Protocol for a prospective neuro-imaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with and without lower urinary tract symptoms

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Protocol for a prospective neuro-imaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with and without lower urinary tract symptoms

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ABSTRACT

Introduction: Lower urinary tract symptoms (LUTS) are highly prevalent, cause an enormous economic burden on health care systems, and significantly impair quality of life (QoL) of affected patients. The dependence of the lower urinary tract (LUT) on complex central neuronal circuits makes it unique in comparison to other visceral functions, such as the gastrointestinal tract, but also more vulnerable to neurological diseases.

Methods & analysis: This is a prospective neuro-imaging study investigating the supraspinal control of LUT function in healthy controls and patients with and without LUTS.
The clinical assessment will include medical history, neuro-urological examination, bladder diary, urine analysis, urodynamic investigations, as well as standardized questionnaires regarding LUTS and QoL.
The acquisition of neuro-imaging data will include structural assessments with diffusion tensor imaging (DTI) and voxel-based morphometry (VBM) as well as functional investigations using blood-oxygen-level-dependent (BOLD) sensitive functional magnetic resonance imaging (fMRI) in a 3T magnetic resonance (MR) scanner. The fMRI will be performed during four different bladder tasks. The first three tasks will consist of automated, repetitive filling of 100mL warm (37°C) saline starting with (1) an empty bladder, (2) a low prefilled bladder volume (100mL), and (3) a high prefilled bladder volume (until a persistent desire to void). The forth task will comprise of automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder.

Ethics & dissemination:
The local ethics committee approved this study (KEK-ZH-Nr. 2011-0346). The findings of the study will be published in peer-reviewed journals and presented at national and international scientific meetings.

Trial registration:
This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

ARTICLE SUMMARY

Study focus

- To demonstrate correlations between alterations in brain structural and functional connectivity in relation to LUT control and LUTS, i.e. urgency with or without urgency urinary incontinence, and urinary frequency.
- To compare clinical correlates of treatment responses in patients receiving treatment for LUTS with the associated changes in brain activity and connectivity.

Key messages

- Although recent neuro-imaging studies in humans and neuro-physiological animal studies have contributed to establish concepts of spinal and supraspinal LUT control, the exact pathophysiological mechanisms involved in human LUTS are still largely unknown.
- Multimodal neuro-imaging approach has considerable potential to broaden our knowledge of the supraspinal LUT control in contrast to the current concepts.

Strengths and limitations of this study

- This will be the first study to correlate findings from structural and functional MRI techniques with clinical measurements to identify abnormalities in brain networks responsible for the supraspinal control of LUT function in healthy controls and patients with and without LUTS.
- Correlations revealed by analysing neuro-imaging data in regard to the clinical context will establish functional biomarkers in patients with and without LUTS.

INTRODUCTION

Lower urinary tract symptoms (LUTS) are highly prevalent, i.e. about 11% in the worldwide population in 2008, and forecasted to increase up to 20% until 2018.[1 2] Moreover, LUTS cause an enormous economic burden on each health care systems,[3 4] i.e. comparable to diabetes mellitus,[5] and significantly impair the quality of life (QoL) of affected patients.[6 7]

For a proper functioning, lower urinary tract (LUT) structures, i.e. bladder, bladder neck, urethra, and urethral sphincter, rely on intact neuronal innervations that are under control of a complex supraspinal network.[8-10] The dependence of the LUT on such complex central neuronal circuits makes it unique in comparison to other visceral functions, e.g. gastrointestinal tract, but also more vulnerable to neurological diseases.[10]

Recent neuro-imaging studies have shown that patients with neurological disorders such as Parkinson's disease (PD)[11-13] and spinal cord injury (SCI)[14] demonstrate different supraspinal activity patterns compared to healthy controls in response to LUT stimulation tasks, which might represent a neural correlate to their LUTS.

Although there are several concepts regarding the human LUT function and neuronal control in normal and pathological conditions, the exact pathophysiological mechanisms involved remain largely unknown.[9] As brain and brainstem are crucial for voluntary LUT control [10 15 16], investigation of the supraspinal regions with high-resolution imaging techniques, i.e. functional magnetic resonance imaging (fMRI), can significantly contribute to increase our understanding on the effects of supraspinal lesions and alterations related to LUTS.[10 17]

In this study, we are aiming to identify supraspinal areas associated with LUT control in healthy controls and in patients with and without LUTS. Blood-oxygen-level-dependent (BOLD) fMRI, resting-state fMRI (RS-fMRI)[18], voxel-based morphometry (VBM)[19], diffusion tensor imaging (DTI)[20], functional connectivity (FC) analysis, and structural integrity evaluation of white matter tracts and gray matter concentration will enable us to identify specific alterations of the supraspinal LUT control to better understand the relevant components and dysfunctions of this network.

METHODS AND ANALYSIS

Study design

This prospective research study will be conducted at the University of Zürich, Zürich, Switzerland.

Study population & recruitment

According to the inclusion and exclusion criteria (Table 1), healthy controls with an unimpaired LUT function and patients with and without LUTS will be investigated.

Patients will be recruited from our own department (Neuro-Urology, Balgrist University Hospital, Zürich) and through our partners at the University Hospital Zürich and the Triemli Hospital Zürich. Eligible patients and healthy controls will be invited to a first visit (screening) during which detailed information about the study, in particular the aims, methods, possible risks, and side effects, will be given. After obtaining written informed consent, the following data will be collected: medical history, a 3-day bladder diary, urine sample to exclude urinary tract infection (UTI) and pregnancy in female subjects, urodynamic parameters, and post void residual measured by ultrasound as well as standardized questionnaires regarding LUTS and QoL. The validated German versions of these questionnaires will be used with permission of the International consultation on incontinence modular questionnaire [(ICIQ), Bristol Urological Institute, Southmead Hospital Bristol, UK], and will address the LUTS (ICIQ-LUTS) in both females (ICIQ-FLUTS) and males (ICIQ-MLUTS) whereas the ICIQ-LUTSQoL will display the QoL in regard to LUTS.

Determination of sample size

Considering our earlier studies[14 21], which provided statistical evidence using small collectives, i.e. 14 and 15 subjects respectively, we estimated the sample size for each group: healthy controls (20), multiple sclerosis (MS) patients with or without LUTS (each 15) and patients with LUTS, but without any underlying neurological disease (20). Other studies confirmed this sample size to enable statistically significant results.[22 23]

Study location

- Neuro-Urology, Spinal Cord Injury Centre & Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland
- MR-Centre, University Hospital Zürich, Zürich, Switzerland

Partners

- NeuroRadiology Clinic, University Hospital Zürich, Zürich, Switzerland
- Departments of Urology, Neurology, and Gynaecology, University Hospital Zürich, Zürich, Switzerland
- Department of Urology and Gynaecology, Triemli Hospital, Zürich, Switzerland

Investigations

Following screening and study inclusion, all subjects will be scheduled for the 2nd and 3rd visits (1st and 2nd fMRI measurement) at the MR-Centre. Patients with LUTS might return for a 4th visit (3rd fMRI measurement) after receiving treatment for LUTS (Figure 1).

MR scanning will be performed using a Philips Ingenia 3.0 Tesla MR scanner (Philips Medical Systems, Best, The Netherlands) with an 8-element head coil. The scan will start with structural MRI including DTI, VBM, and then RS-fMRI. After placement of a transurethral catheter, RS-fMRI will be repeated. Subsequently BOLD fMRI will be acquired to investigate the supraspinal activity during four different bladder tasks (Figure 2). In the first three fMRI tasks, we will examine the effect of visceral bladder sensation by automated, repetitive filling with 100mL warm (37°C) saline starting with (1) an empty bladder, (2) a low prefilled bladder volume (100mL), and (3) a high prefilled bladder volume (until a persistent desire to void). The forth task will consist of automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder to investigate the neural correlates of cold bladder sensation (Figure 3). Subjects will rate their urge to void and also the level of pain using a displayed visual analogue scale (VAS) and an fMRI-compatible, multi-configurable handheld response system.[24]

Safety

The staff involved in this study will be instructed and trained according to the safety regulations of the MR-Centre of the University Hospital Zürich. All subjects will be asked to remove any ferromagnetic items, e.g. bra, earrings, chains, rings, and piercings prior to entering the scanner room. All subjects will be provided with standardised clinical scrubs instead of wearing their own clothes. Before every MR-scan, urine samples will be analysed from every subject in order to exclude UTI or pregnancy. In case of pregnancy, the subject will be excluded from the study and referred to a gynaecologist. In case of UTI, the subject will not undergo the experiment, but will receive immediate antibiotic treatment if the UTI is symptomatic or treatment depending on further microbiological urine analysis in the absence of UTI symptoms. The subject can be reassigned to the study, if the microbiological urine analysis shows no evidence of an UTI or the UTI has been successfully treated.

