

Full title: Investigation of urinary storage symptoms in Parkinson's disease utilising structural MRI techniques

ABSTRACT

Background

Lower urinary tract symptoms occur in 27%-86% of patients with Parkinson's disease (PD), however, the mechanisms responsible for bladder dysfunction are not fully understood. This study utilised magnetic resonance imaging (MRI) to test the hypothesis that key brainstem bladder control areas (including the pontine micturition centre (PMC) and the pontine continence centre (PCC)) and their links with the basal ganglia are important in the development of urinary storage symptoms in PD.

Methods

17 PD patients completed a 'bladder symptom questionnaire' and underwent diffusion-weighted MRI (1.5T). Storage symptom severity and MRI measures of white matter microstructural integrity were correlated using tract-based spatial statistics (TBSS).

Results

Mean diffusivity (MD) in the ventral brainstem correlated significantly with bladder symptom severity, in areas close to the predicted anatomical co-ordinates of the 'pontine continence centre' (PCC). Tracts seeded from these regions passed via areas involved in pelvic floor musculature control and urinary voiding including the cerebellum, pallidum and precentral gyrus.

Conclusion

We used diffusion-weighted MRI to investigate the role of the brainstem and its structural connections in the development of urinary storage symptoms in PD. Our data suggests that

brainstem degenerative change in the vicinity of the PCC may be implicated in the pathogenesis of storage symptoms in these patients.

KEY WORDS

Deep brain stimulation, bladder, Parkinson's disease, incontinence, diffusion tensor imaging, pedunculopontine nucleus, neurogenic lower urinary tract dysfunction

INTRODUCTION

Lower urinary tract symptoms occur in 27%-86% of patients with Parkinson's disease (PD)¹. "Storage" symptoms predominate, and although voiding symptoms also occur, according to a recent study, the most prominent lower urinary tract symptoms in PD, averaging across gender groups, are urinary frequency, nocturia, urgency, and urge incontinence². Bladder dysfunction seriously impairs quality of life however, understanding about the pathophysiology of urinary symptoms in PD is limited. Improved understanding of pathophysiology would facilitate development of treatment strategies.

Major supraspinal/subcortical areas responsible for bladder control exist within the midbrain and pons. These include the periaqueductal grey area (PAG), pontine micturition centre (PMC), and pontine continence centre (PCC)^{3,4}. Neuropathological involvement of the brainstem occurs at an early stage in PD, affecting the majority of sufferers⁵ and a recent study showed that deep brain stimulation (DBS) of the brainstem pedunculopontine nucleus improved bladder capacity in PD patients⁶. Given the importance of the brainstem in bladder control and the frequent involvement of this area in PD pathology, it is important to determine whether changes in brainstem circuitry are involved in bladder symptom development in PD.

Few studies have investigated neural mechanisms of PD-related bladder dysfunction in humans and of these, only one addressed the role of the brainstem⁷. This study used positron emission tomography (PET) to demonstrate lack of significant pontine activation in PD patients during detrusor overactivity, contrasting with previous PET studies in healthy volunteers showing clear activation within the pons during urine storage and voiding⁸, suggesting that the brainstem's response to bladder filling and voiding differs in PD compared with healthy controls, and may contribute to lower urinary tract symptom pathogenesis.

While conventional clinical imaging techniques are not able to detect subtle abnormalities within brainstem white matter, diffusion tensor imaging (DTI) is sensitive in detecting microstructural white matter changes in various

neurodegenerative disorders^{9,10}. DTI indices can be derived from the “diffusion tensor”; a matrix of vectors that describes the magnitude and direction of diffusion within a given voxel. Clinically relevant DTI indices include mean diffusivity (MD), which carries information about the overall diffusion within a voxel, and fractional anisotropy (FA), which provides information about the extent to which diffusion of water is anisotropic across three dimensions. Reduced FA and increased MD have been demonstrated in a host of neurodegenerative disorders and can result from axonal degradation or demyelination¹¹. Cell membranes and myelin in white matter tracts constrain diffusion parallel to the direction of the myelin and reduce diffusion perpendicular to myelinated tracts. This produces anisotropic diffusion, usually reflected by a high FA. Demyelination and axonal degradation result in fewer barriers to diffusion, generating a more isotropic pattern of diffusion (more equal diffusion in all directions), which is equivalent to a lower FA (less anisotropic diffusion) but higher MD (increase in overall diffusion in all directions). We hypothesised that PD-related neurodegeneration within white matter tracts projecting to and from brainstem bladder areas could be implicated in the pathophysiology of urinary symptoms and would be detectable using DTI metrics, including reduced FA and increased MD in areas of interest. We chose to focus specifically on storage symptoms, as they are more common than voiding symptoms in PD, and the pathophysiology of storage and voiding symptoms in PD may be distinct from one another.

