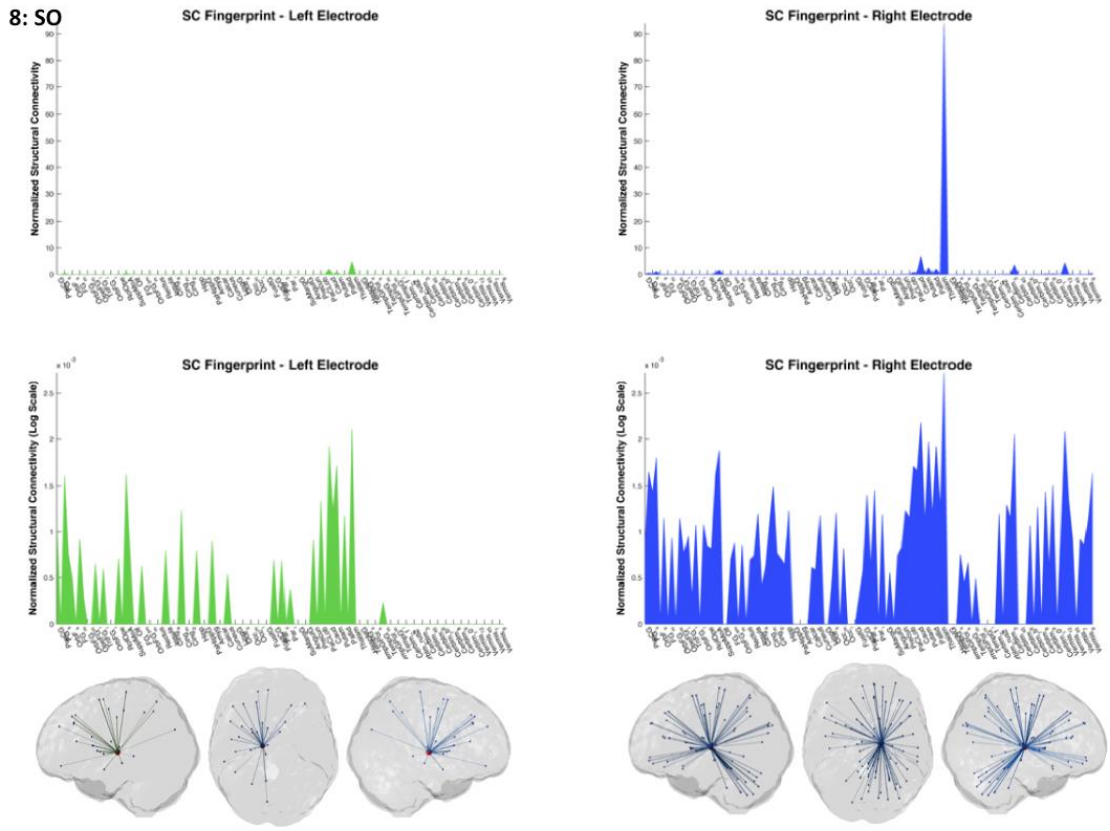


Figure 8: Subject 8. Top: Graph to demonstrate structural connectivity of left and right electrodes for each subject, normal (above) and logarithmic scale (below). Bottom: Brain stimulation networks depicted on glass brain; the thickness of each blue edge represents connection strength (on a logarithmic scale) between the source of stimulation and each AAL target, which is represented by a green sphere. UO: unsuccessful autonomic outcome, SO: successful autonomic outcome

Group-exclusive target areas in STN fingerprint analysis

Connections to the thalamus bilaterally and the left caudate survived a 5% threshold of the maximum connectivity strength of the network in each participant with an improvement in autonomic score (figure 9). There were no group-exclusive target areas in the negative autonomic outcome group.

