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Title

Protocol for a prospective study of cortical lower urinary tract control changes following intradetrusor injection of Botulinum toxin-A in patients with Multiple Sclerosis.

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ABSTRACT

Introduction

Multiple Sclerosis (MS) is a severe debilitating disease that affects patient's quality of life. Up to 90% of patients with MS will develop lower urinary tract dysfunction within the first 18 years of the disease. If anticholinergics, behavioral modifications and pelvic floor physical therapy are unsuccessful, Botulinum toxin A (BTX-A) intradetrusor injection is a highly effective option for these patients. The local effects of BTX-A are well understood, but not much is known of its afferent/sensory effects while treating the end organ. Our study will use fMRI and task-related blood oxygen level dependent (BOLD) signal to evaluate patients with MS and neurogenic detrusor overactivity (NDO) prior to, and after, intradetrusor injection of BTX-A with simultaneous urodynamic evaluation. Urinary concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) will also be collected since it has been shown that patients with overactive bladder have higher concentrations of these neuropeptides.

Methods and analysis

Female patients with MS and lower urinary tract symptoms who previously have undergone urodynamic screening and are refractory to conservative management for neurogenic detrusor over activity and are interested in BTX-A intradetrusor injection will be invited to participate in the study. fMRI scan will be performed pre and post BTX-A intradetrusor injection with simultaneous MRI compatible material urodynamics. Images will be collected and analyzed accordingly.

Ethics and dissemination.

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All of the patients are properly consented before enrolling in this study that has been previously approved by the Institution Review Board.

Results of neural connectivity activation will be presented at national and international meetings and published in scholarly journals.

Strengths and Limitations

This is the first study protocol that will identify activation/deactivation of specific regions of the brain in patients with MS and NDO pre and post intravesical injection of BTX-A using fMRI. These findings will be correlated with urodynamic studies and we can evaluate bladder function during the filling and voiding phase. The imaging results of post intravesical injection of BTX-A will help us better understand the sensory effects that BTX-A has in the brain which is not well known.

A limitation of our study protocol is that we are studying a specific patient population and these findings cannot be generalized to other diseases. Also, given that MS produces multifocal lesions in the brain, not all of the patients will have the same lesions in the brain or spinal cord and symptom severity will vary from patient to patient. During simultaneous UDS and fMRI scan, patients have to void supine and have their bladders filled. This setting could affect our cystogram and brain activation patterns because it is not a physiologic state and this can produce anxiety and discomfort.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic multifocal demyelinating disease that can affect any part of the central nervous system (CNS). Up to 90% of patients with MS will develop lower urinary tract dysfunction within the first 18 years of the disease.[1,2] Classification of voiding dysfunction is based on the degree of inability to store and/or empty urine.[3] Lower urinary tract symptoms (LUTS) experienced by patients with MS can range from urgency to urge urinary incontinence and/or hesitancy and incomplete bladder emptying. Due to the diffuse, multifocal involvement of the CNS in patients with MS, symptom severity may vary from patient to patient. Approximately 70% of patients with MS report that their LUTS have moderate to severe impact on their quality of life.[4] Urgency, frequency, and neurogenic detrusor overactivity (NDO) are the most common urologic findings (34 – 99%) during diagnostic evaluations of patients with MS.[5] Even though anticholinergic or beta agonist drugs have limited effectiveness and adverse side effects, they are the first line pharmacotherapy for patients with NDO if behavioral modifications and pelvic floor physical therapy are unsuccessful.[6] Botulinum toxin-A (BTX-A) intradetrusor injection is a highly effective treatment option for patients with NDO who are refractory to more conservative management.[7–9]

BTX-A blocks the release of acetylcholine at the neuromuscular junction and leads to a temporary chemodenervation of the bladder (paralysis of the muscle). Motor effects of BTX-A on the bladder have been extensively studied and widely reported in the literature, and the US Food and Drug Administration has approved BTX-A for the treatment of detrusor overactivity in neurogenic and non –neurogenic patients. However, the sensory effects of BTX-A injection

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correlating to CNS regional perception/localization of urgency, frequency, and urge incontinence in humans are not well known.

Only a few spinal cord injury animal models have suggested central inhibitory effects of BTX-A. Animal studies have shown that the emergence of C-fibers causes bladder overactivity with induction of non-voiding contractions in rats with spinal injury. Boone *et. al.* have published their observations of the effect on central inhibition of intravesical instillation of BTX-A in animals with spinal cord injury. They were able to show that BTX-A-treated groups demonstrated a significant decrease in L6 (i.e., 67%, $P < 0.001$) and S1 (i.e., 47%, $P < 0.01$) c-fos expression (43%) compared to controls receiving saline solution. This finding demonstrated that intravesical instillation of BXT-A significantly inhibits the response of bladder afferent activated lumbosacral neurons without significantly impairing efferent bladder function.[10] In other animal models of bladder overactivity (i.e., SCI and chronic bladder inflammation), intravesical instillation of BTX-A has also been shown to selectively reduce the bladder's afferent response without significantly affecting efferent function (i.e., bladder contractility).[11,12] Moreover, some studies support the idea of a potential role of sensory purinergic pathways in the development of bladder overactivity and suggest that BTX-A can reduce purinergic transmission.[13,14] Whether or not these BTX-A effects extend to the brain level remains to be elucidated.

Physiologic control of urine storage and micturition relies upon a complex network of neural circuits throughout the brain, spinal cord, and urothelium.[15] For a long time, animal models have been used to study lower urinary tract (LUT) function, but with regard to lower urinary function, they differ in numerous ways (many of them social) from humans. Critical

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distinctions exist between voluntary voiding (humans) and involuntary leakage of urine (animal models) when all the social, emotional, and mechanical criteria for voiding in humans are considered. This distinct intention to void or not to void highlights the need for human research in the area of CNS and LUTS instead of reliance mainly on animal data. The initial afferent stimulus comes from the sensation in the bladder as the desire to void. Afterwards, the forebrain determines a person's social circumstance and whether to proceed with the voiding stimulus. Once it is socially acceptable to void, centers in the brain and spinal cord coordinate to produce bladder contraction and urethral sphincter relaxation.[15,16] In order for the pontine micturition center (PMC) to begin micturition, visceral sensations from passive filling in the bladder are transmitted to the periaqueductal grey (PAG) matter of different structures in the brain such as the thalamus, insula, and anterior cingulate gyrus. Other neural structures associated with the voiding reflex are the motor prefrontal cortex, supplementary motor cortex and parahippocampus.[17–19] All of these structures may be involved during PAG activation to transmit further input to the PMC to start micturition.[20,21] As noted earlier, patients with MS have a multifocal involvement of the CNS including the brain and spinal cord. The severity and presentation of the LUTS depends on the location of the lesions.[2,22]

Over the past decades, functional MRI (fMRI) has been used to study the activation of supraspinal lower urinary tract control centers in healthy subjects during the storage and voiding phases.[17–19,23,24] Previous studies have shown that fMRI with simultaneous video urodynamic testing is feasible and can be used to evaluate or stratify treatment modalities for chronic pelvic pain or urge urinary incontinence.[25] Furthermore, a German group investigated structural brain changes in females after they had undergone 12 weeks of treatment with physical therapy or biofeedback for stress urinary incontinence .[26] In this study the

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investigators identified BOLD responses in primary motor and sensory cortical areas associated with pelvic floor muscles. In a study recently reported by Griffiths et al. the investigators identified two patterns of brain activation sensitive to bladder filling that could predict the response and nonresponse to pelvic floor physical therapy that targeted urinary urge incontinence.[20] This data suggests that treatments targeting the end organ (bladder) can significantly affect CNS neuroplasticity. Given these facts, we are interested in evaluating the role of intradetrusor injection of BTX-A afferent response in patients with MS and NDO.

Using fMRI, our group has already demonstrated the activation of brain networks consisting of interconnected regions of sensorimotor control (cerebellum, thalamus, caudate, lentiform nucleus, red nucleus, supplementary motor area, postcentral gyrus), executive function (left superior frontal gyrus), and emotion processing (anterior and posterior cingulate gyrus and insula), as well as deep brain structures (parahippocampal gyrus, percuneus, cuneus, occipital lobe and pons) during micturition in healthy women.[24] Preliminary data from our group showed that patients with MS have increased activation of brain networks during micturition when compared to healthy controls (data not published). This can be the result of increased bladder afferent input to the brain. High-resolution neuroimaging techniques will help us to further understand how MS affects the bladder-brain controls. Our study will use fMRI and task-related blood oxygen level dependent (BOLD) signal to evaluate patients with MS and NDO prior to, and after, intradetrusor injection of BTX-A with simultaneous urodynamic evaluation. Urinary concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) will also be collected since it has been shown that patients with overactive bladder have higher concentrations of these neuropeptides.[27] In the following, we will describe our

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previously established protocol, which we will use for evaluating brain activity with fMRI during the storage and voiding phases with simultaneous urodynamic study (UDS). [24]

Study Aims

1. To perform fMRI neuroimaging and data analysis of patients with MS and NDO during the act of filling and voiding, pre- and post- intravesical injection of BTX-A, to identify specific regions of the brain that are activated and/or deactivated in this group of patients.
2. To determine whether concurrent UDS data obtained with the fMRI scanner can be correlated to fMRI data in order to elucidate simultaneous bladder function measurements with BOLD signal measurements in this patient population.
3. To evaluate the role of urinary biomarkers associated with bladder overactivity (BDNF and NGF) in patients with MS and NDO, before and after intravesical injection of BTX-A.
4. To determine whether the common validated urgency questionnaires correlate with fMRI findings and urinary biomarker concentration pre- and post- BTX-A injection in patients with MS and NDO.

METHODS AND ANALYSIS

Patient Selection

We propose a prospective research study that has been approved by the Institution Review Board prior to patient selection. Female patients with MS and lower urinary tract symptoms who previously have undergone urodynamic screening and are refractory to conservative management for neurogenic detrusor over activity and are interested in BTX-A intradetrusor injection will be

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invited to participate in the study. A healthy female cohort described in a previous report will be used as controls.[24] Inclusion and exclusion criteria are described in Table 1. To estimate the power of this study we will consider published results from other groups that concluded that 20 patients will be sufficient to detect statistical significance in brain activation/deactivation.[20,28,29] The study will end when the 20th patient is scanned and evaluated after BTX-A injection.

