Introduction

Lower urinary tract symptoms occur in 27% - 86% of patients with PD, the exact figure depending on the population studied and the assessment tool used to determine prevalence (Winge *et al*., 2006). Bladder dysfunction in PD has a negative impact on quality of life (McGrother *et al*., 2004) and represents a significant financial cost to the health care system (Milsom *et al*., 2014). "Storage" symptoms, including urinary frequency, nocturia, urgency and urge incontinence predominate (Araki and Kuno, 2000), although "voiding" symptoms such as difficulty initiating voiding, straining to void and intermittent stream, also occur. An effective treatment for lower urinary tract symptoms would bring substantial benefit in terms of physical and psychosocial well-being for sufferers. However, given the absence of a clear pathophysiological understanding of bladder dysfunction in PD, targeted pharmacological or surgical management is not yet available.

A hallmark pathological feature of PD is degeneration of midbrain dopaminergic neurons in the substantia nigra pars compacta (SNc) and subsequently, reduced dopaminergic input to the striatum (Redgrave *et al*., 2010). According to early PD models, loss of dopaminergic striatal input produces motor dysfunction by promoting activity along the 'indirect' and suppressing activity along the 'direct' basal ganglia pathways, the net effect being overall suppression of movement due to an increase in the inhibitory influence of the GPi/SNr output complex on thalamo-cortical pathways (Albin *et al*., 1989). There is some evidence that dopamine depletion and basal ganglia dysfunction may contribute to the development of bladder symptoms in Parkinson's disease. Two SPECT imaging studies of patients with Parkinson's disease have demonstrated a relationship between bladder symptoms and depletion of dopaminergic neurons in the striatum (Sakakibara *et al*., 2001b; Winge *et al*., 2005). Moreover, intravenous administration of either a D1 receptor antagonist or a D2 receptor agonist, both of which increase inhibitory output from the GPi/SNr, reduces bladder capacity and increases the frequency of bladder contractions in the awake rat (Seki *et al*., 2001). There is also evidence to suggest that the GPi is involved in normal regulation of the micturition reflex in non-Parkinson's disease subjects: pallidal neurons in awake-immobilised cats display a triphasic activity pattern during micturition with increased

firing rates just before the onset of a detrusor contraction (Porter, 1967) and neuroimaging studies in animals and humans confirm that activation of the globus pallidus occurs during urinary voiding (Nour et al., 2000; Tai *et al*., 2009; Shy *et al*., 2014). Furthermore, electrical stimulation of the pallidum inhibits automatic/spontaneous bladder contractions in both anaesthetized cats (Lewin and Porter, 1965) and human subjects (Mordasini *et al*., 2014). However, despite evidence to support the role of the GPi in normal bladder function, no definite link has been established between pallidal dysfunction and the onset of urinary symptoms in PARKINSON'S DISEASE.

The original 'indirect-direct pathway' theory described above is now viewed as an over-simplification of basal ganglia functional anatomy. The basal ganglia are more interconnected both intrinsically and with external subcortical structures than initially proposed (Redgrave *et al*., 2010) and some of these thalamostriatal-subcortical connections may be relevant to the pathophysiology of bladder dysfunction. The role of the brainstem-basal ganglia interactions in the onset of micturition disturbances in PD is important to consider because according to current scientific models of supraspinal micturition control, the major subcortical areas responsible for bladder function are located in the midbrain and pons, namely the periaqueductal grey area, the pontine micturition centre, and the pontine continence centre.

The midbrain periaqueductal grey area (PAG) receives sensory information about bladder fullness via spinal cord afferents (Blok *et al*., 1995), transmits this information to the cortex, probably via the posterior thalamus, and also initiates the switch from 'storage' to 'voiding' mode (Tai *et al*., 2009) when the bladder is full. Deep brain stimulation (DBS) of the subthalamic nucleus (STN) (Herzog *et al*, 2008) increases the degree of correlation between activity in the PAG and posterior thalamus during bladder filling in Parkinson's disease patients and also normalizes bladder capacity, implying that deficits in sensorimotor processing involving the PAG and basal ganglia contribute to bladder dysfunction in Parkinson's disease and can be corrected by neuromodulation at the STN.