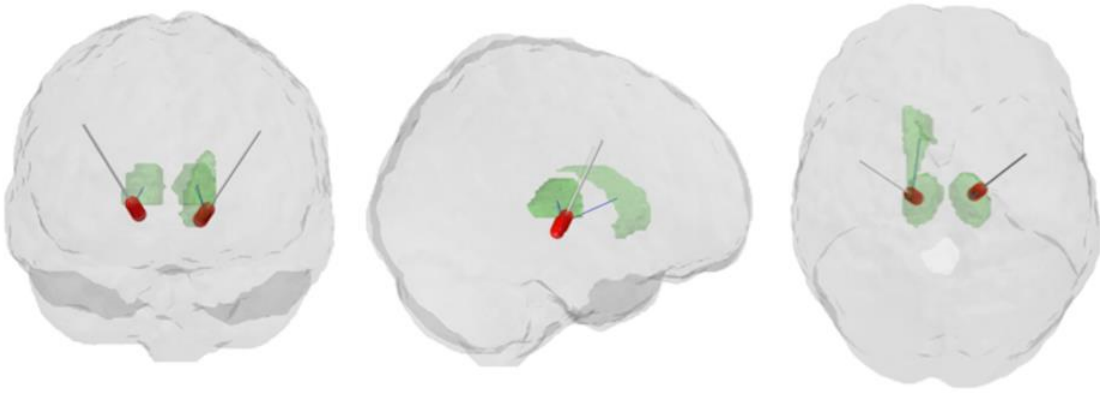


Figure 9: Brain areas common to all subjects in the successful autonomic outcome group in the STN fingerprint analysis after a 5% threshold was applied to the normalised fingerprints. Bilateral thalamus and left caudate depicted (left view, top view, right view).

Figure 10 (i)-(v). Plots showing differences in connection strength between each of the significant network nodes and the successful outcome and unsuccessful outcome groups (i) supramarginal gyrus (ii) postcentral gryrus (iii) cerebellar vermis (iv) left rolandic operculum (v) right cerebellum. Error bars represent standard deviation. Significant differences in structural connectivity between patients with SO and UO ($p < 0.05$), were determined using a permutation-based paired t-test. The absolute value of the structural connectivity difference (SC_{diff}) and the p-value is included beside each bar chart. SC = structural connectivity, SO = successful outcome, UO = unsuccessful outcome