SUBJECTS AND METHODS

Participants

All patients awaiting DBS for PD in Oxford who consented to participate were considered eligible for inclusion. All had to pass a battery of neuropsychological tests including memory and psychiatric scales to qualify for DBS treatment and therefore study participation; hence patients with significant cognitive or psychological impairment were excluded. All patients scored at least 7 on the Dementia Rating Scale-2TM and therefore were considered to not have dementia. Exclusion criteria included: (1) lack of completion of bladder sections of the questionnaire (see below) or, (2) MRI data of insufficient quality for analysis. It was difficult to estimate power because there are relatively few descriptions of studies looking at brainstem DTI indices, using comparable imaging parameters. Furthermore, as the study was designed as a post-hoc analysis using prospectively collected questionnaire data (see below), our sample size was dictated/limited by clinical considerations. However, based on a significance level of 0.05, power of 0.8, and r value of 0.67, (derived from a study which identified correlation between brainstem FA and duration of disease in subjects with Kennedy's disease¹²) the required sample size is calculated to be 14.8.

Questionnaire design

Participants completed a validated autonomic symptom questionnaire (COMPASS-31)¹³ which included general questions about bladder function; along with an additional optional bladder questionnaire. The bladder questionnaire was the King's Health Questionnaire, originally validated in women¹⁴ but used since in mixed populations¹⁵. Data regarding storage symptoms (urinary frequency, nocturia, urinary urgency, and urge incontinence²) were extracted from the questionnaire and summed to give a storage symptom score out of 16 (see supplementary information for questionnaire details). Information about age, sex and recent Unified Parkinson's Disease Rating Scale (UPDRS) assessments were accessed via a clinical database. All patients had MRI scans as described below, as part of the routine work-up for DBS.

All patients gave consent for their information to be used for research. All parts of this study were carried out in accordance with the Declaration of Helsinki and received local ethical approval.

Magnetic Resonance Imaging study

MRI data acquisition

Subjects underwent MRI on a 1.5 tesla Siemens scanner. Diffusion-weighted data was acquired using an echo planar imaging sequence (field of view 256x208 mm², matrix 128x104, slice thickness 2 mm, in plane resolution 2x2 mm², repetition time 15 s, echo time 106.2 ms, 32 directions, b value 1000 s/mm², one image without diffusion weighting was obtained). A high-resolution T1-weighted structural image (voxel size 1x1x1 mm³) was acquired to check for gross structural abnormalities, with a three-dimensional 'FLASH' sequence (TR 12 ms, TE 5.6ms, flip angle 19°, with elliptical sampling of *k*-space, giving a voxel size of 1x1x1 mm³ in 5.05 min).

Image processing

Images were analysed using the FMRIB Software Library (FSL), Release 5.0© 2012¹⁶.

DTI pre-processing

Each individual diffusion-weighted scans was inspected for artefacts and gross distortions, corrected for head motion and eddy currents and brain extracted using BET. FA and MD 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) pre-processing

Individual FA images were aligned to every other FA image, and the “most representative” result 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 skeleton¹⁷. This FA skeleton represented the centres of all tracts common to the group. Each subject’s aligned FA data were then projected onto this skeleton. Right and left brainstem masks were produced using the Juelich Histological Atlas, thresholded at 50%, binarized and applied to the mean TBSS skeleton mask. MD images were subsequently processed in the same way as described above for FA.

Statistical analyses

TBSS correlations between measures of white matter microstructural integrity and storage symptom score

Correlations between storage symptom score and DTI metrics were determined using permutation-based non-parametric testing applied to a voxel-wise general linear model, carried out using the whole brain white matter skeleton and its intersection with the brainstem mask, with age as a covariate of no interest. Essentially, this approach identifies correlations between storage symptom score and values of FA, or MD, for each voxel within the parts of the white matter skeleton being analysed. 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) approach¹⁸. 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’).