Inclusion	Exclusion
18 years or older	Non-ambulatory
Female	Severe debilitating disease (MS)
Patients with MS and NDO	Urinary Tract Infection
	Positive Pregnancy Test
	Contraindication for MRI (i.e. pacemaker, metal implants)
	Male
	History of Incontinence Surgery (i.e sling, MMK, Burch)
	History of LUT surgery (i.e. urethral dilation)
	Other Neurologic diseases (i.e. spinal cord injury, myelomeningiocele, Parkinson disease)

Evaluation

Each patient will provide a detailed history and undergo a complete physical examination. Each will complete the following questionnaires and assessments: Urogenital

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Distress Inventory (UDI-6), Incontinence Impact Questionnaire (IIQ-7), Hamilton Anxiety Rating Scale Assessment (HAM-A), Demographic Form, and MRI Safety Screening Questionnaire. Postvoidal residual volume will be measured and a urine sample will be obtained for urinalysis and neuropeptide evaluation. A baseline video urodynamics will be obtained within a year prior to the baseline neuroimaging scan. Consent and enrollment agreement will be signed during the first visit. (See Figure 1)

Simultaneous Urodynamic testing and fMRI scanning

Prior to entering the MRI room all patients will answer the institutional standard clinical questionnaire for MRI safety and excluded from the study if contraindication to the MRI scan are found. A patient will be asked to wear a gown to avoid image artifacts through ferromagnetic objects in their clothing. Prior to the study, the patient will be asked to void, and post voidal residual (PVR) volume will be measured through catheterization. Double lumen 7 Fr MRI-compatible catheters will be placed in the bladder and rectum. A Phillips Ingenia 3.0T full body MRI scanner with standard 12 channel head coil will be used. Instructions to communicate using right hand signals representing “strong desire”, “voiding”, and “voiding completed” are given to the patient before imaging. To avoid auditory or other extensive brain stimulation not connected to the voiding process, simple signs will be shown to the patient when filling of the bladder is begun and when filling is stopped. Also, in order to keep our noise-to-signal ratio low, all stimulators including any extra visual stimuli and the urodynamic machine will be removed from the MRI scanner room. Total scan times are limited to 45 minutes. After acquisition of a high-resolution 3D brain scan for anatomical reference (turbo fast field, echo, isotropic 3D resolution of 0.7 mm), echo-planar (EPI) scans (repetition time 3 seconds, in-plane resolution 3.4 mm, slice thickness 4 mm) during two functional tasks are performed to record the BOLD signal. First,

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instruction signals from the patient's right hand (Tasks) are performed without actual voiding and scanned separately to obtain a baseline for fMRI activation caused from signaling alone. Afterwards, urodynamic testing and the voiding-task are simultaneously initiated. The bladder is filled at 50mL/min with room-temperature, sterile, normal saline solution until the subject indicates a strong desire to void. Filling will be stopped and subjects will be asked to hold the urine for 30 seconds. Afterwards, patients will be allowed to void into absorbent pads on the scanner table. To create a block-design fMRI task, this sequence will be repeated up to 4 times. Final catheterizable PVR volume will be recorded. This procedure will be performed before treatment and 6-10 weeks after intradetrusor injection of BTX-A. (See Figure 2)

Intradetrusor BTX-A injection

During the second visit, the patient will have a cystoscopy with intradetrusor injection of BTX-A. One hundred or two hundred (patient specific) units of BTX-A will be injected throughout the bladder, including the trigon. Patients will be asked to void to obtain uroflow, if they are spontaneous voiders, and post void residual volume will be measured.

Data Analysis

AFNI software (NIMH, https://afni.nimh.nih.gov/afni/) will be used for the fMRI BOLD analysis. Structural and functional images will be registered to each other. The EPI fMRI images will be motion-corrected. Translational and rotational motion extent will be quantified in this step. Patients with large motion (< 3 mm) will be excluded from analysis. Voxel activation will be identified at the time of strong desire to void and of voiding and BOLD fMRI activation maps will be calculated for both conditions using standard analysis with the generalized linear model (GLM).

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The connectivity MATLAB software CONN (NITRC https://www.nitrc.org/projects/conn,) will be used to perform a functional connectivity analysis. Using the reference 3D anatomical scan, fMRI images will be transferred into MNI space after standard preprocessing steps (detrending, despiking and bandpsss filtering) and parcellated into 132 distinct anatomical location. Strengths of functional connectivity will be calculated between these regions and an average connectivity map will be created pre and post treatment. SPSS (v19.0) will be used to assess statistical significance of changes observed in the clinical data (i.e. Demographic, UDS parameters), the fMRI BOLD and connectivity maps and in the correlation of both.

Safety

Every staff member involved in the collection of data and handling of the patient will be trained and evaluated regarding MRI safety before being allowed to enter the scanner room. Pregnancy tests and urinalysis will exclude patients who are pregnant or have a urinary tract infection. For the latter, after successful treatment of urinary tract infection, patients will be rescheduled.

ETHICS AND DISSEMINATION

Approval of the institutional review board will be obtained for all study procedures**.** **Informed consent will be obtained before** enrolling a patient in the study

Results of neural connectivity activation will be presented at national and international meetings and published in scholarly journals.

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Author Contribution Statement

Rodolfo A. Elizondo and Christof Karmonik contributed to writing and revision of the manuscript.

Timothy B. Boone and Rose Khavari contributed to the study design and manuscript revision.

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Conflict of Interest Statement

None of the authors have conflict of interest.

REFERENCES

1 de Sèze M, Ruffion A, Denys P, *et al.* The neurogenic bladder in multiple sclerosis: review of the literature and proposal of management guidelines. *Mult Scler Houndmills Basingstoke Engl* 2007;**13**:915–28. doi:10.1177/1352458506075651

2 Phé V, Chartier-Kastler E, Panicker JN. Management of neurogenic bladder in patients with multiple sclerosis. *Nat Rev Urol* 2016;**13**:275–88. doi:10.1038/nrurol.2016.53

3 Wein AJ. *Pathophysiology and Classification of Lower Urinary Tract Dysfunction: Overview, In: Campbel-Welsh: Urology.* US: : Elsevier Health Science 2016.

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4 Fowler CJ, Panicker JN, Drake M, *et al.* A UK consensus on the management of the bladder in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2009;**80**:470–7. doi:10.1136/jnnp.2008.159178

5 Wein AJ, Dmochowski RR. *Neuromuscular Dysfunction of the Lower Urinary Tract, In: Campbel-Welsh: Urology.* US: : Elsevier Health Science 2016.

6 Buser N, Ivic S, Kessler TM, *et al.* Efficacy and adverse events of antimuscarinics for treating overactive bladder: network meta-analyses. *Eur Urol* 2012;**62**:1040–60. doi:10.1016/j.eururo.2012.08.060

7 Leone Roberti Maggiore U, Salvatore S, Alessandri F, *et al.* Pharmacokinetics and toxicity of antimuscarinic drugs for overactive bladder treatment in females. *Expert Opin Drug Metab Toxicol* 2012;**8**:1387–408. doi:10.1517/17425255.2012.714365

8 Gormley EA, Lightner DJ, Faraday M, *et al.* Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline amendment. *J Urol* 2015;**193**:1572–80. doi:10.1016/j.juro.2015.01.087

9 Yamaguchi O, Nishizawa O, Takeda M, *et al.* Clinical guidelines for overactive bladder. *Int J Urol* 2009;**16**:126–42. doi:10.1111/j.1442-2042.2008.02177.x

10 Munoz A, Somogyi GT, Boone TB, *et al.* Central inhibitory effect of intravesically applied botulinum toxin A in chronic spinal cord injury. *Neurourol Urodyn* 2011;**30**:1376–81. doi:10.1002/nau.21068

11 Khera M, Somogyi GT, Salas NA, *et al.* In vivo effects of botulinum toxin A on visceral sensory function in chronic spinal cord-injured rats. *Urology* 2005;**66**:208–12. doi:10.1016/j.urology.2005.01.055

12 Smith CP, Vemulakonda VM, Kiss S, *et al.* Enhanced ATP release from rat bladder urothelium during chronic bladder inflammation: effect of botulinum toxin A. *Neurochem Int* 2005;**47**:291–7. doi:10.1016/j.neuint.2005.04.021

13 Apostolidis A, Jacques TS, Freeman A, *et al.* Histological changes in the urothelium and suburothelium of human overactive bladder following intradetrusor injections of botulinum neurotoxin type A for the treatment of neurogenic or idiopathic detrusor overactivity. *Eur Urol* 2008;**53**:1245–53. doi:10.1016/j.eururo.2008.02.037

14 Cheng CL, Liu JC, Chang SY, *et al.* Effect of capsaicin on the micturition reflex in normal and chronic spinal cord-injured cats. *Am J Physiol* 1999;**277**:R786–94.

15 Chai TC, Birder LA. *Physiology and Pharmacology of the Bladder and the Urethra*. US: : Elsevier Health Science 2016.

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16 Griffiths DJ, Fowler CJ. The micturition switch and its forebrain influences. *Acta Physiol Oxf Engl* 2013;**207**:93–109. doi:10.1111/apha.12019

17 Kuhtz-Buschbeck JP, Gilster R, van der Horst C, *et al.* Control of bladder sensations: an fMRI study of brain activity and effective connectivity. *NeuroImage* 2009;**47**:18–27. doi:10.1016/j.neuroimage.2009.04.020

18 Kuhtz-Buschbeck JP, van der Horst C, Wolff S, *et al.* Activation of the supplementary motor area (SMA) during voluntary pelvic floor muscle contractions--an fMRI study. *NeuroImage* 2007;**35**:449–57. doi:10.1016/j.neuroimage.2006.12.032

19 Kuhtz-Buschbeck JP, van der Horst C, Pott C, *et al.* Cortical representation of the urge to void: a functional magnetic resonance imaging study. *J Urol* 2005;**174**:1477–81.

20 Griffiths D, Clarkson B, Tadic SD, *et al.* Brain Mechanisms Underlying Urge Incontinence and its Response to Pelvic Floor Muscle Training. *J Urol* 2015;**194**:708–15. doi:10.1016/j.juro.2015.03.102

21 Panicker JN, Fowler CJ, Kessler TM. Lower urinary tract dysfunction in the neurological patient: clinical assessment and management. *Lancet Neurol* 2015;**14**:720–32. doi:10.1016/S1474-4422(15)00070-8

22 Wintner A, Kim MM, Bechis SK, *et al.* Voiding Dysfunction in Multiple Sclerosis. *Semin Neurol* 2016;**36**:219–20. doi:10.1055/s-0036-1582255

23 Krhut J, Tintera J, Holý P, *et al.* A preliminary report on the use of functional magnetic resonance imaging with simultaneous urodynamics to record brain activity during micturition. *J Urol* 2012;**188**:474–9. doi:10.1016/j.juro.2012.04.004

24 Shy M, Fung S, Boone TB, *et al.* Functional magnetic resonance imaging during urodynamic testing identifies brain structures initiating micturition. *J Urol* 2014;**192**:1149–54. doi:10.1016/j.juro.2014.04.090

25 Borghesi G, Simonetti R, Goldman SM, *et al.* Magnetic resonance imaging urodynamics. Technique development and preliminary results. *Int Braz J Urol Off J Braz Soc Urol* 2006;**32**:336–41; discussion 341.

26 Di Gangi Herms AMR, Veit R, Reisenauer C, *et al.* Functional imaging of stress urinary incontinence. *NeuroImage* 2006;**29**:267–75. doi:10.1016/j.neuroimage.2005.07.018

27 Antunes-Lopes T, Pinto R, Barros SC, *et al.* Urinary neurotrophic factors in healthy individuals and patients with overactive bladder. *J Urol* 2013;**189**:359–65. doi:10.1016/j.juro.2012.08.187

28 Desmond JE, Glover GH. Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. *J Neurosci Methods* 2002;**118**:115–28.