In the situation of an adverse event (AE) or a severe adverse event (SAE), as defined by the international conference on harmonisation (ICH) good clinical practice (GCP) guidelines (E6)[25] and international organization for standardization (ISO, 14155)[26], appropriate actions will be executed and the according body (principle investigator, ethics committee) will be informed. All AEs and SAEs will be followed as long as medically indicated.

Study outcome measures

Primary

BOLD signal intensity changes in supraspinal regions of interest (ROI), e.g. pons, insula, anterior cingulate cortex, thalamus, hypothalamus, supplementary motor area, and prefrontal cortex, in relation to the according fMRI tasks during two (healthy controls and patients without LUTS) or three (patients with LUTS) visits.

Secondary

To analyse structural (SC) and functional connectivity (FC) between supraspinal ROI and to identify specific alterations, i.e. changes of BOLD signal (RS-fMRI), white matter fibre structure (DTI), and gray matter concentration (VBM) data, in patients with LUTS compared to healthy controls and patients without LUTS.

Furthermore, factors like age, bladder volume, urodynamic parameters and level of urge to void during fMRI, will be used as regressors for statistical analysis of SC and FC.

Data analysis

Clinical data will be statistically analysed and compared between groups using IBM's Statistical Package for the Social Sciences (SPSS) version 19.0 (Armonk, New York, U.S.). Urodynamic parameters, urine volume measurement including post void residual (ultrasound), and feedback rating will be presented with means and standard deviations or with medians and interquartile ranges as appropriate. Association of individual (independent) variables on the outcome variables will be reported using correlation coefficients. Results from univariate analysis will inform multivariate modelling. Assessment of causal associations will be performed using multivariate models including potential confounders along with the independent variables of interest.

The neuro-imaging data will be analysed using statistical parametric mapping (SPM) version 8 (Wellcome Department of Imaging Neuroscience, University College London, UK) and toolboxes (e.g. Diffusion II and VBMtools).

ETHICS AND DISSEMINATION

This cohort study will be performed in accordance to the World Medical Association Declaration of Helsinki[27] and the guidelines of the Swiss Academy of Medical Sciences.[28]

This study has been approved by the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH-Nr. 2011-0346). Furthermore, handling of all personal data will strictly comply with the federal law of data protection in Switzerland.[29]

This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

DISCUSSION

This study will investigate supraspinal LUT control in healthy subjects and patients with and without LUTS using a multimodal imaging protocol including fMRI, DTI, VBM, and FC analysis. In addition, effects on the supraspinal LUT control after treatment for LUTS will be investigated. It is expected that this study will provide new insights into the supraspinal neuronal mechanisms and networks responsible for LUT control. The findings will help to verify, amend, or adjust neuronal circuitry models established from findings in healthy subjects, now in the context of patients with or without LUTS. Particularly, the use of newer imaging and evaluation techniques like RS-fMRI, DTI, VBM, and FC analysis have the potential to serve as quantifiable biomarkers for therapy success and provide evidence for non-responders of LUTS treatment.

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Contributors

All authors participated in creating the study design.

MW and UM drafted the manuscript.

LM, SK, PEVK and TMK critically reviewed the manuscript.

UM, SK and TMK obtained the funding of this study.

All the authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Groups	Inclusion criteria	Exclusion criteria
All subjects	▪ Right-handed ▪ Gender (female and male) ▪ Age limits: 18-55 years ▪ MR suitability ▪ Written informed consent	▪ Pregnancy or breast feeding ▪ Any craniocerebral injury or surgery ▪ Any permanent ferromagnetic implant ▪ Any previous surgery of LUT / genitalia ▪ Any anatomical anomaly of LUT / genitalia ▪ Any LUT malignancy ▪ PVR > 150mL

Healthy controls	▪ Unimpaired LUT function
	▪ No LUTS (3-day bladder diary)
	  ▪ ≤ 3 episodes of urinary urgency / week
	  ▪ Urinary frequency < 8 / 24h
		▪ Impaired LUT function
		▪ UTI
		▪ Any LUTS (3-day bladder diary)
		  ▪ ≥ 3 episodes of urinary urgency / week
		  ▪ Urinary frequency > 8 / 24h

MS patients without LUTS	▪ Diagnosis of MS according to the McDonald criteria
	▪ EDSS ≤ 6
	▪ No LUTS (3-day bladder diary)
	  ▪ ≤ 3 episodes of urinary urgency / week
	  ▪ Urinary frequency < 8 / 24h
		▪ Any neurological or psychological disease other than MS
		▪ Any metabolic disease
		▪ Any concomitant treatment for the LUT (e.g. neuromodulation)
		▪ SUI
		▪ UTI
		▪ Any LUTS (3-day bladder diary)
		  ▪ ≥ 3 episodes of urinary urgency / week
		  ▪ Urinary frequency > 8 / 24h

MS patients with LUTS	▪ Diagnosis of MS according to the McDonald criteria
	▪ EDSS ≤ 6
	▪ LUTS > 6 months (3-day bladder diary)
	  ▪ ≥ 3 episodes of urinary urgency / week
	  ▪ Urinary frequency > 8 / 24h
		▪ Any neurological or psychological disease other than MS
		▪ Any metabolic disease
		▪ Any concomitant treatment for the LUT (e.g. neuromodulation)
		▪ SUI
		▪ UTI
		▪ Any condition other than MS that might explain LUTS
		▪ Indwelling catheters or necessity to perform ISC

Patients with non-neurogenic LUTS	▪ LUTS > 6 months (3-day bladder diary)
	  ▪ ≥ 3 episodes of urinary urgency / week
	  ▪ Urinary frequency > 8 / 24h
		▪ Any neurological, psychological, metabolic, or cardiovascular disease
		▪ Any concomitant treatment for the LUT (e.g. neuromodulation)
		▪ SUI
		▪ UTI
		▪ Indwelling catheters or necessity to perform ISC

Table 1 – Inclusion and exclusion criteria for all subjects.

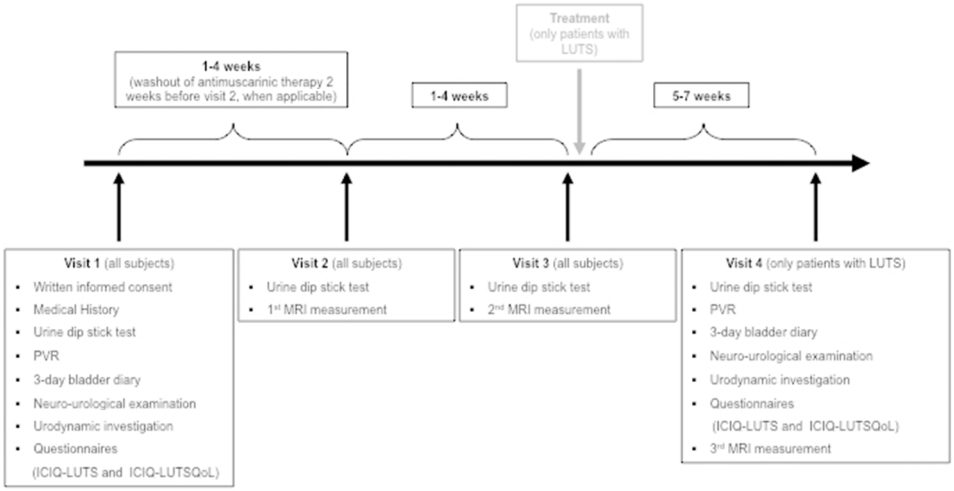
MR = Magnet resonance, LUT = Lower urinary tract, LUTS = LUTS symptoms, MS = Multiple sclerosis, EDSS = Expanded Disability Status Scale, PVR = Post void residual, UTI = Urinary tract infection, SUI = Stress urinary incontinence, ISC = Intermittent self-catheterization

Figure legends

Figure 1 – Timetable and characteristics of all four visits.
PVR = Post void residual, LUTS = Lower urinary tract symptoms, ICIQ = International consultation on incontinence modular questionnaire, QoL = Quality of life

Figure 2 – Schematic protocol of operational sequences of magnetic resonance imaging (MRI) measurements including functional MRI (fMRI): A) 1st and 3rd MRI measurement and B) 2nd MRI measurement.
VBM = Voxel-based morphometry, DTI = Diffusion tensor imaging, RS-fMRI = Resting-State fMRI