Further evidence suggests that the neural deficits underlying bladder dysfunction in Parkinson's disease are not limited to the PAG and its basal ganglia connections.

Abnormally reduced activation in parts of the brainstem (but *not* the PAG) during detrusor overactivity has been reported in Parkinson's disease patients (Kitta *et al*., 2006) and therefore microstructural changes involving pontine bladder control regions must also be considered as a possible contributory factor in the onset of lower urinary tract symptoms, either due to primary degeneration or altered input from abnormal basal ganglia projections. Two pontine areas described by Holstege and co-workers in the 1980s and 1990s (Holstege *et al*., 1986; Griffiths *et al*., 1990; Blok and Holstege, 1994) and subsequently identified in numerous brain imaging studies of voiding (Fukuyama *et al*., 1996; Blok *et al*., 1997; Nour *et al*., 2000; Mehnert *et al*., 2008; Krhut *et al*., 2012;) are important for direct control of the micturition reflex via connections with the sacral parasympathetic nucleus of the spinal cord, which controls the bladder detrusor muscle (Blok and Holstege, 1997), and Onuf's nucleus, located at the first and second sacral level, which contains somatic motor neurons innervating the pelvic floor and external urethral sphincter (Sato *et al*., 1978). The pontine 'M-region' in the dorsolateral pontine tegmentum (otherwise known as 'Barrington's nucleus' or the 'pontine micturition centre') (Blok *et al*, 1995) projects monosynaptically to the sacral parasympathetic nucleus and also to the dorsal grey commissure (Loewy *et al*., 1979; Holstege *et al*., 1986; Blok and Holstege, 1997). It directly excites bladder motorneurons of the sacral parasympathetic nucleus, leading to detrusor muscle contraction, and inhibits external urethral sphincter motorneurons via dorsal grey commissure GABA-ergic interneurons, which innervate Onuf's nucleus (Blok and Holstege, 1999). Electrical stimulation of the 'M-region' leads to relaxation of the pelvic floor and the external urethral sphincter followed by bladder contraction (Holstege *et al*., 1986; Blok and Holstege, 1994); bilateral lesions produce urinary retention (Griffiths *et al*., 1990). A nearby region within the pontine tegmentum, ventral and lateral to the 'M-region', has excitatory projections to Onuf's nucleus (Holstege *et al*., 1986) and is thought to be involved in maintaining sphincter tone during urine storage. This region is known as the pontine 'L-region'. Electrical stimulation of the L-region in animals inhibits micturition and produces strong contraction of the external urethral sphincter (Kuru, 1965; Holstege *et al*., 1986) while bilateral lesions result in severe incontinence (Griffiths *et al*., 1990). Degenerative change and structural reorganisation in either of these bladder control regions could plausibly give rise to abnormal bladder and sphincter activity with associated symptoms of urinary frequency, urgency and incontinence. Measures of brain tissue

microstructural integrity, such as FA and MD, can be obtained using diffusion MRI, which generates information in the form of quantitative diffusion maps based on diffusion properties of water. FA indicates the degree of alignment within white matter tracts while MD provides information about the size and membrane integrity of cellular structures. Altered FA and MD have been associated with tissue breakdown in a number of clinical imaging studies (Rovaris *et al*., 2002; Gallo *et al*., 2005); increased FA and/or reduced MD can signify cell swelling or increased cell density, while reduced FA and/or increased MD can represent demyelination and cell death. In order to investigate the role of the brainstem in the pathophysiology of bladder symptoms in Parkinson's disease we used diffusion MRI to study the relationship between measures of brain grey and white matter structural integrity and lower urinary tract symptoms. We hypothesised that lower urinary tract dysfunction would be associated with structural changes in known bladder control regions of the brainstem, and that the extent of the change would correlate with the severity of bladder symptoms. We also hypothesized that structural connections would exist between abnormal brainstem areas and the basal ganglia, given the established involvement of the basal ganglia in both Parkinson's disease and in bladder control (Porter 1967; Pazo 1976; Sakakibara *et al*., 2001a; Winge *et al*., 2005; Herzog *et al*., 2006; Herzog *et al*., 2008; Mordasini *et al*., 2014), and the fact that there are well described connections between the basal ganglia and various midbrain/brainstem regions (Erro *et al*., 1999; Martinez-Gonzalez *et al*., 2011).