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29 Murphy K, Garavan H. An empirical investigation into the number of subjects required for an event-related fMRI study. *NeuroImage* 2004;**22**:879–85. doi:10.1016/j.neuroimage.2004.02.005

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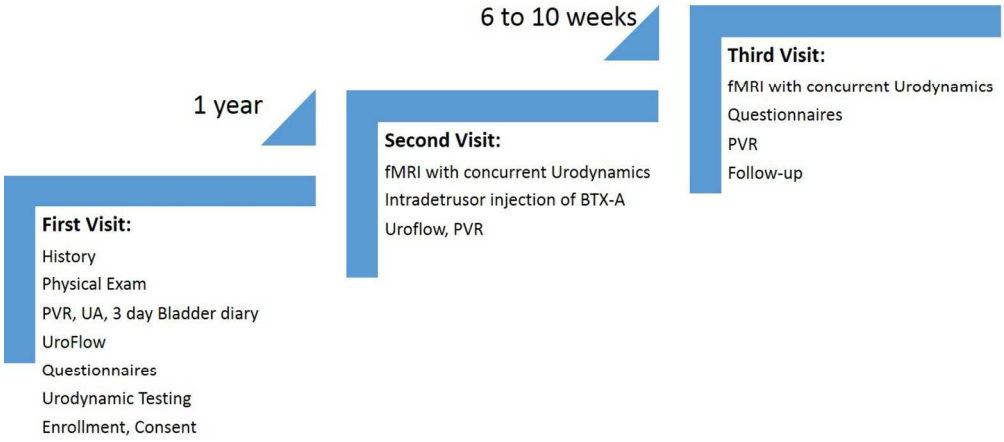

Schematic representation of First, Second and Third clinic visit where data will be collected.
Figure 1
226x150mm (150 x 150 DPI)

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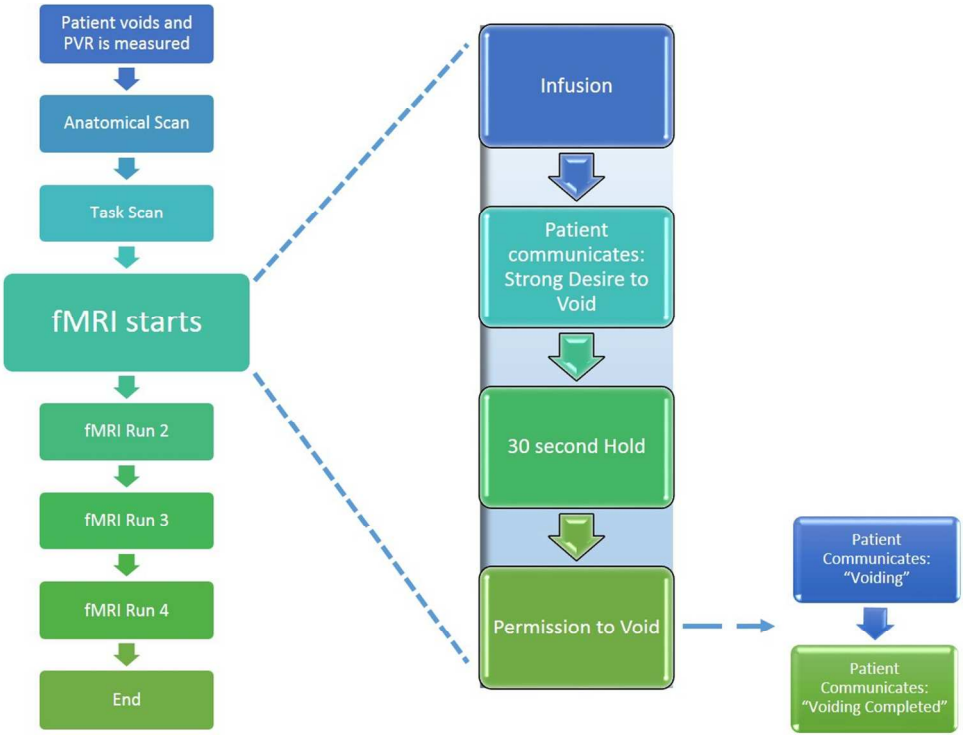

Flow chart of fMRI and simultaneous urodynamic study.
Figure 2
207x151mm (150 x 150 DPI)

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Inclusion	Exclusion
18 years or older	Non-ambulatory
Female	Severe debilitating disease (MS)
Patients with MS and NDO	Urinary Tract Infection
	Positive Pregnancy Test
	Contraindication for MRI (i.e. pacemaker, metal implants)
	Male
	History of Incontinence Surgery (i.e sling, MMK, Burch)
	History of LUT surgery (i.e. urethral dilation)
	Other Neurologic diseases (i.e. spinal cord injury, myelomeningiocele, Parkinson disease)

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Protocol for a prospective observational study of cortical lower urinary tract control changes following intradetrusor injection of Botulinum toxin-A in patients with Multiple Sclerosis.

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Title

Protocol for a prospective observational study of cortical lower urinary tract control changes following intradetrusor injection of Botulinum Toxin-A in patients with Multiple Sclerosis.

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ABSTRACT

Introduction

Multiple Sclerosis (MS) is a severe debilitating disease that affects patients' quality of life. Up to 90% of patients with MS will develop lower urinary tract dysfunction within the first 18 years of the disease. If oral pharmacotherapy with anticholinergics, behavioral modifications and pelvic floor physical therapy are unsuccessful, intradetrusor injection of Botulinum toxin A (BTX-A) (Botox ® Allergan™ plc, Dublin, Ireland) is a highly effective option for these patients. The local effects of BTX-A are well understood, but not much is known of its afferent/sensory effects while treating the end organ. Our study will use fMRI and task-related blood oxygen level dependent (BOLD) signal to evaluate patients with MS and neurogenic detrusor overactivity (NDO) prior to, and after, intradetrusor injection of BTX-A with simultaneous urodynamic evaluation. Urinary concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) will also be collected since it has been shown that patients with overactive bladder have higher concentrations of these neuropeptides.

Methods and analysis

Female patients with MS and lower urinary tract symptoms who previously have undergone urodynamic screening and are refractory to conservative and oral pharmacotherapy management for neurogenic detrusor over activity and are interested in BTX-A intradetrusor injection will be invited to participate in the study. fMRI scan will be performed pre and post BTX-A intradetrusor injection with simultaneous MRI compatible material urodynamics. Images will be collected and analyzed accordingly.

Ethics and dissemination.

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All of the patients are properly consented before enrolling in this study that has been previously approved by the Institutional Review Board.

Results of neural connectivity activation will be presented at national and international meetings and published in scholarly journals.

Strengths and Limitations

Strengths:

- Identifying activation/deactivation of specific regions of the brain in patients with MS and NDO pre and post intravesical injection of BTX-A using fMRI during entire micturition cycle: Filling and voiding.
- Simultaneous correlation of the BOLD signals with urodynamic tracings
- Potentially identifying sensory effects of intradetrusor BTX-A injection that could reveal a novel mechanism of action for BTX-A used in the bladder.

Limitations:

- A specific neurogenic bladder patient population is being studies in this proposal and these findings cannot be generalized to all patients with neurogenic bladder.
- Also, given that MS produces multifocal lesions in the brain, not all of the patients will have the same lesions in the brain or spinal cord and symptom severity will vary from patient to patient.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic multifocal demyelinating disease that can affect any part of the central nervous system (CNS). Up to 90% of patients with MS will develop lower urinary tract dysfunction within the first 18 years of the disease.[1,2] Classification of voiding dysfunction is based on the degree of inability to store and/or empty urine.[3] Lower urinary tract symptoms (LUTS) experienced by patients with MS can range from urgency to urge urinary incontinence and/or hesitancy and incomplete bladder emptying. Due to the diffuse, multifocal involvement of the CNS in patients with MS, symptom severity may vary from patient to patient. Approximately 70% of patients with MS report that their LUTS have moderate to severe impact on their quality of life.[4] Urgency, frequency, and neurogenic detrusor overactivity (NDO) are the most common urologic findings (34 – 99%) during diagnostic evaluations of patients with MS.[5] Even though anticholinergic or beta agonist drugs have limited effectiveness and adverse side effects, they are the first line pharmacotherapy for patients with NDO if behavioral modifications and pelvic floor physical therapy are unsuccessful.[6] Botulinum toxin-A (BTX-A)) (Botox ® Allergan™ plc, Dublin, Ireland) intradetrusor injection is a highly effective treatment option for patients with NDO who are refractory to more conservative management.[7–9]

BTX-A blocks the release of acetylcholine at the neuromuscular junction and leads to a temporary chemodenervation of the bladder (paralysis of the muscle). Motor effects of BTX-A on the bladder have been extensively studied and widely reported in the literature, and the US Food and Drug Administration has approved BTX-A for the treatment of detrusor overactivity in neurogenic and non-neurogenic patients. However, the sensory effects of BTX-A injection

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correlating to CNS regional perception/localization of urgency, frequency, and urge incontinence in humans are not well known.

Only a few spinal cord injury animal models have suggested central inhibitory effects of BTX-A. Animal studies have shown that the emergence of C-fibers causes bladder overactivity with induction of non-voiding contractions in rats with spinal injury. Boone *et. al.* has published their observations of the effect on central inhibition of intravesical instillation of BTX-A in animals with spinal cord injury. They were able to show that BTX-A-treated groups demonstrated a significant decrease in L6 (i.e., 67%, $P < 0.001$) and S1 (i.e., 47%, $P < 0.01$) c-fos expression (43%) compared to controls receiving saline solution. This finding demonstrated that intravesical instillation of BXT-A significantly inhibits the response of bladder afferent activated lumbosacral neurons without significantly impairing efferent bladder function.[10] In other animal models of bladder overactivity (i.e., SCI and chronic bladder inflammation), intravesical instillation of BTX-A has also been shown to selectively reduce the bladder's afferent response without significantly affecting efferent function (i.e., bladder contractility).[11,12] Moreover, some studies support the idea of a potential role of sensory purinergic pathways in the development of bladder overactivity and suggest that BTX-A can reduce purinergic transmission.[13,14] Whether or not these BTX-A effects extend to the brain level remains to be elucidated.

Physiologic control of urine storage and micturition relies upon a complex network of neural circuits throughout the brain, spinal cord, and urothelium.[15] For a long time, animal models have been used to study lower urinary tract (LUT) function, but with regard to lower urinary function, they differ in numerous ways (many of them social) from humans. Critical

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distinctions exist between voluntary voiding (humans) and involuntary leakage of urine (animal models) when all the social, emotional, and mechanical criteria for voiding in humans are considered. This distinct intention to void or not to void highlights the need for human research in the area of CNS and LUTS instead of reliance mainly on animal data. The initial afferent stimulus comes from the sensation in the bladder as the desire to void. Afterwards, the forebrain determines a person's social circumstance and whether to proceed with the voiding stimulus. Once it is socially acceptable to void, centers in the brain and spinal cord coordinate to produce bladder contraction and urethral sphincter relaxation.[15,16] In order for the pontine micturition center (PMC) to begin micturition, visceral sensations from passive filling in the bladder are transmitted to the periaqueductal grey (PAG) matter along with other structures in the brain such as the thalamus, insula, and anterior cingulate gyrus. Additional neural structures associated with the voiding reflex are the motor prefrontal cortex, supplementary motor cortex and parahippocampus.[17–19] All of these structures may be involved during PAG activation to transmit further input to the PMC to start micturition.[20,21] As noted earlier, patients with MS have multifocal lesion involvement of the CNS including the brain and spinal cord. The severity and presentation of the LUTS depends on the location of the lesions.[2,22]

Over the past decades, functional MRI (fMRI) has been used to study the activation of supraspinal lower urinary tract control centers in healthy subjects during the storage and voiding phases.[17–19,23,24] Previous studies have shown that fMRI with simultaneous video urodynamic testing is feasible and can be used to evaluate or stratify treatment modalities for chronic pelvic pain or urge urinary incontinence.[25] Furthermore, a German group investigated structural brain changes in females after they had undergone 12 weeks of treatment with physical therapy or biofeedback for stress urinary incontinence .[26] In this study the

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investigators identified BOLD responses in primary motor and sensory cortical areas associated with pelvic floor muscles. In a study recently reported by Griffiths et al. the investigators identified two patterns of brain activation sensitive to bladder filling that could predict the response and nonresponse to pelvic floor physical therapy that targeted urinary urge incontinence.[20] This data suggests that treatments targeting the end organ (bladder) can significantly affect CNS neuroplasticity. Given these facts, we are interested in evaluating the role of intradetrusor injection of BTX-A afferent response in patients with MS and NDO.