Figure 3 – Schematic diagram of the scan paradigm of four different functional magnetic resonance imaging (fMRI) tasks at visits 2, 3, and 4. All fMRI tasks consist of 8 repetitive blocks, each with either 6 (task 1 and 4) or 7 (task 2 and 3) conditions.
A) Conditions of the 1st fMRI task: 1) rest (no specific stimulus or task is performed), 2) automated infusion of 100mL warm (37°C) saline, 3) rating of desire to void and level of pain, 4) short rest, 5) passive withdrawal to empty the bladder completely, and 6) rest. This task starts with an empty bladder and will be performed in patients with LUTS without any underlying neurologic disease and in MS patients with LUTS at visits 2, 3, and 4 (every MRI measurement), whereas in healthy controls only at visit 3 (2nd MRI measurement).
B and C) Conditions of the 2nd and 3rd fMRI task: 1) rest, 2) automated infusion of 100 mL warm saline, 3) rating of desire to void and level of pain, 4) short rest, 5) automated withdrawal of 100mL, 6) rating of desire to void and level of pain, and 7) rest. The 2nd task starts with a low prefilled bladder volume (100mL) and will be performed in MS patients without LUTS and in healthy controls at visit 2 and 3 (1st and 2nd MRI measurement). The 3rd task starts with a high prefilled bladder volume (until a persistent desire to void) and will be performed in all subjects (patients and healthy controls) during visit 2 and 3 (1st and 2nd MRI measurement). Additionally, this task will be carried out in patients with LUTS without any underlying neurologic disease and MS patients with LUTS at visit 4 (3rd MRI measurement).
D) Conditions of the 4th fMRI task: 1) rest, 2) automated infusion of 100mL cold (4-8°C) saline, 3) rating of desire to void and level of pain, 4) short rest, 5) passive withdrawal to empty the bladder completely, and 6) rest. This task starts with an empty bladder and will be performed in all subjects (patients and healthy controls) during visit 2 and 3 (1st and 2nd MRI measurement). Additionally, this task will be executed in patients with LUTS without any underlying neurologic disease and MS patients with LUTS at visit 4 (3rd MRI measurement).



Timetable and characteristics of all four visits.
PVR = Post void residual, LUTS = Lower urinary tract symptoms, ICIQ = International consultation on incontinence modular questionnaire, QoL = Quality of life
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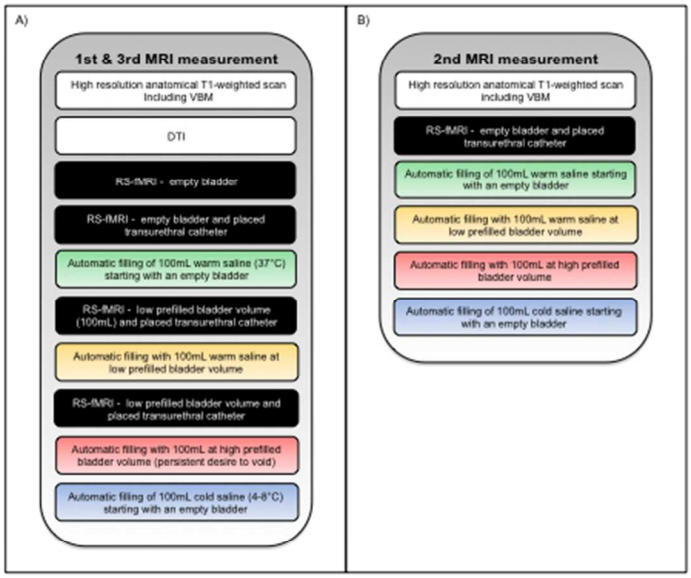
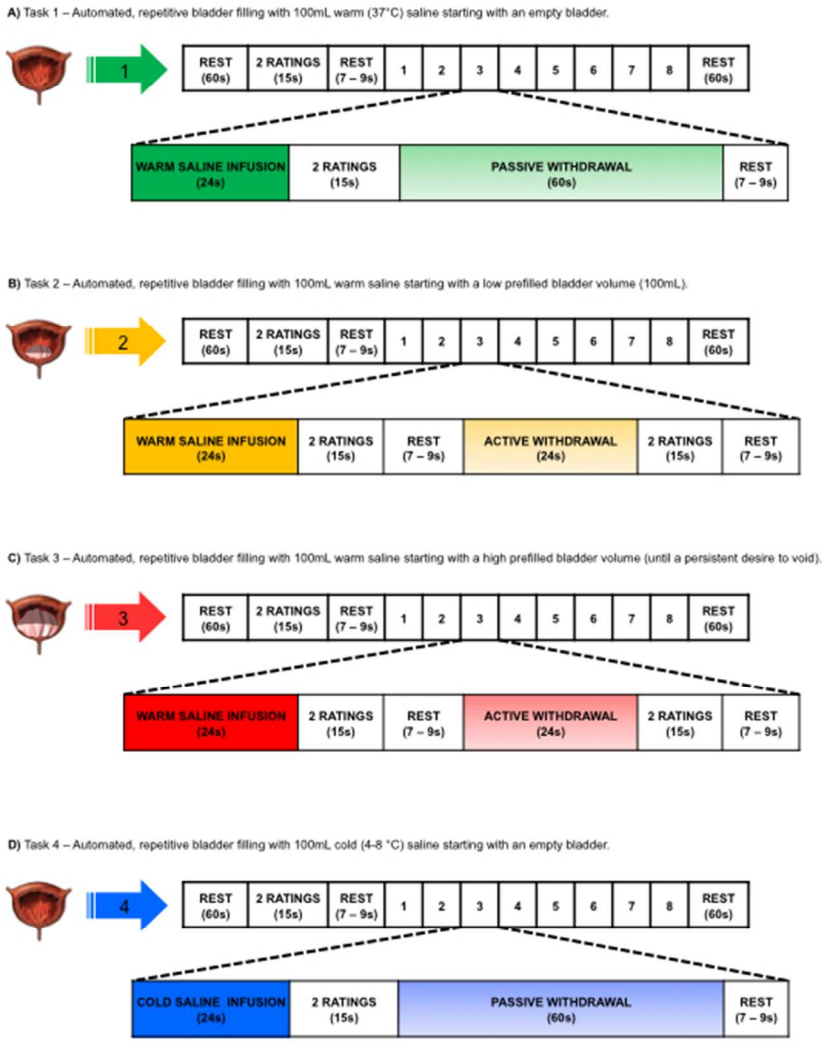


Schematic protocol of operational sequences of magnetic resonance imaging (MRI) measurements including functional MRI (fMRI): A) 1st and 3rd MRI measurement and B) 2nd MRI measurement.
VBM = Voxel-based morphometry, DTI = Diffusion tensor imaging, RS-fMRI = Resting-State fMRI
190x275mm (72 x 72 DPI)



Schematic diagram of the scan paradigm of four different functional magnetic resonance imaging (fMRI) tasks at visits 2, 3, and 4. All fMRI tasks consist of 8 repetitive blocks, each with either 6 (task 1 and 4) or 7 (task 2 and 3) conditions.

A) Conditions of the 1st fMRI task: 1) rest (no specific stimulus or task is performed), 2) automated infusion of 100mL warm (37°C) saline, 3) rating of desire to void and level of pain, 4) short rest, 5) passive withdrawal to empty the bladder completely, and 6) rest. This task starts with an empty bladder and will be performed in patients with LUTS without any underlying neurologic disease and in MS patients with LUTS at visits 2, 3, and 4 (every MRI measurement), whereas in healthy controls only at visit 3 (2nd MRI measurement).

B and C) Conditions of the 2nd and 3rd fMRI task: 1) rest, 2) automated infusion of 100 mL warm saline, 3) rating of desire to void and level of pain, 4) short rest, 5) automated withdrawal of 100mL, 6) rating of desire to void and level of pain, and 7) rest. The 2nd task starts with a low prefilled bladder volume (100mL) and will be performed in MS patients without LUTS and in healthy controls at visit 2 and 3 (1st and 2nd MRI

measurement). The 3rd task starts with a high prefilled bladder volume (until a persistent desire to void) and will be performed in all subjects (patients and healthy controls) during visit 2 and 3 (1st and 2nd MRI measurement). Additionally, this task will be carried out in patients with LUTS without any underlying neurologic disease and MS patients with LUTS at visit 4 (3rd MRI measurement).
D) Conditions of the 4th fMRI task: 1) rest, 2) automated infusion of 100mL cold (4-8°C) saline, 3) rating of desire to void and level of pain, 4) short rest, 5) passive withdrawal to empty the bladder completely, and 6) rest. This task starts with an empty bladder and will be performed in all subjects (patients and healthy controls) during visit 2 and 3 (1st and 2nd MRI measurement). Additionally, this task will be executed in patients with LUTS without any underlying neurologic disease and MS patients with LUTS at visit 4 (3rd MRI measurement).
190x275mm (72 x 72 DPI)

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ABSTRACT

Introduction: Lower urinary tract symptoms (LUTS) are highly prevalent, cause an enormous economic burden on health care systems, and significantly impair quality of life (QoL) of affected patients. The dependence of the lower urinary tract (LUT) on complex central neuronal circuits makes it unique in comparison to other visceral functions, such as the gastrointestinal tract, but also more vulnerable to neurological diseases.