According to a recent case report, a patient experienced detrusor overactivity and symptoms of urinary urgency, frequency and urgency incontinence following deep brain stimulation of the PPN (Aviles-Olmos *et al*., 2011) a diffuse cell group known to undergo degeneration in Parkinson's disease (Zweig *et al*., 1989; Hirsch *et al*., 1987) located in the rostral pontine/caudal mesencephalic tegmentum (Zrinzo *et al*., 2008; Alam *et al*., 2011) and functionally part of the mesencephalic locomotor region (Alam *et al*., 2011) and reticular formation (Ferraye *et al*., 2010). Although PPN DBS has been performed, with varying results, for the treatment of severe gait disturbance in PD (Pahapill and Lozano, 2000; Mazzone *et al*., 2005; Plaha and Gill, 2005), it has been suggested that PPN DBS can alter bladder function due to the anatomical proximity of the PPN and the pontine M-region (Aviles-Olmos *et al*., 2011; Blanco *et al*., 2013). To complement our study of the role of microstructural changes within the brainstem on

bladder symptoms in Parkinson's disease, we decided to select a group of patients with DBS electrodes implanted within the PPN region and investigate the effect of stimulation on bladder function. We planned to determine the anatomical relationship between the stimulating electrode and any areas of microstructural change that might be identified during our diffusion MRI analysis, in order to better understand the mechanism by which PPN DBS affects bladder function in Parkinson's disease.

Materials and Methods

Subjects

Two groups of patients were studied, an 'MRI group' and a 'Urodynamics group' (see table 1 for further details). There was no overlap between groups: i.e. subjects were either in the MRI group or the Urodynamics group but not both.

MRI group

17 patients with Parkinson's disease who were due to have deep brain stimulation (DBS) for their movement disorder symptoms and had completed a pre-operative 'non-motor symptom questionnaire' which included questions about bladder function, were included in this study. All patients underwent pre-operative magnetic resonance imaging (MRI) on a 1.5 Tesla Siemens scanner. During the MRI scan, diffusion-weighted data was acquired using an echo planar imaging sequence (field of view = 256 x 208 mm, matrix = 128 x 104, slice thickness 2 mm, in plane resolution = 2x2 mm^2, repetition time = 15 s, echo time = 106.2 ms, 32 directions, b value = 1000s/mm^2, one image without diffusion weighting was obtained) (Owen *et al*., 2007). Furthermore, a high-resolution T1-weighted structural image (voxel size 1x1x1mm^3) was acquired with a three-dimensional 'FLASH' sequence (TR = 12ms, TE = 5.6ms, flip angle = 19^o, with elliptical sampling of *k*-space, giving a voxel size of 1 x 1 x 1mm^3 in 5.05 min).

Urodynamics group

7 patients with DBS electrodes targeted to the PPN were recruited to undergo urodynamic testing, which took place in a hospital clinic in Oxford. None of these participants had been recruited to the MRI part of the study described above. 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. DBS electrodes were model 3387 (Medtronic) in Oxford patients and 3389 in the London patient - the four circumferential contacts are 1.27mm diameter and 1.5 mm in length and are 1.5mm (3387) and 0.5 mm (3389) apart. Stimulation was either bipolar or monopolar in configuration. Details of the surgical procedure for electrode implantation have been reported elsewhere (Pereira *et al*., 2008; Holl *et al*., 2010). 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. 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 1. For the London patient, electrode placement was guided by stereotactic proton density MRI sequences that were also used to confirm accurate lead placement. For further details refer to Avilés-Olmos *et al*., (2011).

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).

MRI group: Image analysis

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

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, 2002a). FA, MD, axial diffusivity (L1), and radial diffusivity (L2 and L3) images were created by fitting a diffusion tensor model to the diffusion data using DTIFIT within the FMRIB diffusion toolbox (part of FSL).