Post-hoc analyses involving ‘significant’ TBSS results

Proximity of areas of significant correlation to pontine bladder areas

In order to determine the proximity between pontine bladder nuclei and brainstem areas where MD correlated with storage symptom score, we referred to Blok et al. (1997)⁸, which identifies probable locations of the PMC and PCC. The voxel of maximal activation assumed to be the PCC was converted from Talairach to MNI space using GingerALE (<http://brainmap.org/>) 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 PCC is expected to be a bilateral area⁴, we calculated the left sided equivalent by mirroring the co-ordinate in the X dimension. A similar conversion was used to generate an approximate location of the PMC.

Probabilistic tractography

To determine whether brainstem regions in which MD correlated significantly with storage symptom severity projected to other brain regions involved in bladder control, 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, binarized the thresholded output, and averaged these for all subjects to create left and right group maps.

Segmentation of subcortical structures

Subcortical segmentation of basal ganglia grey matter regions intersected by probabilistic tracts seeded from the significant TBSS result was carried out using FMRIB's Integrated Registration and Segmentation Tool (FIRST). To scale subcortical structure volume according to the head size of the individual patient, FMRIB'S SIENAX tool was run for each subject. 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.

RESULTS

Subject information

24 subjects who had completed the bladder questionnaire were initially included, but 7 were excluded due to lack of acceptable quality T1 or DTI imaging. The final study group included 13 male and 4 female subjects. The mean age was 67 years (range 54-75); mean urinary storage symptom score was 8 ± 3 (range 4-16); mean autonomic symptom score was 18 (range 0–46). There was no significant correlation between storage symptom score and age, UPDRS values, or autonomic score. For a summary of patient characteristics, see table 1.

TBSS correlations between measures of white matter microstructural integrity and storage symptom score

TBSS using the entire white matter skeleton did not show any significant correlation between storage symptom scores and MD or FA. However, using the intersection of the right and left brainstem masks with the skeleton as regions-of-interest, we observed bilateral regions in which MD correlated with the storage symptom score (Figure 1A, Figure 2). An additional region in the left brainstem showed a negative correlation between MD and storage symptom score (Figure 1B). No correlation between FA and bladder symptom severity was identified using the brainstem masks.

Post-hoc analysis analyses involving ‘significant’ TBSS results

Anatomical location of pontine bladder areas

As shown in Figure 3, predicted co-ordinates for the PCC were closely related bilaterally to the brainstem areas where MD correlated with storage symptom severity. The predicted location of the PMC was situated more dorsally within the brainstem.

Tractography from significant TBSS results

Probabilistic tractography, using as seed regions the areas where MD significantly correlated with the storage symptom score, produced group maps for the left and the right hemisphere. Tracts on the right passed through the brainstem and cerebellum. Tracts on the left passed through the brainstem, cerebellum, pallidum, putamen, thalamus, precentral gyrus, postcentral gyrus and superior parietal lobule (Figure 4).

Storage symptom score and basal ganglia volume

Subcortical segmentation was carried out for the left pallidum, left putamen and left thalamus as tracts from the significant MD regions bypassed these brain 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 ($R=-0.654$, $p=0.018$ after Bonferroni correction for multiple comparisons) between left pallidal volume (corrected for head size) and bladder score.

DISCUSSION

To our knowledge, this is the largest study to investigate the neural basis of bladder dysfunction in PD using MRI, and the first to employ structural MRI analysis with the brainstem as a region-of-interest in this population. Our analyses revealed areas within the ventral, but not dorsal, brainstem where MD in white matter regions correlated significantly with the severity of urinary storage symptoms. MD is a highly sensitive, though non-specific, marker of underlying tissue organisation, shown to increase in chronic degenerative processes due to loss of barriers to diffusion such as myelin and axonal membranes⁹. Our findings suggest that degenerative changes affecting white matter tracts passing to or from the ventral pons may be relevant to storage symptoms in PD. In addition to bilateral areas where MD correlated positively with storage symptom severity, there was an adjacent left-sided region where MD correlated negatively with symptom severity. Decreased MD values may indicate early cell injury and neurodegenerative change. 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 spaces²⁰. In addition, a rodent study of cerebral infarction directly related decreased MD to markers of acute cell injury²¹.