Methods & analysis: This is a prospective neuro-imaging study investigating the supraspinal control of LUT function in healthy controls and patients with non-neurogenic LUTS.

The clinical assessment will include medical history, neuro-urological examination, bladder diary, urine analysis, urodynamic investigations, as well as standardized questionnaires regarding LUTS and QoL.

The acquisition of neuro-imaging data will include structural assessments (T1-weighted imaging and diffusion tensor imaging (DTI)) as well as functional investigations using blood-oxygen-level-dependent (BOLD) sensitive functional magnetic resonance imaging (fMRI) in a 3 Tesla MR scanner. The fMRI will be performed during four different bladder tasks using an automated MR-compatible and MR-synchronised pump system. The first three task-related fMRIs will consist of automated, repetitive filling of 100mL warm (37°C) saline starting with (1) an empty bladder, (2) a low prefilled bladder volume (100mL), and (3) a high prefilled bladder volume (persistent desire to void). The 4th task-related fMRI will comprise of automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder.

Ethics & dissemination:

The local ethics committee approved this study (KEK-ZH-Nr. 2011-0346). The findings of the study will be published in peer-reviewed journals and presented at national and international scientific meetings.

Trial registration:

This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

ARTICLE SUMMARY

Study focus

- To demonstrate correlations between alterations in brain structural and functional connectivity in relation to LUT control and LUTS, i.e. urgency with or without urgency urinary incontinence, and urinary frequency.
- To compare clinical correlates of treatment efficacy in patients with non-neurogenic LUTS with the associated changes in brain activity and connectivity.

Key messages

- Although recent neuro-imaging studies in humans and neuro-physiological animal studies have contributed to establish concepts of spinal and supraspinal LUT control, the exact pathophysiological mechanisms involved in human LUTS are still largely unknown.
- A multimodal neuro-imaging approach has considerable potential to broaden our knowledge of the supraspinal LUT control, and helps to adapt current concepts of LUT control.

Strengths and limitations of this study

- This will be the first study to identify brain networks of supraspinal LUT control in healthy subjects and abnormalities within such brain networks in patients with non-neurogenic LUTS using structural and functional MRI techniques in correlation with clinical measurements.
- Investigation of test-retest reliability which has not yet been performed for neuroimaging of LUT tasks. However, this is important especially in regard to interpretation of treatment effects.

INTRODUCTION

Lower urinary tract symptoms (LUTS) are highly prevalent, i.e. about 11% in the worldwide population in 2008, and forecasted to increase up to 20% until 2018.[1 2] Moreover, LUTS cause an enormous economic burden on each health care system,[3 4] i.e. comparable to diabetes mellitus,[5] and significantly impair the quality of life (QoL) of affected patients.[6 7]

For a proper functioning, lower urinary tract (LUT) structures, i.e. bladder, bladder neck, urethra, and urethral sphincter, rely on intact neuronal innervations that are under control of a complex supraspinal network.[8-10] The dependence of the LUT on such complex central neuronal circuits makes it unique in comparison to other visceral functions, e.g. gastrointestinal tract, but also more vulnerable to neurological diseases.[10]

Recent neuro-imaging studies have shown that patients with neurological disorders such as Parkinson's disease (PD)[11-13] and spinal cord injury (SCI)[14] demonstrate different supraspinal activity patterns compared to healthy controls in response to LUT stimulation tasks, which might represent a neural correlate to their LUTS.

Although there are several concepts regarding the human LUT function and neuronal control in normal and pathological conditions, the exact pathophysiological mechanisms involved remain largely unknown.[9] Despite the popularity of resting-state functional magnetic resonance imaging (RS-fMRI)[15-20] and diffusion tensor imaging (DTI)[21] in other fields in neuroscience, these techniques have not been applied in the context of supraspinal LUT control. There are only two DTI studies published in regard to LUT control in general: (1) a case report by Theadin et al.[22] studying spinal cord infarctions and clinical symptoms and (2) a prospective study by van der Jagt et al.[23] investigating architectural configuration and microstructural properties of the sacral plexus.

As cortical and sub-cortical (e.g. brainstem) brain regions are crucial for voluntary LUT control[10 24 25], investigation of the supraspinal regions with high-resolution imaging techniques, i.e. fMRI, can significantly contribute to increase our understanding on the effects of supraspinal lesions and alterations related to LUTS.[10 26]

In this study, we are aiming to identify supraspinal areas associated with LUT control in healthy subjects and in patients with non-neurogenic LUTS. Task-related blood-oxygen-level-dependent (BOLD) and RS-fMRI will be applied as well as structural MRI (T1-weighted MRI and DTI). Hence, we will examine if bladder processing is already altered on the structural level, and on baseline (RS-fMRI) functional connectivity. For example, the multiple repetition of the RS-fMRI will help to understand whether manipulation of sensory perception (induced by infusion and withdrawal) will alter the default mode network[20] of the brain. Furthermore, we can examine volumetric parameters (e.g. gray matter concentration) by voxel-based morphometry (VBM)[27], structural integrity and connectivity of white matter tracts (DTI) as well as functional connectivity (FC).

This unique and detailed multimodal imaging protocol should pinpoint to structural and functional processing units involved during supraspinal LUT control and should identify all dysfunctional neuronal components in patients with disturbed LUT control.

Importantly, we will investigate the reliability[28] of BOLD signals in task-related fMRI and RS-fMRI in healthy subjects and patients with non-neurogenic LUTS. The test-retest validation, i.e. the intra-class correlation coefficient (ICC) for absolute or consistent agreement of subject activations from visit to visit, has not been evaluated in regard to the supraspinal LUT control yet.

METHODS AND ANALYSIS

Study design

This prospective research study will be conducted at the University of Zürich, Zürich, Switzerland.

Study population & recruitment

According to the inclusion and exclusion criteria (Table 1), we will investigate patients with non-neurogenic LUTS and healthy controls with an unimpaired LUT function. Subjects of both groups will be matched according to age and gender.

Patients with non-neurogenic LUTS will be recruited from our own department (Neuro-Urology, Balgrist University Hospital Zürich) and through our partners at the University Hospital Zürich and the Triemli Hospital Zürich. Eligible patients with non-neurogenic LUTS and healthy controls will be invited to a first visit (screening) during which detailed information about the study, in particular the aims, methods, possible risks, and side effects, will be given. After obtaining written informed consent, the following data will be collected: medical history, a 3-day bladder diary, urine sample to exclude urinary tract infection (UTI) and pregnancy in female subjects, urodynamic parameters, and post void residual measured by ultrasound as well as standardized questionnaires regarding LUTS and QoL. The validated German versions of these questionnaires will be used with permission of the International consultation on incontinence modular questionnaire [(ICIQ), Bristol Urological Institute, Southmead Hospital Bristol, UK], and will address the LUTS (ICIQ-LUTS) in both females (ICIQ-FLUTS) and males (ICIQ-MLUTS) whereas the ICIQ-LUTSQoL will display the QoL in regard to LUTS.

Determination of sample size

A power analysis was conducted using G*Power (www.gpower.hhu.de). In order to have sufficient power (0.80) to detect a large effect size (0.80) between healthy subjects and

patients with non-neurogenic LUTS (significance level 0.05), at least 21 participants per group need to be recruited. To demonstrate post-treatment effects in patients with non-neurogenic LUTS compared to their baseline using the same power, effect size and significance level, at least 12 participants for each treatment option are necessary. These sample sizes are in line with earlier studies[22 23] including our own[14 29], which provided statistical evidence using small collectives, i.e. between 12 and 21 subjects.

Study location

- Neuro-Urology, Spinal Cord Injury Centre & Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland
- MR-Centre, University Hospital Zürich, Zürich, Switzerland

Partners

- NeuroRadiology Clinic, University Hospital Zürich, Zürich, Switzerland
- Departments of Urology and Gynaecology, University Hospital Zürich, Zürich, Switzerland
- Department of Urology and Gynaecology, Triemli Hospital, Zürich, Switzerland

Investigations

Following screening and study inclusion, all subjects will be scheduled for the 2nd and 3rd visit (1st and 2nd MRI measurement) at the MR-Centre. Patients with non-neurogenic LUTS will return for a 4th visit (3rd MRI measurement), either after receiving treatment for LUTS or without treatment acting as a direct control group within the patient cohort (Figure 1).