Tract-based spatial statistics (TBSS)

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 [52]. This FA skeleton represented the centres of all tracts common to the group.

MD, axial and radial diffusivity images were subsequently processed in the same way as described above for FA.

Voxel-Based Morphometry

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). First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI152 standard space using non-linear registration (Andersson *et al*., 2007). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific grey matter template. Second, all native grey matter images were non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3mm and voxelwise general linear model was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space.

Assessment of Volume and Shape Differences of Subcortical Grey Matter Structures

Grey matter segmentation was carried out on the T1-weighted data of each subject. Putamen, caudate and pallidum were segmented using FMRIB's Integrated Registration and Segmentation Tool (FIRST) (Patenaude *et al*., 2011). In order to scale subcortical structure volume according to the head size of the individual patient, SIENAX was run for each subject (Smith *et al*., 2002b). 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. Additionally, vertex analysis was performed on the putamen, caudate and pallidum, aiming to assess the correlation with bladder score on a per-vertex basis.

Urodynamics group: Urodynamic procedure

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 were asked to void urine in order to fully empty their bladder and urinalysis was performed to exclude a urinary tract infection. Participants then underwent bladder catheterisation with a dual lumen catheter (8Fr), and a rectal catheter (4.5Fr) 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.

Statistical analysis

In order to correlate TBSS and grey matter segmentation results with bladder symptom severity, a "bladder score" was created based on the severity of four symptoms which are most prominent in PD (based on combined gender estimates) according to Sakaibara et al's study in 2001 (Sakakibara *et al*., 2001a), namely urinary frequency, nocturia, urinary urgency and urge incontinence. Severity scores were generated for each of these four variables based on a 1-4 scale, where a score of '1' represented no symptoms at all and '4' represented a significant problem with the symptom in question. Scores were summed to give a total bladder score out of 16, which was then used in subsequent analysis.

MRI group

TBSS correlations

Right and left brainstem masks were produced using the Juelich Histological Atlas, thresholded at 50%, binarized and applied to the mean TBSS skeleton mask.

Correlations between bladder score and DTI metrics were determined using permutation-based non-parametric testing applied to voxel-wise general linear model, carried out using the whole brain white matter skeleton and the brainstem mask, 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) within each region of interest, using the threshold-free cluster enhancement (TFCE) approach (Smith and Nichols, 2009). Pearson's correlation between bladder score and subcortical segmentation volumes corrected for head size was also tested using IBM SPSS

Statistics for Mac (version 20). Results were visualised using TBSS fill. This is an algorithm that 'thickens' the skeletonised results by filling them out to occupy the local tracts seen in the mean FA map.

Subcortical grey matter segmentation

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.

Post-hoc Probabilistic Tractography from 'Significant' TBSS results

We ran probabilistic tractography for all subjects in MNI space from the significant MD results, thresholded each individual tractography output at 10% of the maximum value, binarised the thresholded output, and then averaged these for all subjects to create left and right group masks.

Urodynamics group

ON/OFF Deep Brain Stimulation and Urodynamics

The mean bladder capacity with DBS ON and OFF was calculated for each subject and a Wilcoxon Signed Rank Test was used to assess for a statistically significant difference between the mean bladder capacity with DBS ON and DBS OFF.

To identify the area of maximal effective stimulation for each patient, which we will refer to from now as the 'stimulation site', a mask of the midpoint between the two active contacts in the case of bipolar stimulation, or the active contact itself in the case of monopolar stimulation, was made on the post-operative CT scan (or post-op MRI scan for patient 7) which showed the position of the DBS electrodes. The post-operative scan was registered to the pre-operative T1-weighted scan using FMRIB's Linear Image Registration Tool (FLIRT) (Jenkinson and Smith, 2001; Jenkinson *et al*.,

2002), and the pre-operative T1-weighted scan was then non-linearly registered to the MNI152 brain. Similarly, the transformation used to register the T1-weighted scan to MNI space was used to register the masks of the stimulation site. The stimulation site for each subject was then plotted along with the significant areas of microstructural change identified by the TBSS analysis (see results). Subjects were grouped according to the proximity of their stimulation site and the regions of microstructural change. Subjects whose stimulation site overlapped with or fell within 1mm of the TBSS fill regions in which MD significantly correlated with bladder score, were classified as 'subgroup 1' and subjects whose stimulation site was further away from this region were classified as 'subgroup 2'. The effect of DBS on bladder function was re-analysed for each subgroup, again using a Wilcoxon test.