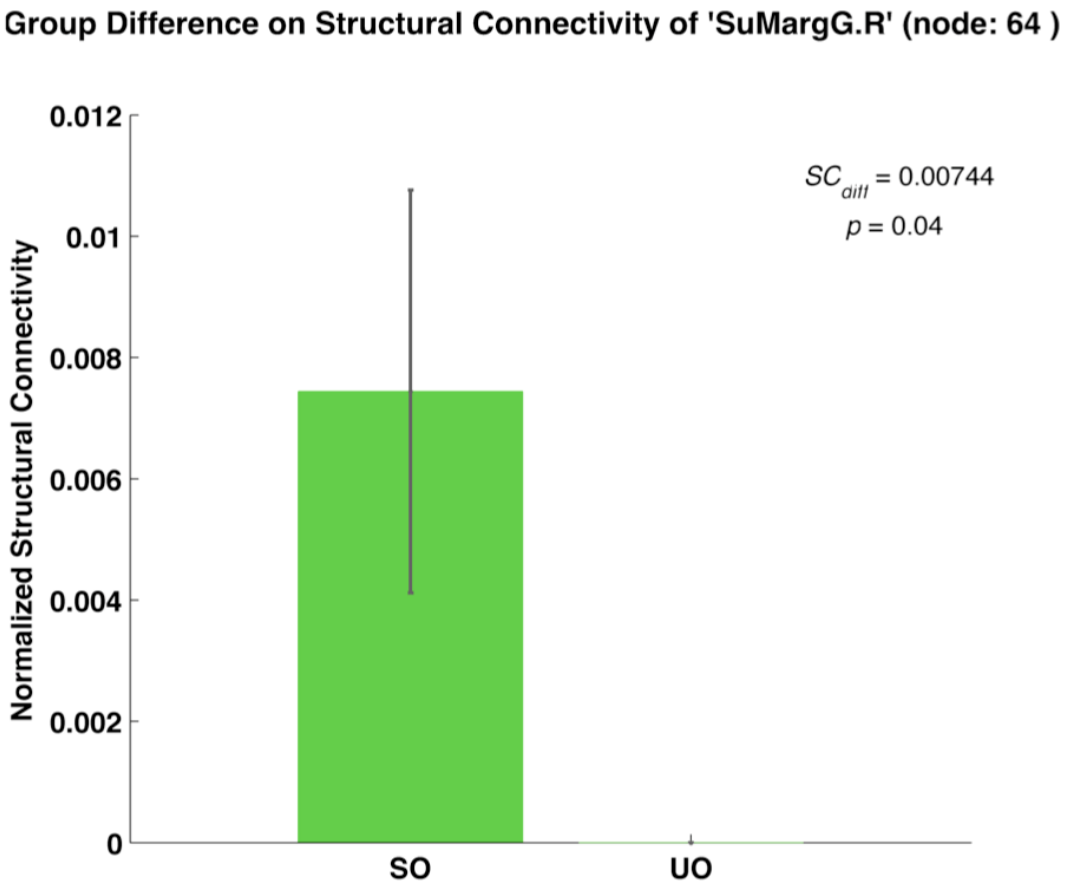


Figure 10 (i): Bar charts showing differences in connection strength between the right supramarginal gyrus and the successful outcome (SO) and unsuccessful outcome (UO) groups.