All MR measurements (Figure 2) will be performed using a Philips Ingenia 3 Tesla MR scanner (Philips Medical Systems, Best, The Netherlands) with a 16-channel head coil. During the 2nd visit, we will acquire the following neuro-imaging data. Structural measurements will contain T1-weighted MRI and DTI. The functional measurements will comprise of RS-fMRI and task-related fMRI. Four different RS-fMRI will be applied, i.e. (1) at baseline with an empty bladder, (2) with an empty bladder plus transurethral catheter, (3) with a low prefilled bladder (100mL saline, body warm) prior to task-related fMRI, and (4) after a task-related fMRI, to understand whether manipulation of sensory perception (induced by catheter or prefilling) will alter the default mode network.[20] The task-related fMRI will be acquired during four different bladder tasks (Figure 3). In order to precisely fill and drain the bladder (i.e. specific volume and duration of time), we designed an automated MR-compatible and MR-synchronised pump system. In the first three task-related fMRI, we will examine the effect of visceral bladder sensation by automated, repetitive filling with 100mL body warm saline starting with (1) an empty bladder, (2) a low prefilled bladder, and (3) a high prefilled bladder (persistent desire to void). The 4th task-related fMRI will consist of

automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder to investigate the neural correlates of cold bladder sensation (Figure 3). Subjects will rate their desire to void and their level of pain using a displayed visual analogue scale and an fMRI-compatible handheld response system.[30]

During the 2nd MRI measurement (3rd visit, 1 to 4 weeks later), we will utilise a selection of MRI measurements for the purpose of reliability analysis [28], i.e. RS-fMRI (baseline with an empty bladder plus transurethral catheter), and task-related fMRI to compare within and between groups.

The 3rd MRI measurement will be identical to the 1st MRI measurement to evaluate post versus pre-treatment effects in patients with non-neurogenic LUTS. The time from the start of treatment to the 4th visit, i.e. 5 to 7 weeks (Figure 1), is necessary to let clinical improvements develop.[31 32]

Safety

The staff involved in this study will be instructed and trained according to the safety regulations of the MR-Centre of the University Hospital Zürich. All subjects will be asked to remove any ferromagnetic items, e.g. bra, earrings, chains, rings, and piercings prior to entering the scanner room. All subjects will be provided with standardised clinical scrubs instead of wearing their own clothes. Before every MR measurement (visit 2, 3 and 4), urine samples will be analysed from every subject in order to exclude UTI or pregnancy. In case of pregnancy, the subject will be excluded from the study and referred to a gynaecologist. In case of UTI, the subject will not undergo the experiment, but will receive immediate antibiotic treatment if the UTI is symptomatic or treatment depending on further microbiological urine analysis in the absence of UTI symptoms. The subject can be reassigned to the study, if the microbiological urine analysis shows no evidence of an UTI or the UTI has been successfully treated.

In the situation of an adverse event (AE) or a severe adverse event (SAE), as defined by the international conference on harmonisation (ICH) good clinical practice (GCP) guidelines (E6)[33] and international organization for standardization (ISO, 14155)[34], appropriate actions will be executed and the according body (principle investigator, ethics committee) will be informed. All AEs and SAEs will be followed as long as medically indicated.

Study outcome measures

Primary

(A) BOLD signal intensity changes in a priori supraspinal regions of interest (ROI), e.g. pons, insula, anterior cingulate cortex, thalamus, hypothalamus, supplementary motor area, and prefrontal cortex, during task-related fMRI in relation to the specific

condition, i.e. infusion or to a contrast, i.e. low versus full bladder volume, during two (healthy controls) or three (patients with non-neurogenic LUTS) visits.

The precise selection of ROIs will be based on the coordinates of the peak activations during task-related fMRI, i.e. taken from the MNI space.

(B) Reliability of BOLD signal changes during RS-fMRI and task-related fMRI across visits (e.g. 2nd and 3rd visit) in healthy subjects and patients with non-neurogenic LUTS.

(C) BOLD signal changes in supraspinal ROIs during task-related fMRI in patients with non-neurogenic LUTS before and after treatment to quantify the link between BOLD signal changes and treatment efficacy.

Secondary

(A) Structural differences, i.e. between healthy subjects and patients with non-neurogenic LUTS, and changes, i.e. post vs pre-treatment state, of gray matter concentration using VBM.

(B) Structural (SC) and functional connectivity (FC) between supraspinal ROIs, e.g. insula, cingulate, and prefrontal cortices, and to identify specific alterations with DTI[35], i.e. whole-brain fractional anisotropy (FA) and mean diffusivity (MD) comparison between healthy subjects and patients with non-neurogenic LUTS (incl. post vs pre-treatment changes) as well as probabilistic tractography between ROIs (white matter fibre structure).

(C) Differences of BOLD signals during RS-fMRI between healthy subjects and patients with non-neurogenic LUTS (incl. post vs pre-treatment changes), i.e. intra- and interhemispheric connectivity[36] whether these signals already differ at baseline, are influenced by the presence of a catheter, by prefilling and/or by task-related fMRI.

(D) Clinical scores (e.g. bladder volume, urodynamic parameters, and level of desire to void during fMRI) will be correlated to BOLD signal changes as well as to structural markers (e.g. grey matter volume or number of white matter tracts between ROIs) using regression analyses.

Data analysis

Clinical data, e.g. urodynamic parameters, 3-day bladder diary outcome, and questionnaires scores, will be statistically analysed and compared between groups using IBM's Statistical Package for the Social Sciences (SPSS) version 19.0 or newer (Armonk, New York, U.S.) and will be presented with means and standard deviations or with medians and interquartile ranges as appropriate.

The neuro-imaging data will be analysed using statistical parametric mapping (SPM) version 12 (Wellcome Department of Imaging Neuroscience, University College London, UK). Preprocessing of functional data from each task-related fMRI will be done for each participant individually. The images will be realigned to the first scan, unwarped to control for movement- and susceptibility-induced image distortions[37], spatially coregistered to the T1-weighted image, and normalized to the Montreal Neurologic Institute (MNI) anatomical standard space. At last, the functional data will be smoothed spatially with an isotropic Gaussian kernel. Thereafter, first-level analysis using the general linear model (GLM) will performed to create contrasts of interest, e.g. low vs full bladder or infusion vs withdrawal.[38] The six movement parameters will be modeled as additional regressors to control for potential head motion.

Second-level factorial design will include at least (1) one-sample t-tests to compute a mean for each group, (2) two-sample t-tests to compare healthy subjects and patients with non-neurogenic LUTS and (3) paired t-tests to evaluate post- vs. pre-treatment effects in patients with non-neurogenic LUTS. The ICC for task-related and RS-fMRI reliability will be analyzed using the SPM-compatible ICC toolbox (http://www.kcl.ac.uk/iop/depts/neuroimaging/research/imaginganalysis/Software/ICC-Toolbox.aspx).

Association of individual (clinical) variables with BOLD signal changes will be assessed by whole-brain and ROIs-based correlation analyses (correlation coefficients will be reported).

DTI data will be analysed using TBSS (http://fsl.fmrib.ox.ac.uk/fsl/fsl4.0/tbss/index) and BrainVoyager (http://www.brainvoyager.com/downloads/downloads.html) with the following established DTI analyses: Whole-brain fractional anisotropy (FA) and mean diffusivity (MD) comparison between groups as well as probabilistic tractography between ROIs. Seed and target regions, i.e. ROIs will be defined (1) a priori using anatomic coordinates, e.g. from the SPM toolbox WFU_PickAtlas (http://fmri.wfubmc.edu/software/PickAtlas) and (2) from peak activations acquired from task-related fMRI and RS-fMRI on the standard MNI space. Volumetric changes in gray and white matter will be analyzed using voxel-based morphometry (VBM), e.g. the VBM toolbox (http://dbm.neuro.uni-jena.de/vbm) in SPM.

ETHICS AND DISSEMINATION

This cohort study will be performed in accordance to the World Medical Association Declaration of Helsinki[39] and the guidelines of the Swiss Academy of Medical Sciences.[40]

This study has been approved by the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH-Nr. 2011-0346). Furthermore, handling of all personal data will strictly comply with the federal law of data protection in Switzerland.[41]

This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

DISCUSSION

This study will investigate supraspinal LUT control in healthy subjects and patients with non-neurogenic LUTS using a multimodal imaging protocol, i.e. structural (T1-weighted and DTI) and fMRI (RS-fMRI and task-related fMRI), to examine hemodynamic responses to LUT stimulation. From the acquired neuro-imaging data, structural (SC) and functional connectivity (FC), and structural integrity evaluation of white matter tracts and gray matter concentration (VBM) will be analysed to identify specific alterations of the supraspinal LUT control. In addition, effects on the supraspinal LUT control after treatment for LUTS (e.g. antimuscarinergics or botulinum toxin) will be investigated. It is expected that this study will provide new insights into the supraspinal neuronal mechanisms and networks responsible for LUT control. The findings will help to verify, amend, or adjust neuronal circuitry models established from findings in healthy subjects, now in the context of patients with non-neurogenic LUTS. Particularly, the use of newer imaging and evaluation techniques has the potential to serve as quantifiable outcome measures for therapy success and provide evidence for non-responders of LUTS treatment.