Long-term bladder symptom follow-up

Two patients from the urodynamic part of the study completed bladder symptom questionnaires pre-operatively and six months post-operatively in order for us to gain preliminary information about whether the urodynamic response to PPN DBS predicted clinical response over the long term.

Results

MRI group

Clinical information

There were 13 male and 4 female subjects within the MRI group and the mean age at the time of MRI scan was 67.34 ± 5.97 years (range 54-75). The mean bladder score was 8.12 ± 3.38 (range 4-16). There was no significant correlation between bladder score and age, or bladder score and pre-operative Unified PD Rating Scale (UPDRS) values. Further clinical information is given in table 2.

Tract-based spatial statistics (TBSS)

TBSS using the entire white matter skeleton did not show any significant correlation between bladder scores and DTI measures of white matter microstructural integrity. However, using the intersection of the right and left brainstem masks with the skeleton as regions-of-interest, there were regions in which MD (left and right brainstem), axial diffusivity (left brainstem) and radial diffusivity (right brainstem) correlated with bladder score (Figures 2A and 2C). There was also a separate but nearby area in the left brainstem where MD and radial diffusivity were inversely correlated with bladder score (Figure 2B).

Anatomical location of pontine bladder areas

In order to determine the proximity between brainstem areas in which MD correlated with bladder score and known pontine bladder regions, we referred to Blok et al's 1997 PET study, which identifies neural activations thought to represent activation of the pontine M-region and pontine L-region based on comparisons between neural activity during 'urine withholding', 'micturition' and 'empty bladder' states. The voxel of maximal activation assumed to be the pontine 'L-region' or 'continence centre' was converted from Talairach to MNI space using GingerALE (http://brainmap.org/) (Eickhoff *et al*., 2009; Eickhoff *et al*., 2012) and overlaid onto the images showing areas where MD correlated significantly with bladder symptom severity (figure 3A). As the region identified in Blok's study was on the right side only, whereas the pontine L-region is expected to be a bilateral area based on animal studies (Griffiths *et al*., 1990), we calculated the left sided equivalent by inverting the co-ordinate in the X dimension. A similar conversion from Blok's 1997 study was used to generate an approximate location of the pontine M-region.

As shown in figure 3A, Blok's 'L-region' overlapped with the left-sided regions in which MD correlated with bladder symptom severity. There was no overlap between the location of the pontine M-region and the areas where MD correlated with bladder score (figure 3B).

Volumes and shape of subcortical grey matter structures

One subject was excluded from this part of the analysis due to poor automated segmentation. Analysis of the remaining 16 subjects revealed a significant negative correlation ($R = -0.654$, $p < 0.05$) between left pallidal volume (corrected for head size) and bladder score, which survived Bonferroni correction for multiple comparisons (Figure 4A). Vertex-wise analysis of the pallidum, caudate and putamen did not reveal any significant correlation with bladder score on a per-vertex basis.

Tractography from significant TBSS results

Probabilistic tractography using the areas where MD significantly correlated with bladder score as seed regions produced group maps for the left and the right hemisphere. Tracts on the right passed through the right brainstem and right cerebellum. Tracts on the left passed to the left brainstem, left cerebellum, left pallidum and left precentral gyrus (figure 4B).

Urodynamic study

Clinical information: Urodynamic group

All 7 patients who underwent the urodynamic testing were male. 6 had DBS electrodes targeted bilaterally to the pedunculopontine nucleus. One patient (patient 3) also had bilateral STN DBS electrodes which were switched off throughout testing. One patient (patient 1) had a right-sided PPN electrode only. Information about patient demographics and stimulation parameters is given in table 3.