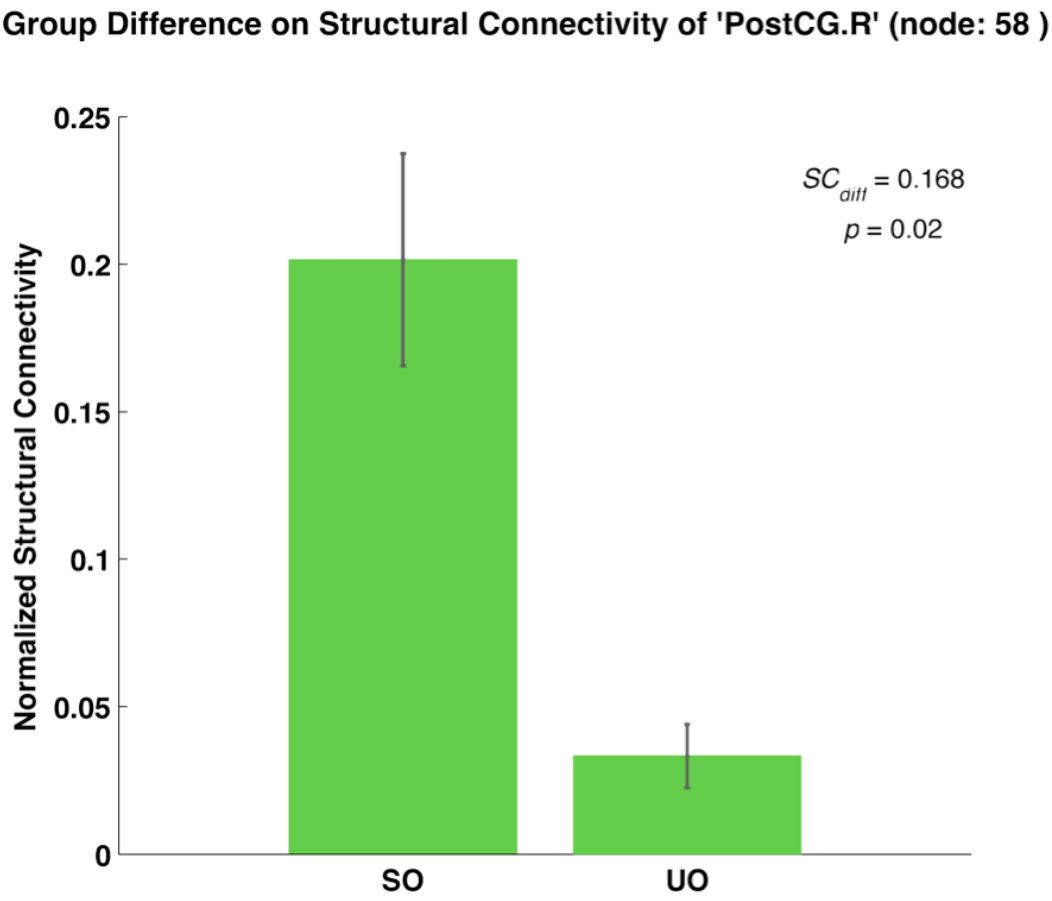


Figure 10 (ii): Bar charts showing differences in connection strength between the right post-central gyrus and the successful outcome (SO) and unsuccessful outcome (UO) groups.