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Contributors

All authors participated in creating the study design.

MW and UM drafted the manuscript.

LM, SK, PEVK and TMK critically reviewed the manuscript.

UM, SK and TMK obtained the funding of this study.

All the authors read and approved the final manuscript.

Competing interests

There are no competing interests.

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Figure legends

Figure 1 – Timetable and characteristics of all four visits.
PVR = Post void residual, LUTS = Lower urinary tract symptoms, QoL = Quality of life

Figure 2 – Schematic protocol of operational sequences of magnetic resonance imaging (MRI) measurements including functional MRI (fMRI): A) 1st MRI measurement, B) 2nd MRI measurement, and C) 3rd MRI measurement.

Figure 3 – Schematic diagram of the scan paradigm of four different task-related functional magnetic resonance imaging (fMRI) at visits 2, 3, and 4. All task-related fMRIs identically start with a "baseline" rest (60s, no specific stimulus or task is performed), a "baseline" rating of desire to void and level of pain, a short rest jittered between 7 to 9s in which blood-level-oxygen-dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition, and conclude with a "last" rest (60s, no specific stimulus or task is performed).
All task-related fMRIs consist of 8 repetitive blocks, each with either 5 (1st and 4th fMRI) or 8 (2nd and 3rd fMRI) conditions.

A) Conditions of the 1st task-related fMRI: 1) automated infusion of 100mL body warm saline, 2) plateau phase (bladder distention after infusion is perceived), 3) rating of desire to void and level of pain, 4) passive withdrawal to empty the bladder completely, and 5) short rest jittered between 7 to 9s. This task-related fMRI starts with an empty bladder and will be performed in patients with non-neurogenic LUTS in visit 2, 3, and 4, while in healthy controls only at visit 3 (2nd MRI measurement).
B and C) Conditions of the 2nd and 3rd task-related fMRI: 1) automated infusion of 100 mL warm saline, 2) plateau phase (bladder distention after infusion is perceived), 3) rating of desire to void and level of pain, 4) short rest jittered between 7 to 9s in which blood-level-oxygen-dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition, 5) automated withdrawal of 100mL, 6) plateau phase (bladder distention after withdrawal is perceived), 7) rating of desire to void and level of pain, and 8) short rest jittered between 7 to 9s in which blood-level-oxygen-dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition. The 2nd task-related fMRI (B) starts with a low prefilled bladder volume (100mL) and will be performed only in healthy controls at visit 2 and 3 (1st and 2nd MRI measurement). The 3rd

task-related fMRI (C) starts with a high prefilled bladder volume (persistent desire to void) and will be performed in all subjects (patients with non-neurogenic LUTS and healthy controls) during visit 2 and 3 (1st and 2nd MRI measurement). Additionally, this task-related fMRI will be carried out in patients with non-neurogenic LUTS at visit 4 (3rd MRI measurement).

D) Conditions of the 4th task-related fMRI task: 1) automated infusion of 100mL cold (4-8°C) saline, 2) plateau phase (bladder distention after infusion is perceived), 3) rating of desire to void and level of pain, 4) passive withdrawal to empty the bladder completely, and 5) short rest jittered between 7 to 9s. This task-related fMRI starts with an empty bladder and will be performed in all subjects (patient

Table 1 – Inclusion and exclusion criteria for all subjects.

Groups	Inclusion criteria	Exclusion criteria
All subjects	▪ Right-handed ▪ Gender (female and male) ▪ Age limits: 18-55 years ▪ MR suitability ▪ Written informed consent	▪ Pregnancy or breast feeding ▪ Any craniocerebral injury or surgery ▪ Any permanent ferromagnetic implant ▪ Any previous surgery of LUT / genitalia ▪ Any anatomical anomaly of LUT / genitalia ▪ Any LUT malignancy ▪ PVR > 150mL ▪ UTI
Healthy controls	▪ Unimpaired LUT function ▪ No LUTS (3-day bladder diary) ▪ No episode of urinary urgency / week ▪ Urinary frequency < 8 / 24h	▪ Impaired LUT function ▪ Any LUTS (3-day bladder diary) ▪ Any number of episodes of urinary urgency / week ▪ Urinary frequency > 8 / 24h
Patients with non-neurogenic LUTS	▪ LUTS > 6 months (3-day bladder diary) ▪ ≥ 2 episodes of urinary urgency / week ▪ Urinary frequency > 8 / 24h	▪ Any neurological, psychological, metabolic, or cardiovascular disease ▪ Any concomitant treatment for the LUT (e.g. neuromodulation) ▪ SUI ▪ Indwelling catheters or necessity to perform ISC

MR = Magnet resonance, LUT = Lower urinary tract, LUTS = Lower urinary tract symptoms, PVR = Post void residual, UTI = Urinary tract infection, SUI = Stress urinary incontinence, ISC = Intermittent self-catheterization

Protocol for a prospective neuro-imaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with non-neurogenic lower urinary tract symptoms

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ABSTRACT

Introduction: Lower urinary tract symptoms (LUTS) are highly prevalent, cause an enormous economic burden on health care systems, and significantly impair quality of life (QoL) of affected patients. The dependence of the lower urinary tract (LUT) on complex central neuronal circuits makes it unique in comparison to other visceral functions, such as the gastrointestinal tract, but also more vulnerable to neurological diseases.

Methods & analysis: This is a prospective neuro-imaging study investigating the supraspinal control of LUT function in healthy controls and patients with non-neurogenic LUTS.

The clinical assessment will include medical history, neuro-urological examination, bladder diary, urine analysis, urodynamic investigations, as well as standardized questionnaires regarding LUTS and QoL.

The acquisition of neuro-imaging data will include structural assessments (T1-weighted imaging and diffusion tensor imaging (DTI)) as well as functional investigations using blood-oxygen-level-dependent (BOLD) sensitive functional magnetic resonance imaging (fMRI) in a 3 Tesla MR scanner. The fMRI will be performed during four different bladder tasks using an automated MR-compatible and MR-synchronised pump system. The first three task-related fMRIs will consist of automated, repetitive filling of 100mL warm (37°C) saline starting with (1) an empty bladder, (2) a low prefilled bladder volume (100mL), and (3) a high prefilled bladder volume (persistent desire to void). The 4th task-related fMRI will comprise of automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder.

Ethics & dissemination:

The local ethics committee approved this study (KEK-ZH-Nr. 2011-0346). The findings of the study will be published in peer-reviewed journals and presented at national and international scientific meetings.

Trial registration:

This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

ARTICLE SUMMARY

Study focus

- To demonstrate correlations between alterations in brain structural and functional connectivity in relation to LUT control and LUTS, i.e. urgency with or without urgency urinary incontinence, and urinary frequency.
- To compare clinical correlates of treatment efficacy in patients with non-neurogenic LUTS with the associated changes in brain activity and connectivity.

Key messages

- Although recent neuro-imaging studies in humans and neuro-physiological animal studies have contributed to establish concepts of spinal and supraspinal LUT control, the exact pathophysiological mechanisms involved in human LUTS are still largely unknown.
- A multimodal neuro-imaging approach has considerable potential to broaden our knowledge of the supraspinal LUT control, and helps to adapt current concepts of LUT control.

Strengths and limitations of this study

- This will be the first study to identify brain networks of supraspinal LUT control in healthy subjects and abnormalities within such brain networks in patients with non-neurogenic LUTS using structural and functional MRI techniques in correlation with clinical measurements.
- Investigation of test-retest reliability which has not yet been performed for neuroimaging of LUT tasks. However, this is important especially in regard to interpretation of treatment effects.