Whole group analysis

Comparison of mean bladder capacity OFF vs ON PPN DBS revealed no significant effect on bladder capacity of PPN stimulation, although there was a trend towards an increased bladder capacity with stimulation ON (OFF DBS: 137 mls (range 39-254) vs ON DBS: 188 mls (range 40-441). The mean maximal bladder capacity ON and OFF DBS for each patient is given in table 3.

Subgroup analysis based on location of stimulation site

The stimulation site for each patient was located in relation to the brainstem regions identified in the TBSS analysis (figure 5A).

For two patients (subgroup 2), the stimulation site was distant from the regions of microstructural change and there was no significant effect of DBS on bladder capacity (OFF DBS: 176 mls (range 52-300); ON DBS: 159 mls (range 40-279).

For five patients (subgroup 1), the stimulation site closely approximated the regions of brainstem microstructural change identified in the TBSS analysis (figure 2). Of these, four patients (patients 3-6) had stimulation sites that directly overlapped with the regions of microstructural change presented with TBSS fill, and one patient (patient 7) was included within this group because his stimulation site was within 1mm of the regions in which MD correlated with bladder score. Furthermore, on a less conservative estimate of stimulation site, there was found to be overlap with his stimulation site and the regions of microstructural change. In all five patients within subgroup 1, the overlap between stimulation site and microstructural change occurred on the left but not the right, although in one patient the right-sided electrode came close to the significant regions. Statistical analysis showed that PPN DBS significantly increased mean maximal bladder capacity in subgroup 1 patients (OFF DBS: 131 mls (range 39-230); ON DBS: 199 mls (range 103-440), $p < 0.05$, Wilcoxon test) (figure 5B).

Relationship between gait and bladder symptom improvements

The follow-up data on gait and freezing episodes shows no clear relationship between gait/freezing improvement and bladder capacity improvement with stimulation (table 3). Both patients in subgroup 2 (no improvement in bladder capacity with DBS) had improved gait and freezing symptoms post-operatively. The subgroup 1 patients had a mixed response to DBS: three patients showed improvements in both gait and freezing measures post-operatively, one patient experienced no change in either gait or freezing and one patient had worsening of his gait symptoms but no increase in the amount or

severity of freezing. We note that, the patient whose gait symptoms worsened at 6-month follow-up (patient 5), was the patient with the most dramatic increase in bladder capacity with DBS.

Long-term follow-up of 2 DBS patients

A patient from subgroup 1 (improvement in bladder capacity with DBS) and a patient from subgroup 2 (no improvement in bladder capacity with DBS) completed bladder symptom questionnaires pre-operatively and six months post-operatively. For the patient from subgroup 1, bladder symptoms improved 6 months after surgery (bladder score improved from 12 to 8). For the patient from subgroup 2, bladder symptoms worsened (bladder score worsened from 8 to 10) (see Figure 5C).

Discussion

To investigate the neural basis of lower urinary tract symptoms in Parkinson's disease, we studied the association between microstructural anatomical changes within the brainstem and the severity of bladder symptoms in a group of Parkinson's disease patients. Given the importance of the pons in the control of the micturition reflex, we hypothesised that lower urinary tract dysfunction in Parkinson's disease would be related to structural changes within pontine bladder control regions. In support of our hypothesis, we found areas bilaterally within the brainstem in which MD, a measure derived from diffusion-weighted MRI data, correlated significantly with bladder symptom severity. Increased MD can signify cell death, and reduced MD can occur in areas of cell swelling or increased cell density. Our analysis indicated that there were bilateral areas in which MD correlated positively with bladder symptom severity, and an adjacent left-sided region where MD was negatively correlated with bladder symptom severity. One interpretation of this finding is that the two areas of significant but opposite correlation between MD and bladder symptom severity could represent populations of two different neuronal cell types responding with different structural changes to a single pathological process, with either the primary process or the resultant changes having a detrimental effect on bladder function.