Using fMRI, our group has already demonstrated the activation of brain networks consisting of interconnected regions of sensorimotor control (cerebellum, thalamus, caudate, lentiform nucleus, red nucleus, supplementary motor area, postcentral gyrus), executive function (left superior frontal gyrus), and emotion processing (anterior and posterior cingulate gyrus and insula), as well as deep brain structures (parahippocampal gyrus, percuneus, cuneus, occipital lobe and pons) during micturition in healthy women.[24] We have also shown that patients with MS have distinct supraspinal control activation/deactivation patterns during micturition cycle when compared to healthy controls. [27] This can be the result of increased bladder afferent input to the brain. High-resolution neuroimaging techniques will help us understand how MS affects the bladder-brain controls over micturition. Our study will use fMRI and task-related blood oxygen level dependent (BOLD) signal to evaluate patients with MS and NDO prior to, and after, intradetrusor injection of BTX-A with simultaneous urodynamic evaluation. Urinary concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) will also be collected since it has been shown that patients with overactive bladder have higher concentrations of these neuropeptides.[28] In the following, we will describe our previously

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established protocol, which we will use for evaluating brain activity with fMRI during the storage and voiding phases with simultaneous urodynamic study (UDS). [24]

Study Aims

1. To perform fMRI neuroimaging and data analysis in patients with MS and NDO during the act of filling and voiding, pre- and post- intravesical injection of BTX-A and, to identify specific regions of the brain that are activated and/or deactivated in this group of patients.
2. To determine whether concurrent UDS data obtained with the fMRI scanner can be correlated to fMRI data in order to elucidate simultaneous bladder function measurements with BOLD signal measurements in this patient population.
3. To evaluate the role of urinary biomarkers associated with bladder overactivity (BDNF and NGF) in patients with MS and NDO, before and after intravesical injection of BTX-A.
4. To determine whether the common validated urgency questionnaires correlate with fMRI findings and urinary biomarker concentration pre- and post- BTX-A injection in patients with MS and NDO.

METHODS AND ANALYSIS

Patient Selection and Recruitment

We propose an observational prospective research study that has been approved by our Institutional Review Board prior to patient selection. Inclusion criteria consist of adult female patients with clinically stable MS for ≥ 3 months before screening and an Expanded Disability

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Status Score (EDSS) ≤ 6.5 with lower urinary tract symptoms (urinary frequency or urgency) who previously have undergone a clinic urodynamic screening and are refractory to conservative and oral pharmacological management for neurogenic detrusor over activity and are interested in BTX-A intradetrusor injection, will be invited to participate in the study. Men will be excluded from the study to avoid confounding by prostate pathology. (Table 1) Other exclusion criteria include: severe debilitating disease, pregnancy or planning to become pregnant, nursing, MRI contraindications, history of stress urinary incontinence surgery (e.g. sling, urethral suspension), history of augmentation cystoplasty, and presence of other neurological diseases. Patients with active UTI can be treated and subsequently screened to determine their participation in this study.(Table 1) A healthy female cohort described in a previous report will be used as controls.[24]

Patient recruitment is performed at the Houston Methodist Neurourology Clinic in Houston, Texas, USA by three fulltime fellowship-trained Urologist in the field of Neurourology and Pelvic Reconstructive Surgery who work closely with MS Neurologist from all institutions in the Texas Medical Center. RK serves as the current clinical director of the Neurourology clinic and her practice is focused on patients with neurogenic bladder and pelvic reconstruction surgery in males and females. Patients will be recruited in our clinic and assessed by RK. Recruitment will be initiated in March 2016 and will conclude when we have data from 30 patients.

Inclusion	Exclusion
18 years or older	Non-ambulatory
Female	Severe debilitating disease (MS)
Patients with stable MS ≥ 3 months before screening	Urinary Tract Infection

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Neurogenic detrusor overactivity	Positive Pregnancy Test
LUTS (urinary frequency, urgency)	Contraindication for MRI (i.e. pacemaker, metal implants)
Positive clinic UDS for neurogenic detrusor over activity	Male
Refractory to conservative and oral pharmacotherapy	History of Incontinence Surgery (i.e sling, MMK, Burch)
	History of LUT surgery (i.e. urethral dilation)
	Other Neurologic diseases (i.e. spinal cord injury, myelomeningiocele, Parkinson disease)

Evaluation

Each patient will provide a detailed history and undergo a complete physical examination. Each will complete the following questionnaires and assessments: Expanded Disability Status Scale and Incontinence Quality of Life (I-QOL), Patient Perception of Bladder Control (PPBC), Bladder Control Scale (BLCS), Pelvic Organ Prolapse Distress Inventory 6 (POPDI-6), Demographic Form, and MRI Safety Screening Questionnaire. Post voidal residual volume will be measured and a urine sample will be obtained for urinalysis and neuropeptide evaluation. A baseline video urodynamic study will be obtained within a year prior to the baseline neuroimaging scan. Consent and enrollment agreement will be signed during the first visit. (See Figure 1) Patients will be seen a total of 4 times: initial visit, fMRI control and BTX-A injection, follow-up in the clinic at 2 and 6 weeks following BTX-A treatment for symptom

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evaluation, urinalysis and post voidal residual volume. All questionnaires will be completed after BTX-A treatment as well as a simple patient reported perception of their treatment benefit using a 4-point treatment based scale (TBS). If there are clinical signs of increased post voidal residual volume or urinary retention, clean intermittent catheterizations (CIC) will be initiated.

Simultaneous Urodynamic testing and fMRI scanning

Prior to entering the MRI room all patients will answer the institutional standard clinical questionnaire for MRI safety and excluded from the study if contraindication to the MRI scan are found. A patient will be asked to wear a gown to avoid image artifacts through ferromagnetic objects in their clothing. Prior to the study, the patient will be asked to void, and post voidal residual (PVR) volume will be measured through catheterization. Double lumen 7 Fr MRI-compatible catheters will be placed in the bladder and rectum. A Phillips Ingenia 3.0T full body MRI scanner with standard 12 channel head coil will be used. Instructions to communicate using right hand signals representing “strong desire”, “voiding”, and “voiding completed” are given to the patient before imaging. To avoid auditory or other extensive brain stimulation not connected to the voiding process, simple signs will be shown to the patient when filling of the bladder is begun and when filling is stopped. Also, in order to keep our noise-to-signal ratio low, all stimulators including any extra visual stimuli and the urodynamic machine will be removed from the MRI scanner room. Total scan times are limited to 45 minutes. After acquisition of a high-resolution 3D brain scan for anatomical reference (turbo fast field, echo, isotropic 3D resolution of 0.7 mm), echo-planar (EPI) scans (repetition time 3 seconds, in-plane resolution 3.4 mm, slice thickness 4 mm) during two functional tasks are performed to record the BOLD signal. First, instruction signals from the patients’ right hand (Tasks) are performed without actual voiding and scanned separately to obtain a baseline for fMRI activation caused from signaling alone.

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Afterwards, urodynamic testing and the voiding-task are simultaneously initiated. The bladder is filled at 50mL/min with room-temperature, sterile, normal saline solution until the subject indicates a strong desire to void. Filling will be stopped and subjects will be asked to hold the urine for 30 seconds. Afterwards, patients will be allowed to void into absorbent pads on the scanner table. To create a block-design fMRI task, this sequence will be repeated up to 4 times. Final catheterizable PVR volume will be recorded. This procedure will be performed before treatment and 6-10 weeks after intradetrusor injection of BTX-A. (See Figure 2)

Intradetrusor BTX-A injection

During the second visit, the patient will have a cystoscopy with intradetrusor injection of BTX-A performed by RK. One hundred (in spontaneous voiders) or 200 (CIC dependent patients) units of BTX-A will be injected throughout the bladder, including the trigone. Patients will be asked to void to obtain uroflow, if they are spontaneous voiders, and post void residual volume will be measured.

Data Analysis and Statistics

Power Analysis

Careful application of power calculation is done in the early phases of designing a study to prevent spending time and money on an experiment that is under powered. This is especially important when more costly studies such as the current one are being designed. However, various and limited methods exist to calculate the fMRI power.

A study from 2002 presented an approach for group analysis in a fMRI model while accounting for intra and inter-subject variability. They concluded that approximately 12 subjects were required to achieve 80% power at the single voxel level for typical activations. [29]

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However, at more realistic thresholds after correcting for multiple comparisons, 24 subjects should be used to maintain this level of power. A study by Murphy et al performed an empirical investigation in data of 58 subjects with go/nogo studies. The correlation coefficients were calculated within the regions of interest defined by the 58-subject map threshold at the strictest P value of 0.000001 were there was a 0.8 correlation when 24 subjects were included. [30] To estimate the power of this study we would take into account the 10% exclusions for movement artifacts and 20% dropout rates where patients may not return for post BTX-A scans and we concluded to study a total of 30 patients. The study will end when the 30th patient is scanned and evaluated after BTX-A injection.

Three dimensional structural images will be obtained from a T1-weighted sequence; (sagittal direction, 0.7 in-plane resolution). Functional images will be collected afterwards by means of simultaneous urodynamic analysis (axial echo-planar, TR= 3,000ms, 4.0mm slice thickness, 3.38mm in-plane resolution). AFNI software (NIMH, https://afni.nimh.nih.gov/afni/) will be used for the fMRI BOLD analysis. Structural and functional images will be registered to each other. The EPI fMRI images will be motion-corrected. Translational and rotational motion extent will be quantified in this step. Patients with large motion (< 4.5 mm) will be excluded from analysis. Voxel activation will be identified at the time of "strong desire to void" and "the initiation voiding"; and BOLD fMRI activation maps will be calculated for both conditions using standard analysis with the generalized linear model (GLM). Group analysis will be performed by transforming data into Talairach space, and significantly activated voxels will be identified using a Student's T-test. Comparisons will be drawn between MS subjects comparing baseline scans to the post BTX-A treatment. Subgroup analysis will be performed comparing subjects with and

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without Detrusor Sphincter Dysinergia (DSD). We will use SPSS (v19.0) to perform statistical analysis of clinical data (i.e. Demographic, UDS parameters).

Functional Connectivity analysis will be performed using CONN (NITRC https://www.nitrc.org/projects/conn,), a functional connectivity toolbox for MATLAB57, Version 15a, which utilizes the statistical parametric mapping (SPM) MATLAB toolbox. fMRI images acquired during the voiding task together with their corresponding anatomical datasets will be processed using the default-mni preprocessing option to enable group analysis in Montreal Neurological Institute (MNI) space. A region-based connectivity analysis will be performed for each patient group pre and post BTX-A injection based on regions of interest (ROI, n=4) defined in a brain atlas available from the toolbox. Functional connectivity (FC) is the connectivity between brain regions that share functional properties. It can be defined as the temporal correlation between spatially remote neurophysiological events assessed by their respective BOLD signal time courses. FC will be quantified by T-values for a p-value < 0.05 (two-sided, FDR-corrected).

DTI images will be acquired (32 directions, one B0 image) using the standard MRI pulse sequence available on the Philips 3.0 T Ingenia scanner. The original Diffusion Tensor Imaging (DTI) images as well as the fractional anisotropy (FA) and mean diffusivity (MD) calculated on the scanner will be transferred to on offline workstation for further processing. The software packages TackVis (version 0.6.0.1) and the Diffusion Toolkit (version 0.6.3, trackvis.org) will be used to calculate and extract selected white matter tracts of interest. This software also enables calculating FA and MD values for the segmented white matter tracts.

Safety

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Every staff member involved in the collection of data and handling of the patient will be trained and evaluated regarding MRI safety before being allowed to enter the scanner room. Pregnancy tests and urinalysis will exclude patients who are pregnant or have a urinary tract infection. For the latter, after successful treatment of urinary tract infection, patients will be rescheduled.

Limitations

Since this study protocol focus on a specific neurogenic bladder patient population these findings cannot be generalized to all patients with neurogenic bladder. Also, given that MS produces multifocal lesions in the brain, not all of the patients will have the same lesions in the brain or spinal cord and symptom severity will vary from patient to patient. This limitating factors will be thoroughly described when we present our results in a future manuscript submission.