INTRODUCTION

Lower urinary tract symptoms (LUTS) are highly prevalent, i.e. about 11% in the worldwide population in 2008, and forecasted to increase up to 20% until 2018.[1 2] Moreover, LUTS cause an enormous economic burden on each health care system,[3 4] i.e. comparable to diabetes mellitus,[5] and significantly impair the quality of life (QoL) of affected patients.[6 7]

For a proper functioning, lower urinary tract (LUT) structures, i.e. bladder, bladder neck, urethra, and urethral sphincter, rely on intact neuronal innervations that are under control of a complex supraspinal network.[8-10] The dependence of the LUT on such complex central neuronal circuits makes it unique in comparison to other visceral functions, e.g. gastrointestinal tract, but also more vulnerable to neurological diseases.[10]

Recent neuro-imaging studies have shown that patients with neurological disorders such as Parkinson's disease (PD)[11-13] and spinal cord injury (SCI)[14] demonstrate different supraspinal activity patterns compared to healthy controls in response to LUT stimulation tasks, which might represent a neural correlate to their LUTS.

Although there are several concepts regarding the human LUT function and neuronal control in normal and pathological conditions, the exact pathophysiological mechanisms involved remain largely unknown.[9] Despite the popularity of resting-state functional magnetic resonance imaging (RS-fMRI)[15-20] and diffusion tensor imaging (DTI)[21] in other fields in neuroscience, these techniques have not been applied in the context of supraspinal LUT control. There are only two DTI studies published in regard to LUT control in general: (1) a case report by Theadin et al.[22] studying spinal cord infarctions and clinical symptoms and (2) a prospective study by van der Jagt et al.[23] investigating architectural configuration and microstructural properties of the sacral plexus.

As cortical and sub-cortical (e.g. brainstem) brain regions are crucial for voluntary LUT control[10 24 25], investigation of the supraspinal regions with high-resolution imaging techniques, i.e. fMRI, can significantly contribute to increase our understanding on the effects of supraspinal lesions and alterations related to LUTS.[10 26]

In this study, we are aiming to identify supraspinal areas associated with LUT control in healthy subjects and in patients with non-neurogenic LUTS. Task-related blood-oxygen-level-dependent (BOLD) and RS-fMRI will be applied as well as structural MRI (T1-weighted MRI and DTI). Hence, we will examine if bladder processing is already altered on the structural level, and on baseline (RS-fMRI) functional connectivity. For example, the multiple repetition of the RS-fMRI will help to understand whether manipulation of sensory perception (induced by infusion and withdrawal) will alter the default mode network[20] of the brain. Furthermore, we can examine volumetric parameters (e.g. gray matter concentration) by voxel-based morphometry (VBM)[27], structural integrity and connectivity of white matter tracts (DTI) as well as functional connectivity (FC).

This unique and detailed multimodal imaging protocol should pinpoint to structural and functional processing units involved during supraspinal LUT control and should identify all dysfunctional neuronal components in patients with disturbed LUT control.

Importantly, we will investigate the reliability[28] of BOLD signals in task-related fMRI and RS-fMRI in healthy subjects and patients with non-neurogenic LUTS. The test-retest validation, i.e. the intra-class correlation coefficient (ICC) for absolute or consistent agreement of subject activations from visit to visit, has not been evaluated in regard to the supraspinal LUT control yet.

METHODS AND ANALYSIS

Study design

This prospective research study will be conducted at the University of Zürich, Zürich, Switzerland.

Study population & recruitment

According to the inclusion and exclusion criteria (Table 1), we will investigate patients with non-neurogenic LUTS and healthy controls with an unimpaired LUT function. Subjects of both groups will be matched according to age and gender.

Patients with non-neurogenic LUTS will be recruited from our own department (Neuro-Urology, Balgrist University Hospital Zürich) and through our partners at the University Hospital Zürich and the Triemli Hospital Zürich. Eligible patients with non-neurogenic LUTS and healthy controls will be invited to a first visit (screening) during which detailed information about the study, in particular the aims, methods, possible risks, and side effects, will be given. After obtaining written informed consent, the following data will be collected: medical history, a 3-day bladder diary, urine sample to exclude urinary tract infection (UTI) and pregnancy in female subjects, urodynamic parameters, and post void residual measured by ultrasound as well as standardized questionnaires regarding LUTS and QoL. The validated German versions of these questionnaires will be used with permission of the International consultation on incontinence modular questionnaire [(ICIQ), Bristol Urological Institute, Southmead Hospital Bristol, UK], and will address the LUTS (ICIQ-LUTS) in both females (ICIQ-FLUTS) and males (ICIQ-MLUTS) whereas the ICIQ-LUTSQoL will display the QoL in regard to LUTS.

Determination of sample size

A power analysis was conducted using G*Power (www.gpower.hhu.de). In order to have sufficient power (0.80) to detect a large effect size (0.80) between healthy subjects and

patients with non-neurogenic LUTS (significance level 0.05), at least 21 participants per group need to be recruited. To demonstrate post-treatment effects in patients with non-neurogenic LUTS compared to their baseline using the same power, effect size and significance level, at least 12 participants for each treatment option are necessary. These sample sizes are in line with earlier studies[22 23] including our own[14 29], which provided statistical evidence using small collectives, i.e. between 12 and 21 subjects.

Study location

- Neuro-Urology, Spinal Cord Injury Centre & Research, University of Zürich, Balgrist University Hospital, Zürich, Switzerland
- MR-Centre, University Hospital Zürich, Zürich, Switzerland

Partners

- NeuroRadiology Clinic, University Hospital Zürich, Zürich, Switzerland
- Departments of Urology and Gynaecology, University Hospital Zürich, Zürich, Switzerland
- Department of Urology and Gynaecology, Triemli Hospital, Zürich, Switzerland

Investigations

Following screening and study inclusion, all subjects will be scheduled for the 2nd and 3rd visit (1st and 2nd MRI measurement) at the MR-Centre. Patients with non-neurogenic LUTS will return for a 4th visit (3rd MRI measurement), either after receiving treatment for LUTS or without treatment acting as a direct control group within the patient cohort (Figure 1).

All MR measurements (Figure 2) will be performed using a Philips Ingenia 3 Tesla MR scanner (Philips Medical Systems, Best, The Netherlands) with a 16-channel head coil. During the 2nd visit, we will acquire the following neuro-imaging data. Structural measurements will contain T1-weighted MRI and DTI. The functional measurements will comprise of RS-fMRI and task-related fMRI. Four different RS-fMRI will be applied, i.e. (1) at baseline with an empty bladder, (2) with an empty bladder plus transurethral catheter, (3) with a low prefilled bladder (100mL saline, body warm) prior to task-related fMRI, and (4) after a task-related fMRI, to understand whether manipulation of sensory perception (induced by catheter or prefilling) will alter the default mode network.[20] The task-related fMRI will be acquired during four different bladder tasks (Figure 3). In order to precisely fill and drain the bladder (i.e. specific volume and duration of time), we designed an automated MR-compatible and MR-synchronised pump system. In the first three task-related fMRI, we will examine the effect of visceral bladder sensation by automated, repetitive filling with 100mL body warm saline starting with (1) an empty bladder, (2) a low prefilled bladder, and (3) a high prefilled bladder (persistent desire to void). The 4th task-related fMRI will consist of

automated, repetitive filling of 100mL cold (4-8°C) saline starting with an empty bladder to investigate the neural correlates of cold bladder sensation (Figure 3). Subjects will rate their desire to void and their level of pain using a displayed visual analogue scale and an fMRI-compatible handheld response system.[30]

During the 2nd MRI measurement (3rd visit, 1 to 4 weeks later), we will utilise a selection of MRI measurements for the purpose of reliability analysis [28], i.e. RS-fMRI (baseline with an empty bladder plus transurethral catheter), and task-related fMRI to compare within and between groups.

The 3rd MRI measurement will be identical to the 1st MRI measurement to evaluate post versus pre-treatment effects in patients with non-neurogenic LUTS. The time from the start of treatment to the 4th visit, i.e. 5 to 7 weeks (Figure 1), is necessary to let clinical improvements develop.[31 32]

Safety

The staff involved in this study will be instructed and trained according to the safety regulations of the MR-Centre of the University Hospital Zürich. All subjects will be asked to remove any ferromagnetic items, e.g. bra, earrings, chains, rings, and piercings prior to entering the scanner room. All subjects will be provided with standardised clinical scrubs instead of wearing their own clothes. Before every MR measurement (visit 2, 3 and 4), urine samples will be analysed from every subject in order to exclude UTI or pregnancy. In case of pregnancy, the subject will be excluded from the study and referred to a gynaecologist. In case of UTI, the subject will not undergo the experiment, but will receive immediate antibiotic treatment if the UTI is symptomatic or treatment depending on further microbiological urine analysis in the absence of UTI symptoms. The subject can be reassigned to the study, if the microbiological urine analysis shows no evidence of an UTI or the UTI has been successfully treated.