In order to understand the structural changes revealed by our analysis more fully, we compared the location of the areas in which microstructural change correlated with bladder dysfunction with the predicted location of known pontine bladder control areas, namely the pontine 'M-region' (pontine micturition centre) and the pontine 'L-region' (pontine continence centre). Co-ordinates provided by a seminal PET study on bladder control by Blok *et al.*, 1997 were used to plot the location of the M-region and L-region alongside our data. We found that the pontine L-region, but not the M-region, was in close proximity to the areas we had identified. This suggests that structural changes in the pontine L-region in Parkinson's disease could be associated with the development of lower urinary tract symptoms. The pontine L-region is thought to facilitate continence by maintaining contraction of the external urethral sphincter and pelvic floor during bladder storage. Bilateral lesions give rise to severe incontinence (Griffiths *et al*., 1990), and so it is highly plausible that incomplete destruction of this area, as may be indicated by our results, would give rise to milder symptoms of frequency, nocturia, urgency and urge incontinence; the symptoms assessed by our bladder score.

Abnormal basal ganglia function is a central pathophysiological feature of Parkinson's disease (Albin *et al*., 1989), and based on this understanding, along with evidence from previous studies that deep brain stimulation at the subthalamic nucleus, part of the basal ganglia, can improve bladder function in Parkinson's disease (Finazzi-Agro *et al*., 2003; Herzog *et al*., 2006; Herzog *et al*., 2008), we assessed the relationship between structural changes in the basal ganglia and lower urinary tract symptoms. We identified volume reduction within the left pallidum that correlated with bladder symptom severity and furthermore, we found that tracts seeded from the brainstem areas where MD correlated significantly with bladder symptom severity projected to the left pallidum, raising the possibility that the microstructural changes within the two areas could be related. Previous experimental evidence has shown that pallidal activity is modulated by bladder filling and urinary voiding (Porter, 1967) and that electrical stimulation of the globus pallidus reduces the frequency of spontaneous detrusor muscle contractions (Lewin and Porter, 1965; Pazo, 1976; Mordasini *et al*., 2014). Single unit recording studies in the 1970s identified similar triphasic patterns of neuronal activity in the globus pallidus and the anterior brainstem during urinary voiding, implying a functional connection between these regions in the co-ordination

of micturition (Porter *et al*., 1971). Finally, surgical lesioning of the GPi in a patient with Parkinson's disease was shown to produce degeneration of pallido-tegmental neurons and selective degeneration of a 2mm cell region dorsolateral to the decussation of the superior cerebellar peduncle thought to be in the region of the PPN but "largely dorsal and medial to the majority of large, intensely AchE-positive" neurons which make up the cholinergic sub-division of the PPN (Rye *et al*., 1996). Overall, this evidence supports the view that a functional unit comprised of the GPi and parts of the brainstem are involved in co-ordinating urinary voiding, and that there are structural connections between the two regions that are susceptible to neurodegeneration. Our present structural findings support this possibility. We suggest that we may have described Parkinson's disease -related degeneration within a single functional pathway responsible for lower urinary tract control, which involves the globus pallidus and the pontine L-region.

Implications for future management of lower urinary tract symptoms

Having identified changes within this putative brainstem-basal ganglia continence pathway, we investigated whether deep brain stimulation targeting the system at the level of the brainstem could improve lower urinary tract symptoms. Filling cystometry in seven patients with deep brain stimulation electrodes implanted in the region of the PPN revealed that stimulation increased bladder capacity in a subgroup of patients whose stimulation sites co-incided with left-sided brainstem regions where MD correlated with bladder symptom severity, implying that stimulation restored function in these areas. Furthermore, long-term follow up of a patient from the subgroup whose bladder capacity improved with DBS showed that he had improved lower urinary tract symptoms 6 months post-operatively. These follow-up findings suggest that the urodynamic improvement in bladder capacity may predict clinical improvements in continence, although an adequately powered prospective clinical study will be needed to evaluate this properly.