It is widely accepted that a PMC within the dorsolateral pons is responsible for the initiation of micturition⁴. In a PET study⁸ and a more recent fMRI study²³, significant activation of the dorsal pons occurred during micturition. However, there is also evidence for the presence of a PCC, which is important for maintaining contraction of the external urethral sphincter and pelvic floor during bladder storage. This area is situated more ventrally within the pons, where bilateral lesions produce severe incontinence⁴. In seven human subjects, a right-sided ventral pontine region was identified during urine withholding⁸ and furthermore, ventral pontine activation has been associated with pelvic floor contraction and relaxation, a motor function relevant to continence²². Although Seseke et al. (2008)²² describe the locus of activation in their pelvic floor fMRI study as the pontine micturition centre, the neural activity is positioned fairly ventrally within the pons, where one might expect to find the PCC^{4,8}. Thus, although the number of studies is small, ventral pontine regions are implicated in processes related to continence. In our cohort, structural changes in this continence-promoting region of the ventral brainstem may be associated with the development of urinary storage symptoms.

Trajectory of white matter tracts seeded from affected brainstem areas

If the brainstem regions of significant correlation between MD and storage symptom severity have a role in continence, we would expect to find evidence of structural connectivity with other brain regions important for continence and lower urinary tract control. Probabilistic tractography using as seed regions the brainstem areas of significant correlation between MD and storage symptom severity revealed tracts passing via the brainstem and cerebellum on the right, and the brainstem, cerebellum, pallidum, putamen, thalamus, precentral gyrus, postcentral gyrus and superior parietal lobule on the left. All of the above regions have been implicated variously in studies of bladder filling, detrusor overactivity, urinary voiding, and pelvic floor control^{7,22,24}. While tractography has a number of limitations and is strictly speaking not able to

answer the question whether two brain regions connect, the findings are complemented by the results of the volumetric analysis of left pallidal volume indicating a significant inverse correlation between left pallidal volume and bladder symptom severity. Electrical stimulation of the pallidum appears to reduce contractile behaviour of the bladder²⁵ and recording from the pallidum demonstrates patterns of activity corresponding to voiding²⁴. Thus, based on existing experimental evidence, degeneration of the pallidum could conceivably impair regulation of detrusor activity, resulting in urinary symptoms.

Limitations

Although DTI-based analyses allow non-invasive quantification of microstructural anatomy, there are inherent limitations associated with the technique, especially when investigating the brainstem. Brainstem pulsatility renders diffusion-weighted scans of this region susceptible to artefacts, particularly if, as was the case in this study, cardiac gating is not used during the acquisition. Echo planar imaging-related geometric distortion and reduction in local sensitivity due to signal drop-out are also possible factors, which can give rise to imprecision. Further work to overcome some of these limitations would include recruiting larger numbers of subjects and using more state-of-the-art diffusion-weighted imaging to address the above mentioned artefact sources.

We acknowledge the lack of a control group, which results from the fact that all MRI and clinical data emerged from routine clinical work-up. We are therefore unable to exclude the possibility that the brainstem changes correlating with bladder symptom severity were a result of disease-unrelated individual variation rather than PD. A subsequent study is needed to address the issue and clearly differentiate between PD and age-related changes. However, the strong emphasis of this study is in its identification of brainstem regions where white matter changes correlated with bladder symptom severity, thus highlighting the brainstem as an important area for further study in the field of brain-bladder imaging in both PD and elderly populations. It also strengthens the case for the existence of a PCC.

To our knowledge there are no post-mortem studies correlating brainstem Lewy body load with bladder symptom severity in PD. This would be an interesting line of investigation that could strengthen the present findings.

CONCLUSION

Using diffusion-weighted MRI, we have identified brainstem areas, close to the predicted location of the PCC, where structural change may be associated with urinary storage symptoms in PD. These findings support the view that the brainstem may play a key role in the pathophysiology of storage symptoms and that a brainstem PCC may be implicated.

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