ETHICS AND DISSEMINATION

Approval of the institutional review board (Houston Methodist Research Institute) will be obtained for all study procedures. Informed consent will be obtained before enrolling a patient in the study. Our informed consent states that participation is completely voluntary and patients can withdraw at any point and this will not affect their relationship with the physician and their treatment course. All staff members involved in the collection of data and handling of patients will have proper privileges and training by our Research Institute and MRI Core. Before proceeding with fMRI testing, patients will be asked to remove all clothing and items containing

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metal. All materials used during the fMRI/UDS scans are MRI compatible. Subjects will be provided with gowns during the imaging study. No minor or vulnerable individuals will be recruited for the study. If an adverse effect occurs, the principal investigator and appropriate authorities will be informed and proper actions will be taken based on Good Clinical Practice. Outcomes of this research will be presented at national and international meetings and published in scholarly journals.

Author Contribution Statement

Rodolfo A. Elizondo and Christof Karmonik contributed to writing and revision of the manuscript.

Timothy B. Boone and Rose Khavari contributed to the study design and manuscript revision.

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Conflict of Interest Statement

None of the authors have conflict of interest.

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REFERENCES

1 de Sèze M, Ruffion A, Denys P, *et al.* The neurogenic bladder in multiple sclerosis: review of the literature and proposal of management guidelines. *Mult Scler Houndmills Basingstoke Engl* 2007;**13**:915–28. doi:10.1177/1352458506075651

2 Phé V, Chartier-Kastler E, Panicker JN. Management of neurogenic bladder in patients with multiple sclerosis. *Nat Rev Urol* 2016;**13**:275–88. doi:10.1038/nrurol.2016.53

3 Wein AJ. *Pathophysiology and Classification of Lower Urinary Tract Dysfunction: Overview, In: Campbel-Welsh: Urology.* US: : Elsevier Health Science 2016.

4 Fowler CJ, Panicker JN, Drake M, *et al.* A UK consensus on the management of the bladder in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2009;**80**:470–7. doi:10.1136/jnnp.2008.159178

5 Wein AJ, Dmochowski RR. *Neuromuscular Dysfunction of the Lower Urinary Tract, In: Campbel-Welsh: Urology.* US: : Elsevier Health Science 2016.

6 Buser N, Ivic S, Kessler TM, *et al.* Efficacy and adverse events of antimuscarinics for treating overactive bladder: network meta-analyses. *Eur Urol* 2012;**62**:1040–60. doi:10.1016/j.eururo.2012.08.060

7 Leone Roberti Maggiore U, Salvatore S, Alessandri F, *et al.* Pharmacokinetics and toxicity of antimuscarinic drugs for overactive bladder treatment in females. *Expert Opin Drug Metab Toxicol* 2012;**8**:1387–408. doi:10.1517/17425255.2012.714365

8 Gormley EA, Lightner DJ, Faraday M, *et al.* Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline amendment. *J Urol* 2015;**193**:1572–80. doi:10.1016/j.juro.2015.01.087

9 Yamaguchi O, Nishizawa O, Takeda M, *et al.* Clinical guidelines for overactive bladder. *Int J Urol* 2009;**16**:126–42. doi:10.1111/j.1442-2042.2008.02177.x

10 Munoz A, Somogyi GT, Boone TB, *et al.* Central inhibitory effect of intravesically applied botulinum toxin A in chronic spinal cord injury. *Neurourol Urodyn* 2011;**30**:1376–81. doi:10.1002/nau.21068

11 Khera M, Somogyi GT, Salas NA, *et al.* In vivo effects of botulinum toxin A on visceral sensory function in chronic spinal cord-injured rats. *Urology* 2005;**66**:208–12. doi:10.1016/j.urology.2005.01.055

12 Smith CP, Vemulakonda VM, Kiss S, *et al.* Enhanced ATP release from rat bladder urothelium during chronic bladder inflammation: effect of botulinum toxin A. *Neurochem Int* 2005;**47**:291–7. doi:10.1016/j.neuint.2005.04.021

13 Apostolidis A, Jacques TS, Freeman A, *et al.* Histological changes in the urothelium and suburothelium of human overactive bladder following intradetrusor injections of botulinum neurotoxin type A for the treatment of neurogenic or idiopathic detrusor overactivity. *Eur Urol* 2008;**53**:1245–53. doi:10.1016/j.eururo.2008.02.037

14 Cheng CL, Liu JC, Chang SY, *et al.* Effect of capsaicin on the micturition reflex in normal and chronic spinal cord-injured cats. *Am J Physiol* 1999;**277**:R786–94.

15 Chai TC, Birder LA. *Physiology and Pharmacology of the Bladder and the Urethra*. US: : Elsevier Health Science 2016.

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16 Griffiths DJ, Fowler CJ. The micturition switch and its forebrain influences. *Acta Physiol Oxf Engl* 2013;**207**:93–109. doi:10.1111/apha.12019
17 Kuhtz-Buschbeck JP, Gilster R, van der Horst C, *et al.* Control of bladder sensations: an fMRI study of brain activity and effective connectivity. *NeuroImage* 2009;**47**:18–27. doi:10.1016/j.neuroimage.2009.04.020
18 Kuhtz-Buschbeck JP, van der Horst C, Wolff S, *et al.* Activation of the supplementary motor area (SMA) during voluntary pelvic floor muscle contractions--an fMRI study. *NeuroImage* 2007;**35**:449–57. doi:10.1016/j.neuroimage.2006.12.032
19 Kuhtz-Buschbeck JP, van der Horst C, Pott C, *et al.* Cortical representation of the urge to void: a functional magnetic resonance imaging study. *J Urol* 2005;**174**:1477–81.
20 Griffiths D, Clarkson B, Tadic SD, *et al.* Brain Mechanisms Underlying Urge Incontinence and its Response to Pelvic Floor Muscle Training. *J Urol* 2015;**194**:708–15. doi:10.1016/j.juro.2015.03.102
21 Panicker JN, Fowler CJ, Kessler TM. Lower urinary tract dysfunction in the neurological patient: clinical assessment and management. *Lancet Neurol* 2015;**14**:720–32. doi:10.1016/S1474-4422(15)00070-8
22 Wintner A, Kim MM, Bechis SK, *et al.* Voiding Dysfunction in Multiple Sclerosis. *Semin Neurol* 2016;**36**:219–20. doi:10.1055/s-0036-1582255
23 Krhut J, Tintera J, Holý P, *et al.* A preliminary report on the use of functional magnetic resonance imaging with simultaneous urodynamics to record brain activity during micturition. *J Urol* 2012;**188**:474–9. doi:10.1016/j.juro.2012.04.004
24 Shy M, Fung S, Boone TB, *et al.* Functional magnetic resonance imaging during urodynamic testing identifies brain structures initiating micturition. *J Urol* 2014;**192**:1149–54. doi:10.1016/j.juro.2014.04.090
25 Borghesi G, Simonetti R, Goldman SM, *et al.* Magnetic resonance imaging urodynamics. Technique development and preliminary results. *Int Braz J Urol Off J Braz Soc Urol* 2006;**32**:336–41; discussion 341.
26 Di Gangi Herms AMR, Veit R, Reisenauer C, *et al.* Functional imaging of stress urinary incontinence. *NeuroImage* 2006;**29**:267–75. doi:10.1016/j.neuroimage.2005.07.018
27 Khavari R, Karmonik C, Fletcher S, et al. Functional Magnetic Resonance Imaging with Concurrent Urodynamic Testing Identifies Brain Structures Involved in Micturition Cycle in Patients with Multiple Sclerosis. *J Urol* 2016.doi:10.1016/j.juro.2016.09.077
28 Antunes-Lopes T, Pinto R, Barros SC, *et al.* Urinary neurotrophic factors in healthy individuals and patients with overactive bladder. *J Urol* 2013;**189**:359–65. doi:10.1016/j.juro.2012.08.187
29 Desmond JE, Glover GH. Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. *J Neurosci Methods* 2002;**118**:115–28.
30 Murphy K, Garavan H. An empirical investigation into the number of subjects required for an event-related fMRI study. *NeuroImage* 2004;**22**:879–85. doi:10.1016/j.neuroimage.2004.02.005

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Figure Legends

Figure 1. Schematic representation of First, Second and Third clinic visit where data will be collected. Fourth clinic visit at 6 weeks will not include fMRI scan.

Figure 2. Flow chart of fMRI and simultaneous urodynamic study.

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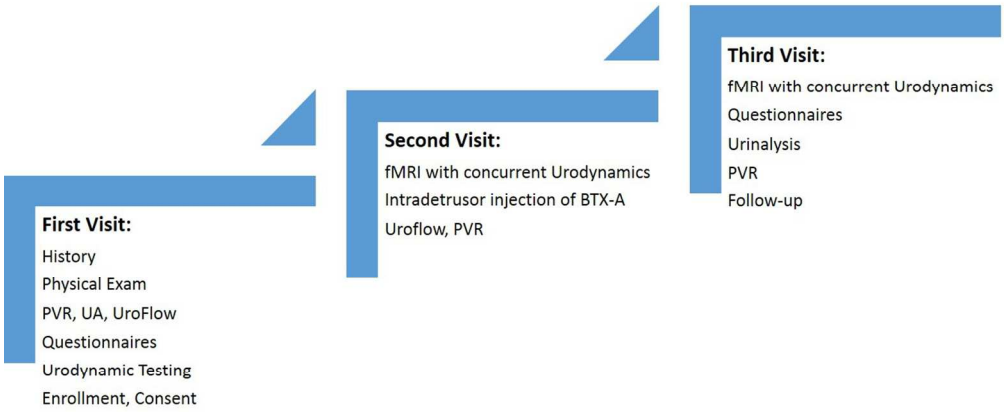

Figure 1. Schematic representation of First, Second and Third clinic visit where data will be collected. Fourth clinic visit at 6 weeks will not include fMRI scan.

Figure 1
226x150mm (150 x 150 DPI)

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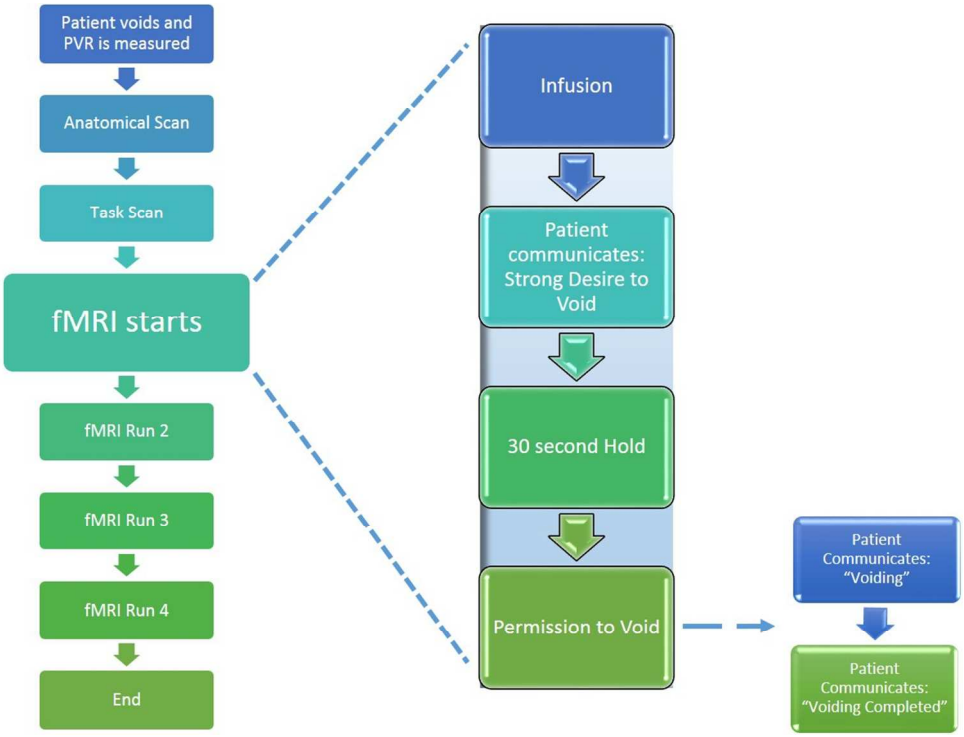

Flow chart of fMRI and simultaneous urodynamic study.
Figure 2
207x151mm (150 x 150 DPI)

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Inclusion	Exclusion
18 years or older	Non-ambulatory
Female	Severe debilitating disease (MS)
Patients with stable MS ≥ 3 months before screening	Urinary Tract Infection
Neurogenic detrusor overactivity	Positive Pregnancy Test
LUTS (urinary frequency, urgency)	Contraindication for MRI (i.e. pacemaker, metal implants)
Positive clinic UDS for neurogenic detrusor over activity	Male
Refractory to conservative and oral pharmacotherapy	History of Incontinence Surgery (i.e sling, MMK, Burch)
	History of LUT surgery (i.e. urethral dilation)
	Other Neurologic diseases (i.e. spinal cord injury, myelomeningiocele, Parkinson disease)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2 & 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4 – 7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	8 – 11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9 & 10
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	8
		(*b*) For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	12 – 14
Bias	9	Describe any efforts to address potential sources of bias	15
Study size	10	Explain how the study size was arrived at	12 & 13
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12 – 14
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how matching of cases and controls was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	NA
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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BMJ Open

Protocol for a prospective observational study of cortical lower urinary tract control changes following intradetrusor injection of Botulinum toxin-A in patients with Multiple Sclerosis.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-013225.R2
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Primary Subject Heading:	Urology
Secondary Subject Heading:	Radiology and imaging, Obstetrics and gynaecology, Neurology, Research methods
Keywords:	Neurogenic Bladder, LUTS, Multiple sclerosis < NEUROLOGY, fMRI, Botox

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Title

Protocol for a prospective observational study of cortical lower urinary tract control changes following intradetrusor injection of Botulinum Toxin-A in patients with Multiple Sclerosis.