Anatomical relationship between effective stimulation sites and the pedunculopontine nucleus

Although all patients recruited to this study had received unilateral or bilateral deep brain stimulation of the PPN region, the anatomical distribution of stimulation sites was fairly wide, and most sites were caudal and slightly ventral to the defined boundaries of the PPN reported by one group (Zrinzo *et al*., 2008; Zrinzo *et al*., 2011). There exists a degree of variability in surgical methods for identifying and targeting of the PPN (Plaha and Gill, 2005; Pereira *et al*., 2008; Ferraye *et al*., 2010; Zrinzo *et al*., 2011); also, as a reticular structure, it can be argued that its boundaries are difficult to accurately define (Ferraye *et al*., 2010). The majority of patients experienced improvements in gait and freezing symptoms post-operatively, suggesting that the neuromodulation was effective in targeting the mesencephalic locomotor area. However, as there was no obvious direct relationship between gait/freezing improvement and bladder response to DBS, the single optimal stimulation site for gait/freezing is probably different to the ideal target for bladder symptom control. Moreover, as there is likely to be controversy about whether all of the electrodes were placed within the PPN proper, we cannot draw any conclusions about the effect of strictly anatomically defined "PPN DBS" on bladder capacity. Rather, the electrodes in this particular group of subjects happened to be placed in such a way as to have an effect on areas of the brainstem where microstructural metrics correlated with bladder function; areas that are highly likely to be linked to the pontine L-region. Finally, we acknowledge that the brainstem is a highly pulsatile structure and that therefore MRI scans of this region are susceptible to motion artefact, which could have influenced the results presented here, particularly as pulse gating was not used during the acquisition. It is clear that further investigation, using optimised scan protocols, is needed to examine whether there is an "ideal target" for DBS for lower urinary tract symptoms in Parkinson's disease and whether it a clinically useful intervention in terms of quality of life, and to determine the relationship between this area and the optimal stimulation site that may improve gait. Further MRI studies should be carried out using pulse gating an

Left-sided predominance of effects

An interesting feature of this study is the fact that the effective stimulation sites were all on the left side of the brainstem. Based on original animal experiments, the pontine

L-region is expected to be bilateral (Griffiths *et al*., 1990), and in functional imaging experiments, midline, right-sided and left-sided pontine sites associated with urinary control have all been identified (Fukuyama *et al*., 1996; Blok *et al*., 1997; Nour *et al*., 2000; Mehnert *et al*., 2008; Krhut *et al*., 2012). Our relatively small sample size prevents us from drawing any definitive conclusions. However, questions about the side of effective stimulation and the side of degenerative changes in Parkinson's disease giving rise to bladder dysfunction should be directly addressed in future studies.

Worsening of bladder symptoms

We note that a previous report has been published regarding a patient with PPN DBS who developed incontinence following electrode implantation (Aviles-Olmos *et al*., 2011). The two patients in the present study whose stimulation sites did not overlap with the regions where MD correlated with bladder function (subgroup 2) each experienced reduced bladder capacity with stimulation turned ON, and one of these patients also reported a deterioration in bladder function at 6-months follow-up. These 2 patients had electrodes implanted at opposite ends of the rostro-caudal axis range occupied by the 7 patient electrodes, which means that there does not appear to be a simple anatomical explanation for the effect. However, it is important to recognise the risk of causing bladder symptoms when implanting electrodes into the brainstem. A greater awareness of the functional anatomy of this region and the way in which it changes in Parkinson's disease would improve our ability to avoid these side-effects following DBS.

Summary

Our data suggests a possible basal ganglia-brainstem pathway involving the GPi and the pontine L-region whose degeneration appears to contribute to lower urinary tract symptoms in Parkinson's disease. This raises the possibility of DBS modulation of bladder function at the level of the brainstem.

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Supplementary Materials

Bladder questions

Patients were asked to select how much each of the following problems affected them:

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 pass urine
D. Urge incontinence: urinary leakage accompanied by strong desire to pass urine

Options were: *(1) Never (2) A little (3) Moderately (4) A lot*

This was taken from a more comprehensive non-motor symptom questionnaire administered to all patients prior to DBS surgery. These particular questions are drawn from the King's Health Quality of Life Questionnaire (Kelleher *et al*., 1997).

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