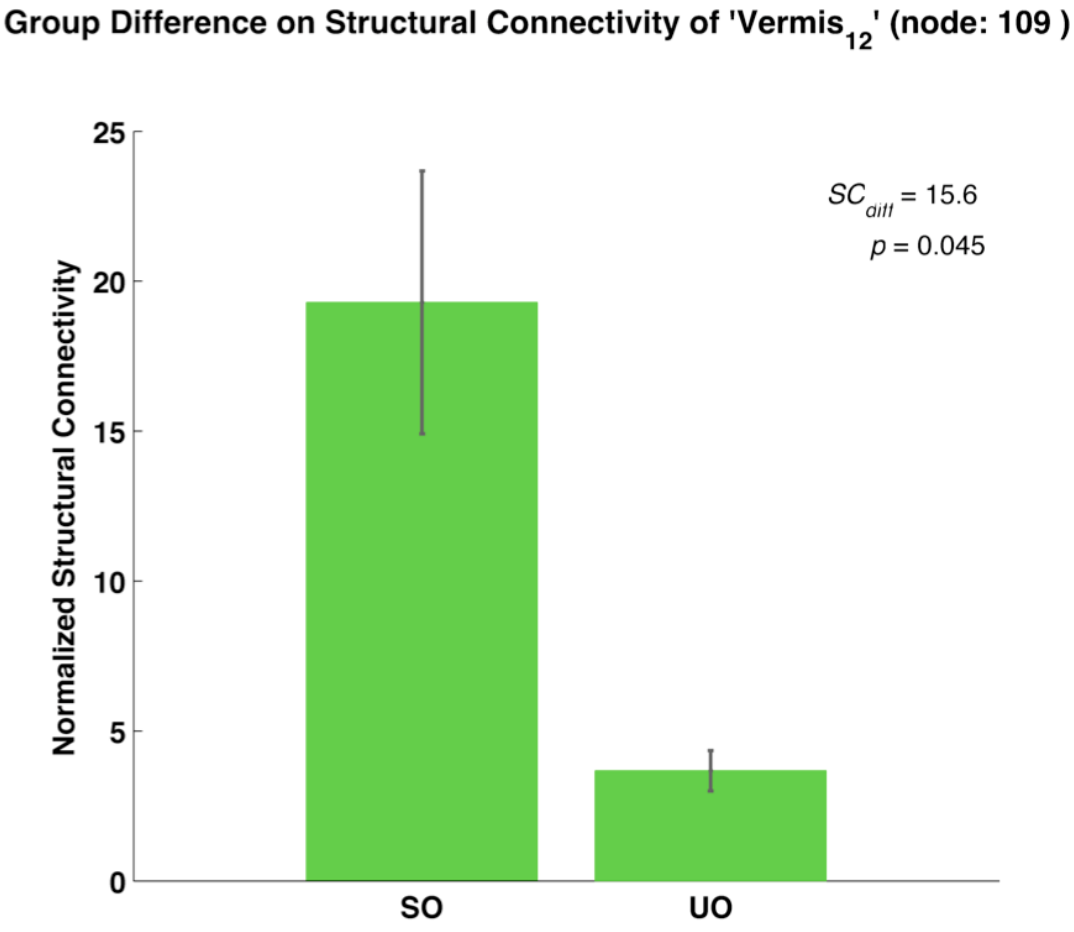


Figure 10 (iii): Bar charts showing differences in connection strength between the left rolandic operculum and the successful outcome (SO) and unsuccessful outcome (UO) groups.

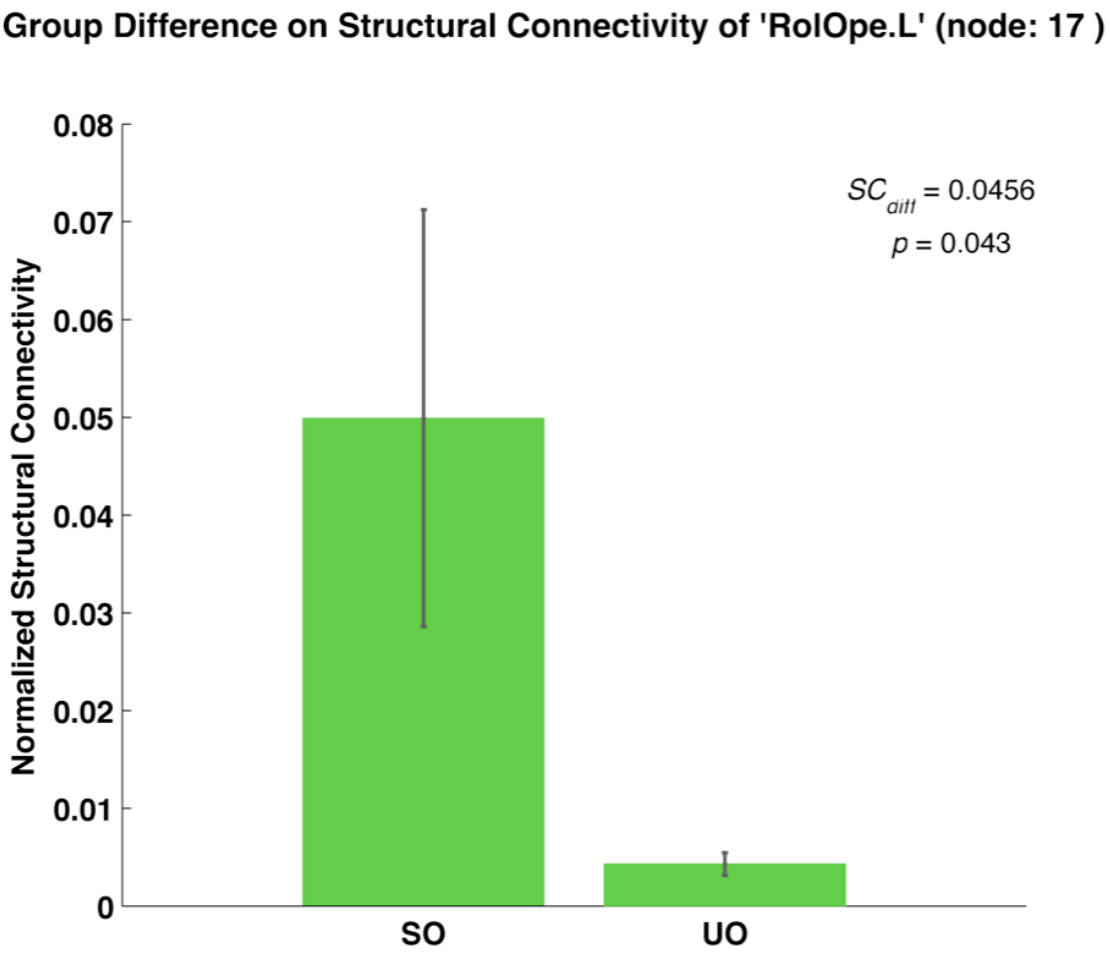


Figure 10 (iv): Bar charts showing differences in connection strength between the left rolandic operculum and the successful outcome (SO) and unsuccessful outcome (UO) groups.