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Keywords

Neurogenic Bladder, Multiple Sclerosis, fMRI, Botox, LUTS

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ABSTRACT

Introduction

Multiple Sclerosis (MS) is a severe debilitating disease that affects patients' quality of life. Up to 90% of patients with MS will develop lower urinary tract dysfunction within the first 18 years of the disease. If oral pharmacotherapy with anticholinergics, behavioral modifications and pelvic floor physical therapy are unsuccessful, intradetrusor injection of Botulinum toxin A (OnaBotA) (Botox ® Allergan™ plc, Dublin, Ireland) is a highly effective option for these patients. The local effects of OnaBotA are well understood, but not much is known of its afferent/sensory effects while treating the end organ. Our study will use fMRI and task-related blood oxygen level dependent (BOLD) signal to evaluate patients with MS and neurogenic detrusor overactivity (NDO) prior to, and after, intradetrusor injection of OnaBotA with simultaneous urodynamic evaluation. Urinary concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) will also be collected since it has been shown that patients with overactive bladder have higher concentrations of these neuropeptides.

Methods and analysis

Female patients with MS and lower urinary tract symptoms who previously have undergone urodynamic screening and are refractory to conservative and oral pharmacotherapy management for neurogenic detrusor over activity and are interested in OnaBotA intradetrusor injection will be invited to participate in the study. fMRI scan will be performed pre and post OnaBotA intradetrusor injection with simultaneous MRI compatible material urodynamics. Images will be collected and analyzed accordingly.

Ethics and dissemination.

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All of the patients are properly consented before enrolling in this study that has been previously approved by the Institutional Review Board.

Results of neural connectivity activation will be presented at national and international meetings and published in scholarly journals.

Strengths and Limitations

Strengths:

- Identifying activation/deactivation of specific regions of the brain in patients with MS and NDO pre and post intravesical injection of OnaBotA using fMRI during entire micturition cycle: Filling and voiding.
- Simultaneous correlation of the BOLD signals with urodynamic tracings
- Potentially identifying sensory effects of intradetrusor OnaBotA injection that could reveal a novel mechanism of action for OnaBotAused in the bladder.

Limitations:

- A specific neurogenic bladder patient population is being studies in this proposal and these findings cannot be generalized to all patients with neurogenic bladder.
- Also, given that MS produces multifocal lesions in the brain, not all of the patients will have the same lesions in the brain or spinal cord and symptom severity will vary from patient to patient.

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INTRODUCTION

Multiple sclerosis (MS) is a chronic multifocal demyelinating disease that can affect any part of the central nervous system (CNS). Up to 90% of patients with MS will develop lower urinary tract dysfunction within the first 18 years of the disease.[1,2] Classification of voiding dysfunction is based on the degree of inability to store and/or empty urine.[3] Lower urinary tract symptoms (LUTS) experienced by patients with MS can range from urgency to urge urinary incontinence and/or hesitancy and incomplete bladder emptying. Due to the diffuse, multifocal involvement of the CNS in patients with MS, symptom severity may vary from patient to patient. Approximately 70% of patients with MS report that their LUTS have moderate to severe impact on their quality of life.[4] Urgency, frequency, and neurogenic detrusor overactivity (NDO) are the most common urologic findings (34 – 99%) during diagnostic evaluations of patients with MS.[5] Even though anticholinergic or beta agonist drugs have limited effectiveness and adverse side effects, they are the first line pharmacotherapy for patients with NDO if behavioral modifications and pelvic floor physical therapy are unsuccessful.[6] Botulinum toxin-A (OnaBotA)) (Botox ® Allergan™ plc, Dublin, Ireland) intradetrusor injection is a highly effective treatment option for patients with NDO who are refractory to more conservative management.[7–9]

OnaBotA blocks the release of acetylcholine at the neuromuscular junction and leads to a temporary chemodenervation of the bladder (paralysis of the muscle). Motor effects of OnaBotA on the bladder have been extensively studied and widely reported in the literature, and the US Food and Drug Administration has approved OnaBotA for the treatment of detrusor overactivity in neurogenic and non-neurogenic patients. However, the sensory effects of OnaBotA injection

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correlating to CNS regional perception/localization of urgency, frequency, and urge incontinence in humans are not well known.

Only a few spinal cord injury animal models have suggested central inhibitory effects of OnaBotA. Animal studies have shown that the emergence of C-fibers causes bladder overactivity with induction of non-voiding contractions in rats with spinal injury. Boone *et. al.* has published their observations of the effect on central inhibition of intravesical instillation of OnaBotA in animals with spinal cord injury. They were able to show that OnaBotA-treated groups demonstrated a significant decrease in L6 (i.e., 67%, $P < 0.001$) and S1 (i.e., 47%, $P < 0.01$) c-fos expression (43%) compared to controls receiving saline solution. This finding demonstrated that intravesical instillation of BXT-A significantly inhibits the response of bladder afferent activated lumbosacral neurons without significantly impairing efferent bladder function.[10] In other animal models of bladder overactivity (i.e., SCI and chronic bladder inflammation), intravesical instillation of OnaBotA has also been shown to selectively reduce the bladder's afferent response without significantly affecting efferent function (i.e., bladder contractility).[11,12] Moreover, some studies support the idea of a potential role of sensory purinergic pathways in the development of bladder overactivity and suggest that OnaBotA can reduce purinergic transmission.[13,14] Whether or not these OnaBotA effects extend to the brain level remains to be elucidated.

Physiologic control of urine storage and micturition relies upon a complex network of neural circuits throughout the brain, spinal cord, and urothelium.[15] For a long time, animal models have been used to study lower urinary tract (LUT) function, but with regard to lower urinary function, they differ in numerous ways (many of them social) from humans. Critical

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distinctions exist between voluntary voiding (humans) and involuntary leakage of urine (animal models) when all the social, emotional, and mechanical criteria for voiding in humans are considered. This distinct intention to void or not to void highlights the need for human research in the area of CNS and LUTS instead of reliance mainly on animal data. The initial afferent stimulus comes from the sensation in the bladder as the desire to void. Afterwards, the forebrain determines a person's social circumstance and whether to proceed with the voiding stimulus. Once it is socially acceptable to void, centers in the brain and spinal cord coordinate to produce bladder contraction and urethral sphincter relaxation.[15,16] In order for the pontine micturition center (PMC) to begin micturition, visceral sensations from passive filling in the bladder are transmitted to the periaqueductal grey (PAG) matter along with other structures in the brain such as the thalamus, insula, and anterior cingulate gyrus. Additional neural structures associated with the voiding reflex are the motor prefrontal cortex, supplementary motor cortex and parahippocampus.[17–19] All of these structures may be involved during PAG activation to transmit further input to the PMC to start micturition.[20,21] As noted earlier, patients with MS have multifocal lesion involvement of the CNS including the brain and spinal cord. The severity and presentation of the LUTS depends on the location of the lesions.[2,22]

Over the past decades, functional MRI (fMRI) has been used to study the activation of supraspinal lower urinary tract control centers in healthy subjects during the storage and voiding phases.[17–19,23,24] Previous studies have shown that fMRI with simultaneous video urodynamic testing is feasible and can be used to evaluate or stratify treatment modalities for chronic pelvic pain or urge urinary incontinence.[25] Furthermore, a German group investigated structural brain changes in females after they had undergone 12 weeks of treatment with physical therapy or biofeedback for stress urinary incontinence .[26] In this study the

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investigators identified BOLD responses in primary motor and sensory cortical areas associated with pelvic floor muscles. In a study recently reported by Griffiths et al. the investigators identified two patterns of brain activation sensitive to bladder filling that could predict the response and nonresponse to pelvic floor physical therapy that targeted urinary urge incontinence.[20] This data suggests that treatments targeting the end organ (bladder) can significantly affect CNS neuroplasticity. Given these facts, we are interested in evaluating the role of intradetrusor injection of OnaBotA afferent response in patients with MS and NDO.

Using fMRI, our group has already demonstrated the activation of brain networks consisting of interconnected regions of sensorimotor control (cerebellum, thalamus, caudate, lentiform nucleus, red nucleus, supplementary motor area, postcentral gyrus), executive function (left superior frontal gyrus), and emotion processing (anterior and posterior cingulate gyrus and insula), as well as deep brain structures (parahippocampal gyrus, percuneus, cuneus, occipital lobe and pons) during micturition in healthy women.[24] We have also shown that patients with MS have distinct supraspinal control activation/deactivation patterns during micturition cycle when compared to healthy controls. [27] This can be the result of increased bladder afferent input to the brain. High-resolution neuroimaging techniques will help us understand how MS affects the bladder-brain controls over micturition. Our study will use fMRI and task-related blood oxygen level dependent (BOLD) signal to evaluate patients with MS and NDO prior to, and after, intradetrusor injection of OnaBotA with simultaneous urodynamic evaluation. Urinary concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) will also be collected since it has been shown that patients with overactive bladder have higher concentrations of these neuropeptides.[28] In the following, we will describe our previously

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established protocol, which we will use for evaluating brain activity with fMRI during the storage and voiding phases with simultaneous urodynamic study (UDS). [24]

Study Aims

1. To perform fMRI neuroimaging and data analysis in patients with MS and NDO during the act of filling and voiding, pre- and post- intravesical injection of OnaBotA and, to identify specific regions of the brain that are activated and/or deactivated in this group of patients.
2. To determine whether concurrent UDS data obtained with the fMRI scanner can be correlated to fMRI data in order to elucidate simultaneous bladder function measurements with BOLD signal measurements in this patient population.
3. To evaluate the role of urinary biomarkers associated with bladder overactivity (BDNF and NGF) in patients with MS and NDO, before and after intravesical injection of OnaBotA.
4. To determine whether the common validated urgency questionnaires correlate with fMRI findings and urinary biomarker concentration pre- and post- OnaBotA injection in patients with MS and NDO.