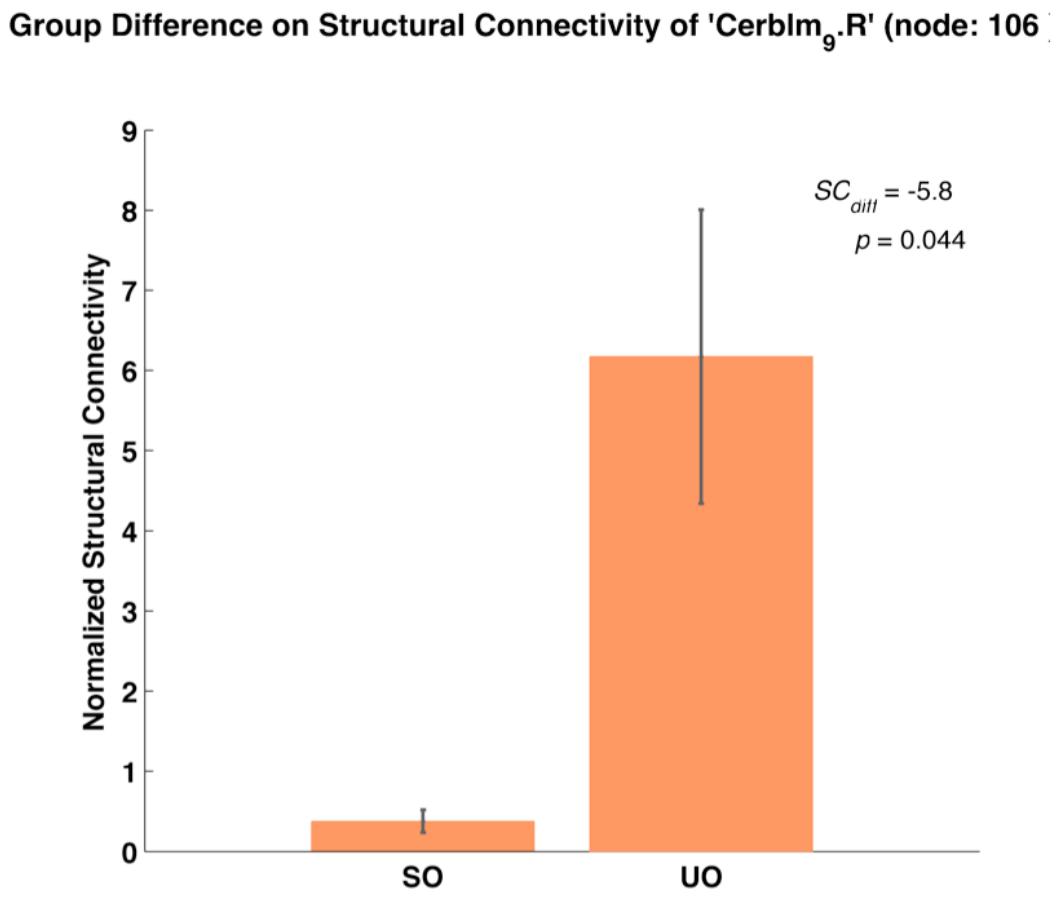


Figure 10 (v): Bar charts showing differences in connection strength between the left rolandic operculum and the successful outcome (SO) and unsuccessful outcome (UO) groups.