METHODS AND ANALYSIS

Patient Selection and Recruitment

We propose an observational prospective research study that has been approved by our Institutional Review Board prior to patient selection. Inclusion criteria consist of adult female patients with clinically stable MS for ≥ 3 months before screening and an Expanded Disability

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Status Score (EDSS) ≤ 6.5 with lower urinary tract symptoms (urinary frequency or urgency) who previously have undergone a clinic urodynamic screening and are refractory to conservative and oral pharmacological management for neurogenic detrusor over activity and are interested in OnaBotA intradetrusor injection, will be invited to participate in the study. Men will be excluded from the study to avoid confounding by prostate pathology. (Table 1) Other exclusion criteria include: severe debilitating disease, pregnancy or planning to become pregnant, nursing, MRI contraindications, history of stress urinary incontinence surgery (e.g. sling, urethral suspension), history of augmentation cystoplasty, and presence of other neurological diseases. Patients with active UTI can be treated and subsequently screened to determine their participation in this study.(Table 1) A healthy female cohort described in a previous report will be used as controls.[24]

Patient recruitment is performed at the Houston Methodist Neurourology Clinic in Houston, Texas, USA by three fulltime fellowship-trained Urologist in the field of Neurourology and Pelvic Reconstructive Surgery who work closely with MS Neurologist from all institutions in the Texas Medical Center. RK serves as the current clinical director of the Neurourology clinic and her practice is focused on patients with neurogenic bladder and pelvic reconstruction surgery in males and females. Patients will be recruited in our clinic and assessed by RK. Recruitment will be initiated in March 2016 and will conclude when we have data from 30 patients.

Inclusion	Exclusion
18 years or older	Non-ambulatory
Female	Severe debilitating disease (MS)
Patients with stable MS ≥ 3 months before screening	Urinary Tract Infection

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Neurogenic detrusor overactivity	Positive Pregnancy Test
LUTS (urinary frequency, urgency)	Contraindication for MRI (i.e. pacemaker, metal implants)
Positive clinic UDS for neurogenic detrusor over activity	Male
Refractory to conservative and oral pharmacotherapy	History of Incontinence Surgery (i.e sling, MMK, Burch)
	History of LUT surgery (i.e. urethral dilation)
	Other Neurologic diseases (i.e. spinal cord injury, myelomeningiocele, Parkinson disease)

Evaluation

Each patient will provide a detailed history and undergo a complete physical examination. Each will complete the following questionnaires and assessments: Expanded Disability Status Scale and Incontinence Quality of Life (I-QOL), Patient Perception of Bladder Control (PPBC), Bladder Control Scale (BLCS), Pelvic Organ Prolapse Distress Inventory 6 (POPDI-6), Demographic Form, and MRI Safety Screening Questionnaire. Post voidal residual volume will be measured and a urine sample will be obtained for urinalysis and neuropeptide evaluation. A baseline video urodynamic study will be obtained within a year prior to the baseline neuroimaging scan. Consent and enrollment agreement will be signed during the first visit. (See Figure 1) Patients will be seen a total of 4 times: initial visit, fMRI control and OnaBotA injection, follow-up in the clinic at 2 and 6 weeks following OnaBotA treatment for

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symptom evaluation, urinalysis and post voidal residual volume. All questionnaires will be completed after OnaBotA treatment as well as a simple patient reported perception of their treatment benefit using a 4-point treatment based scale (TBS). If there are clinical signs of increased post voidal residual volume or urinary retention, clean intermittent catheterizations (CIC) will be initiated.

Simultaneous Urodynamic testing and fMRI scanning

Prior to entering the MRI room all patients will answer the institutional standard clinical questionnaire for MRI safety and excluded from the study if contraindication to the MRI scan are found. A patient will be asked to wear a gown to avoid image artifacts through ferromagnetic objects in their clothing. Prior to the study, the patient will be asked to void, and post voidal residual (PVR) volume will be measured through catheterization. Double lumen 7 Fr MRI-compatible catheters will be placed in the bladder and rectum. A Phillips Ingenia 3.0T full body MRI scanner with standard 12 channel head coil will be used. Instructions to communicate using right hand signals representing “strong desire”, “voiding”, and “voiding completed” are given to the patient before imaging. To avoid auditory or other extensive brain stimulation not connected to the voiding process, simple signs will be shown to the patient when filling of the bladder is begun and when filling is stopped. Also, in order to keep our noise-to-signal ratio low, all stimulators including any extra visual stimuli and the urodynamic machine will be removed from the MRI scanner room. Total scan times are limited to 45 minutes. After acquisition of a high-resolution 3D brain scan for anatomical reference (turbo fast field, echo, isotropic 3D resolution of 0.7 mm), echo-planar (EPI) scans (repetition time 3 seconds, in-plane resolution 3.4 mm, slice thickness 4 mm) during two functional tasks are performed to record the BOLD signal. First, instruction signals from the patients’ right hand (Tasks) are performed without actual voiding

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and scanned separately to obtain a baseline for fMRI activation caused from signaling alone. Afterwards, urodynamic testing and the voiding-task are simultaneously initiated. The bladder is filled at 50mL/min with room-temperature, sterile, normal saline solution until the subject indicates a strong desire to void. Filling will be stopped and subjects will be asked to hold the urine for 30 seconds. Afterwards, patients will be allowed to void into absorbent pads on the scanner table. To create a block-design fMRI task, this sequence will be repeated up to 4 times. Final catheterizable PVR volume will be recorded. This procedure will be performed before treatment and 6-10 weeks after intradetrusor injection of OnaBotA. (See Figure 2)

Intradetrusor OnaBotA *injection*

During the second visit, the patient will have a cystoscopy with intradetrusor injection of OnaBotA performed by RK. One hundred (in spontaneous voiders) or 200 (CIC dependent patients) units of OnaBotA will be injected throughout the bladder, including the trigone. Patients will be asked to void to obtain uroflow, if they are spontaneous voiders, and post void residual volume will be measured.

Data Analysis and Statistics

Power Analysis

Careful application of power calculation is done in the early phases of designing a study to prevent spending time and money on an experiment that is under powered. This is especially important when more costly studies such as the current one are being designed. However, various and limited methods exist to calculate the fMRI power.

A study from 2002 presented an approach for group analysis in a fMRI model while accounting for intra and inter-subject variability. They concluded that approximately 12 subjects

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were required to achieve 80% power at the single voxel level for typical activations. [29] However, at more realistic thresholds after correcting for multiple comparisons, 24 subjects should be used to maintain this level of power. A study by Murphy et al performed an empirical investigation in data of 58 subjects with go/nogo studies. The correlation coefficients were calculated within the regions of interest defined by the 58-subject map threshold at the strictest P value of 0.000001 were there was a 0.8 correlation when 24 subjects were included. [30] To estimate the power of this study we would take into account the 10% exclusions for movement artifacts and 20% dropout rates where patients may not return for post OnaBotA scans and we concluded to study a total of 30 patients. The study will end when the 30th patient is scanned and evaluated after OnaBotA injection.

Three dimensional structural images will be obtained from a T1-weighted sequence; (sagittal direction, 0.7 in-plane resolution). Functional images will be collected afterwards by means of simultaneous urodynamic analysis (axial echo-planar, TR= 3,000ms, 4.0mm slice thickness, 3.38mm in-plane resolution). AFNI software (NIMH, https://afni.nimh.nih.gov/afni/) will be used for the fMRI BOLD analysis. Structural and functional images will be registered to each other. The EPI fMRI images will be motion-corrected. Translational and rotational motion extent will be quantified in this step. Patients with large motion (< 4.5 mm) will be excluded from analysis. Voxel activation will be identified at the time of "strong desire to void" and "the initiation voiding"; and BOLD fMRI activation maps will be calculated for both conditions using standard analysis with the generalized linear model (GLM). Group analysis will be performed by transforming data into Talairach space, and significantly activated voxels will be identified using a Student's T-test. Comparisons will be drawn between MS subjects comparing baseline scans to

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the post OnaBotA treatment. Subgroup analysis will be performed comparing subjects with and without Detrusor Sphincter Dysinergia (DSD). We will use SPSS (v19.0) to perform statistical analysis of clinical data (i.e. Demographic, UDS parameters).

Functional Connectivity analysis will be performed using CONN (NITRC https://www.nitrc.org/projects/conn,), a functional connectivity toolbox for MATLAB57, Version 15a, which utilizes the statistical parametric mapping (SPM) MATLAB toolbox. fMRI images acquired during the voiding task together with their corresponding anatomical datasets will be processed using the default-mni preprocessing option to enable group analysis in Montreal Neurological Institute (MNI) space. A region-based connectivity analysis will be performed for each patient group pre and post OnaBotA injection based on regions of interest (ROI, n=4) defined in a brain atlas available from the toolbox. Functional connectivity (FC) is the connectivity between brain regions that share functional properties. It can be defined as the temporal correlation between spatially remote neurophysiological events assessed by their respective BOLD signal time courses. FC will be quantified by T-values for a p-value < 0.05 (two-sided, FDR-corrected).

DTI images will be acquired (32 directions, one B0 image) using the standard MRI pulse sequence available on the Philips 3.0 T Ingenia scanner. The original Diffusion Tensor Imaging (DTI) images as well as the fractional anisotropy (FA) and mean diffusivity (MD) calculated on the scanner will be transferred to on offline workstation for further processing. The software packages TackVis (version 0.6.0.1) and the Diffusion Toolkit (version 0.6.3, trackvis.org) will be used to calculate and extract selected white matter tracts of interest. This software also enables calculating FA and MD values for the segmented white matter tracts.

Safety

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Every staff member involved in the collection of data and handling of the patient will be trained and evaluated regarding MRI safety before being allowed to enter the scanner room. Pregnancy tests and urinalysis will exclude patients who are pregnant or have a urinary tract infection. For the latter, after successful treatment of urinary tract infection, patients will be rescheduled.

Limitations

Since this study protocol focus on a specific neurogenic bladder patient population these findings cannot be generalized to all patients with neurogenic bladder. Also, given that MS produces multifocal lesions in the brain, not all of the patients will have the same lesions in the brain or spinal cord and symptom severity will vary from patient to patient. This limitating factors will be thoroughly described when we present our results in a future manuscript submission.

ETHICS AND DISSEMINATION

Approval of the institutional review board (Houston Methodist Research Institute) will be obtained for all study procedures. Informed consent will be obtained before enrolling a patient in the study. Our informed consent states that participation is completely voluntary and patients can withdraw at any point and this will not affect their relationship with the physician and their treatment course. All staff members involved in the collection of data and handling of patients will have proper privileges and training by our Research Institute and MRI Core. Before proceeding with fMRI testing, patients will be asked to remove all clothing and items containing

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metal. All materials used during the fMRI/UDS scans are MRI compatible. Subjects will be provided with gowns during the imaging study. No minor or vulnerable individuals will be recruited for the study. If an adverse effect occurs, the principal investigator and appropriate authorities will be informed and proper actions will be taken based on Good Clinical Practice. Outcomes of this research will be presented at national and international meetings and published in scholarly journals.

Author Contribution Statement

Rodolfo A. Elizondo and Christof Karmonik contributed to writing and revision of the manuscript.

Timothy B. Boone and Rose Khavari contributed to the study design and manuscript revision.

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Conflict of Interest Statement

None of the authors have conflict of interest.

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REFERENCES

1 de Sèze M, Ruffion A, Denys P, *et al.* The neurogenic bladder in multiple sclerosis: review of the literature and proposal of management guidelines. *Mult Scler Houndmills Basingstoke Engl* 2007;**13**:915–28. doi:10.1177/1352458506075651

2 Phé V, Chartier-Kastler E, Panicker JN. Management of neurogenic bladder in patients with multiple sclerosis. *Nat Rev Urol* 2016;**13**:275–88. doi:10.1038/nrurol.2016.53

3 Wein AJ. *Pathophysiology and Classification of Lower Urinary Tract Dysfunction: Overview, In: Campbel-Welsh: Urology.* US: : Elsevier Health Science 2016.

4 Fowler CJ, Panicker JN, Drake M, *et al.* A UK consensus on the management of the bladder in multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2009;**80**:470–7. doi:10.1136/jnnp.2008.159178

5 Wein AJ, Dmochowski RR. *Neuromuscular Dysfunction of the Lower Urinary Tract, In: Campbel-Welsh: Urology.* US: : Elsevier Health Science 2016.

6 Buser N, Ivic S, Kessler TM, *et al.* Efficacy and adverse events of antimuscarinics for treating overactive bladder: network meta-analyses. *Eur Urol* 2012;**62**:1040–60. doi:10.1016/j.eururo.2012.08.060

7 Leone Roberti Maggiore U, Salvatore S, Alessandri F, *et al.* Pharmacokinetics and toxicity of antimuscarinic drugs for overactive bladder treatment in females. *Expert Opin Drug Metab Toxicol* 2012;**8**:1387–408. doi:10.1517/17425255.2012.714365

8 Gormley EA, Lightner DJ, Faraday M, *et al.* Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline amendment. *J Urol* 2015;**193**:1572–80. doi:10.1016/j.juro.2015.01.087

9 Yamaguchi O, Nishizawa O, Takeda M, *et al.* Clinical guidelines for overactive bladder. *Int J Urol* 2009;**16**:126–42. doi:10.1111/j.1442-2042.2008.02177.x

10 Munoz A, Somogyi GT, Boone TB, *et al.* Central inhibitory effect of intravesically applied botulinum toxin A in chronic spinal cord injury. *Neurourol Urodyn* 2011;**30**:1376–81. doi:10.1002/nau.21068

11 Khera M, Somogyi GT, Salas NA, *et al.* In vivo effects of botulinum toxin A on visceral sensory function in chronic spinal cord-injured rats. *Urology* 2005;**66**:208–12. doi:10.1016/j.urology.2005.01.055

12 Smith CP, Vemulakonda VM, Kiss S, *et al.* Enhanced ATP release from rat bladder urothelium during chronic bladder inflammation: effect of botulinum toxin A. *Neurochem Int* 2005;**47**:291–7. doi:10.1016/j.neuint.2005.04.021

13 Apostolidis A, Jacques TS, Freeman A, *et al.* Histological changes in the urothelium and suburothelium of human overactive bladder following intradetrusor injections of botulinum neurotoxin type A for the treatment of neurogenic or idiopathic detrusor overactivity. *Eur Urol* 2008;**53**:1245–53. doi:10.1016/j.eururo.2008.02.037

14 Cheng CL, Liu JC, Chang SY, *et al.* Effect of capsaicin on the micturition reflex in normal and chronic spinal cord-injured cats. *Am J Physiol* 1999;**277**:R786–94.

15 Chai TC, Birder LA. *Physiology and Pharmacology of the Bladder and the Urethra*. US: : Elsevier Health Science 2016.

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16 Griffiths DJ, Fowler CJ. The micturition switch and its forebrain influences. *Acta Physiol Oxf Engl* 2013;**207**:93–109. doi:10.1111/apha.12019
17 Kuhtz-Buschbeck JP, Gilster R, van der Horst C, *et al.* Control of bladder sensations: an fMRI study of brain activity and effective connectivity. *NeuroImage* 2009;**47**:18–27. doi:10.1016/j.neuroimage.2009.04.020
18 Kuhtz-Buschbeck JP, van der Horst C, Wolff S, *et al.* Activation of the supplementary motor area (SMA) during voluntary pelvic floor muscle contractions--an fMRI study. *NeuroImage* 2007;**35**:449–57. doi:10.1016/j.neuroimage.2006.12.032
19 Kuhtz-Buschbeck JP, van der Horst C, Pott C, *et al.* Cortical representation of the urge to void: a functional magnetic resonance imaging study. *J Urol* 2005;**174**:1477–81.
20 Griffiths D, Clarkson B, Tadic SD, *et al.* Brain Mechanisms Underlying Urge Incontinence and its Response to Pelvic Floor Muscle Training. *J Urol* 2015;**194**:708–15. doi:10.1016/j.juro.2015.03.102
21 Panicker JN, Fowler CJ, Kessler TM. Lower urinary tract dysfunction in the neurological patient: clinical assessment and management. *Lancet Neurol* 2015;**14**:720–32. doi:10.1016/S1474-4422(15)00070-8
22 Wintner A, Kim MM, Bechis SK, *et al.* Voiding Dysfunction in Multiple Sclerosis. *Semin Neurol* 2016;**36**:219–20. doi:10.1055/s-0036-1582255
23 Krhut J, Tintera J, Holý P, *et al.* A preliminary report on the use of functional magnetic resonance imaging with simultaneous urodynamics to record brain activity during micturition. *J Urol* 2012;**188**:474–9. doi:10.1016/j.juro.2012.04.004
24 Shy M, Fung S, Boone TB, *et al.* Functional magnetic resonance imaging during urodynamic testing identifies brain structures initiating micturition. *J Urol* 2014;**192**:1149–54. doi:10.1016/j.juro.2014.04.090
25 Borghesi G, Simonetti R, Goldman SM, *et al.* Magnetic resonance imaging urodynamics. Technique development and preliminary results. *Int Braz J Urol Off J Braz Soc Urol* 2006;**32**:336–41; discussion 341.
26 Di Gangi Herms AMR, Veit R, Reisenauer C, *et al.* Functional imaging of stress urinary incontinence. *NeuroImage* 2006;**29**:267–75. doi:10.1016/j.neuroimage.2005.07.018
27 Khavari R, Karmonik C, Fletcher S, et al. Functional Magnetic Resonance Imaging with Concurrent Urodynamic Testing Identifies Brain Structures Involved in Micturition Cycle in Patients with Multiple Sclerosis. *J Urol* 2016.doi:10.1016/j.juro.2016.09.077
28 Antunes-Lopes T, Pinto R, Barros SC, *et al.* Urinary neurotrophic factors in healthy individuals and patients with overactive bladder. *J Urol* 2013;**189**:359–65. doi:10.1016/j.juro.2012.08.187
29 Desmond JE, Glover GH. Estimating sample size in functional MRI (fMRI) neuroimaging studies: statistical power analyses. *J Neurosci Methods* 2002;**118**:115–28.
30 Murphy K, Garavan H. An empirical investigation into the number of subjects required for an event-related fMRI study. *NeuroImage* 2004;**22**:879–85. doi:10.1016/j.neuroimage.2004.02.005

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Figure Legends

Figure 1. Schematic representation of First, Second and Third clinic visit where data will be collected. Fourth clinic visit at 6 weeks will not include fMRI scan.

Figure 2. Flow chart of fMRI and simultaneous urodynamic study.

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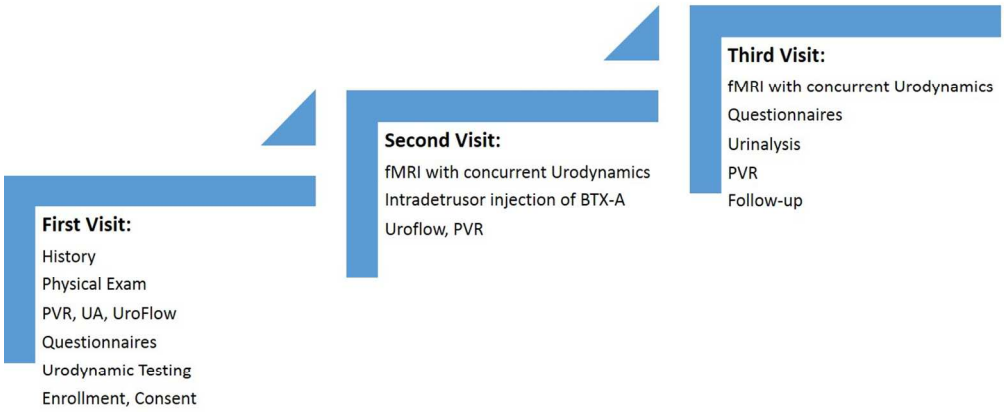

Figure 1. Schematic representation of First, Second and Third clinic visit where data will be collected. Fourth clinic visit at 6 weeks will not include fMRI scan.

Figure 1
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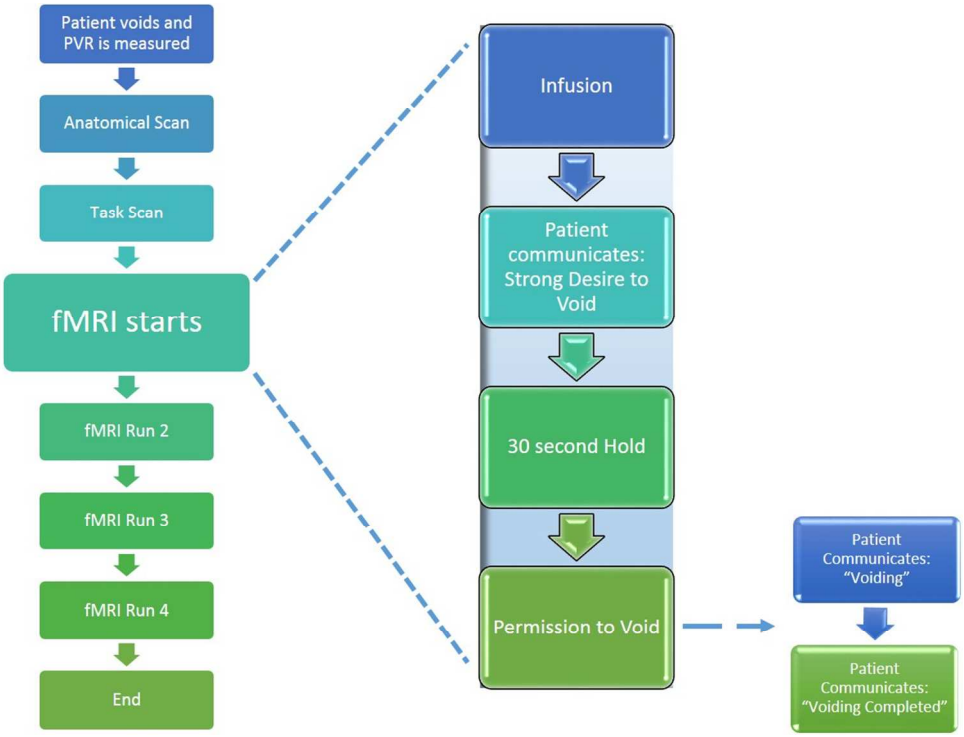

Figure 2. Flow chart of fMRI and simultaneous urodynamic study.

Figure 2
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Inclusion	Exclusion
18 years or older	Non-ambulatory
Female	Severe debilitating disease (MS)
Patients with stable MS ≥ 3 months before screening	Urinary Tract Infection
Neurogenic detrusor overactivity	Positive Pregnancy Test
LUTS (urinary frequency, urgency)	Contraindication for MRI (i.e. pacemaker, metal implants)
Positive clinic UDS for neurogenic detrusor over activity	Male
Refractory to conservative and oral pharmacotherapy	History of Incontinence Surgery (i.e sling, MMK, Burch)
	History of LUT surgery (i.e. urethral dilation)
	Other Neurologic diseases (i.e. spinal cord injury, myelomeningiocele, Parkinson disease)

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *case-control studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2 & 3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4 – 7
Objectives	3	State specific objectives, including any prespecified hypotheses	8
Methods			
Study design	4	Present key elements of study design early in the paper	8 – 11
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	9 & 10
Participants	6	*(*a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	8
		(*b*) For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	12 – 14
Bias	9	Describe any efforts to address potential sources of bias	15
Study size	10	Explain how the study size was arrived at	12 & 13
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	12 – 14
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how matching of cases and controls was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

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Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	NA
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	NA
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	NA
		(*b*) Report category boundaries when continuous variables were categorized	NA
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	NA
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	NA
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	NA
Generalisability	21	Discuss the generalisability (external validity) of the study results	NA
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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