In the situation of an adverse event (AE) or a severe adverse event (SAE), as defined by the international conference on harmonisation (ICH) good clinical practice (GCP) guidelines (E6)[33] and international organization for standardization (ISO, 14155)[34], appropriate actions will be executed and the according body (principle investigator, ethics committee) will be informed. All AEs and SAEs will be followed as long as medically indicated.

Study outcome measures

Primary

(A) BOLD signal intensity changes in a priori supraspinal regions of interest (ROI), e.g. pons, insula, anterior cingulate cortex, thalamus, hypothalamus, supplementary motor area, and prefrontal cortex, during task-related fMRI in relation to the specific

condition, i.e. infusion or to a contrast, i.e. low versus full bladder volume, during two (healthy controls) or three (patients with non-neurogenic LUTS) visits.

The precise selection of ROIs will be based on the coordinates of the peak activations during task-related fMRI, i.e. taken from the MNI space.

(B) Reliability of BOLD signal changes during RS-fMRI and task-related fMRI across visits (e.g. 2nd and 3rd visit) in healthy subjects and patients with non-neurogenic LUTS.

(C) BOLD signal changes in supraspinal ROIs during task-related fMRI in patients with non-neurogenic LUTS before and after treatment to quantify the link between BOLD signal changes and treatment efficacy.

Secondary

(A) Structural differences, i.e. between healthy subjects and patients with non-neurogenic LUTS, and changes, i.e. post vs pre-treatment state, of gray matter concentration using VBM.

(B) Structural (SC) and functional connectivity (FC) between supraspinal ROIs, e.g. insula, cingulate, and prefrontal cortices, and to identify specific alterations with DTI[35], i.e. whole-brain fractional anisotropy (FA) and mean diffusivity (MD) comparison between healthy subjects and patients with non-neurogenic LUTS (incl. post vs pre-treatment changes) as well as probabilistic tractography between ROIs (white matter fibre structure).

(C) Differences of BOLD signals during RS-fMRI between healthy subjects and patients with non-neurogenic LUTS (incl. post vs pre-treatment changes), i.e. intra- and interhemispheric connectivity[36] whether these signals already differ at baseline, are influenced by the presence of a catheter, by prefilling and/or by task-related fMRI.

(D) Clinical scores (e.g. bladder volume, urodynamic parameters, and level of desire to void during fMRI) will be correlated to BOLD signal changes as well as to structural markers (e.g. grey matter volume or number of white matter tracts between ROIs) using regression analyses.

Data analysis

Clinical data, e.g. urodynamic parameters, 3-day bladder diary outcome, and questionnaires scores, will be statistically analysed and compared between groups using IBM's Statistical Package for the Social Sciences (SPSS) version 19.0 or newer (Armonk, New York, U.S.) and will be presented with means and standard deviations or with medians and interquartile ranges as appropriate.

The neuro-imaging data will be analysed using statistical parametric mapping (SPM) version 12 (Wellcome Department of Imaging Neuroscience, University College London, UK). Preprocessing of functional data from each task-related fMRI will be done for each participant individually. The images will be realigned to the first scan, unwarped to control for movement- and susceptibility-induced image distortions[37], spatially coregistered to the T1-weighted image, and normalized to the Montreal Neurologic Institute (MNI) anatomical standard space. At last, the functional data will be smoothed spatially with an isotropic Gaussian kernel. Thereafter, first-level analysis using the general linear model (GLM) will performed to create contrasts of interest, e.g. low vs full bladder or infusion vs withdrawal.[38] The six movement parameters will be modeled as additional regressors to control for potential head motion.

Second-level factorial design will include at least (1) one-sample t-tests to compute a mean for each group, (2) two-sample t-tests to compare healthy subjects and patients with non-neurogenic LUTS and (3) paired t-tests to evaluate post- vs. pre-treatment effects in patients with non-neurogenic LUTS. The ICC for task-related and RS-fMRI reliability will be analyzed using the SPM-compatible ICC toolbox (http://www.kcl.ac.uk/iop/depts/neuroimaging/research/imaginganalysis/Software/ICC-Toolbox.aspx).

Association of individual (clinical) variables with BOLD signal changes will be assessed by whole-brain and ROIs-based correlation analyses (correlation coefficients will be reported).

DTI data will be analysed using TBSS (http://fsl.fmrib.ox.ac.uk/fsl/fsl4.0/tbss/index) and BrainVoyager (http://www.brainvoyager.com/downloads/downloads.html) with the following established DTI analyses: Whole-brain fractional anisotropy (FA) and mean diffusivity (MD) comparison between groups as well as probabilistic tractography between ROIs. Seed and target regions, i.e. ROIs will be defined (1) a priori using anatomic coordinates, e.g. from the SPM toolbox WFU_PickAtlas (http://fmri.wfubmc.edu/software/PickAtlas) and (2) from peak activations acquired from task-related fMRI and RS-fMRI on the standard MNI space. Volumetric changes in gray and white matter will be analyzed using voxel-based morphometry (VBM), e.g. the VBM toolbox (http://dbm.neuro.uni-jena.de/vbm) in SPM.

ETHICS AND DISSEMINATION

This cohort study will be performed in accordance to the World Medical Association Declaration of Helsinki[39] and the guidelines of the Swiss Academy of Medical Sciences.[40]

This study has been approved by the local ethics committee (Kantonale Ethikkommission Zürich, KEK-ZH-Nr. 2011-0346). Furthermore, handling of all personal data will strictly comply with the federal law of data protection in Switzerland.[41]

This study has been registered at clinicaltrials.gov (http://www.clinicaltrials.gov/ct2/show/NCT01768910).

DISCUSSION

This study will investigate supraspinal LUT control in healthy subjects and patients with non-neurogenic LUTS using a multimodal imaging protocol, i.e. structural (T1-weighted and DTI) and fMRI (RS-fMRI and task-related fMRI), to examine hemodynamic responses to LUT stimulation. From the acquired neuro-imaging data, structural (SC) and functional connectivity (FC), and structural integrity evaluation of white matter tracts and gray matter concentration (VBM) will be analysed to identify specific alterations of the supraspinal LUT control. In addition, effects on the supraspinal LUT control after treatment for LUTS (e.g. antimuscarinergics or botulinum toxin) will be investigated. It is expected that this study will provide new insights into the supraspinal neuronal mechanisms and networks responsible for LUT control. The findings will help to verify, amend, or adjust neuronal circuitry models established from findings in healthy subjects, now in the context of patients with non-neurogenic LUTS. Particularly, the use of newer imaging and evaluation techniques has the potential to serve as quantifiable outcome measures for therapy success and provide evidence for non-responders of LUTS treatment.

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Contributors

All authors participated in creating the study design.

MW and UM drafted the manuscript.

LM, SK, PEVK and TMK critically reviewed the manuscript.

UM, SK and TMK obtained the funding of this study.

All the authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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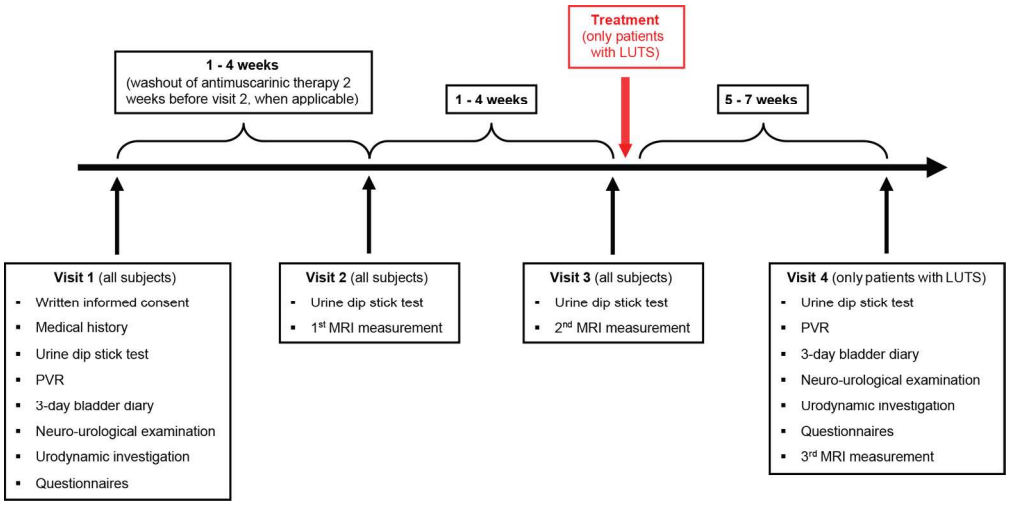

Figure 1 – Timetable and characteristics of all four visits.
PVR = Post void residual, LUTS = Lower urinary tract symptoms, QoL = Quality of life

169x83mm (300 x 300 DPI)

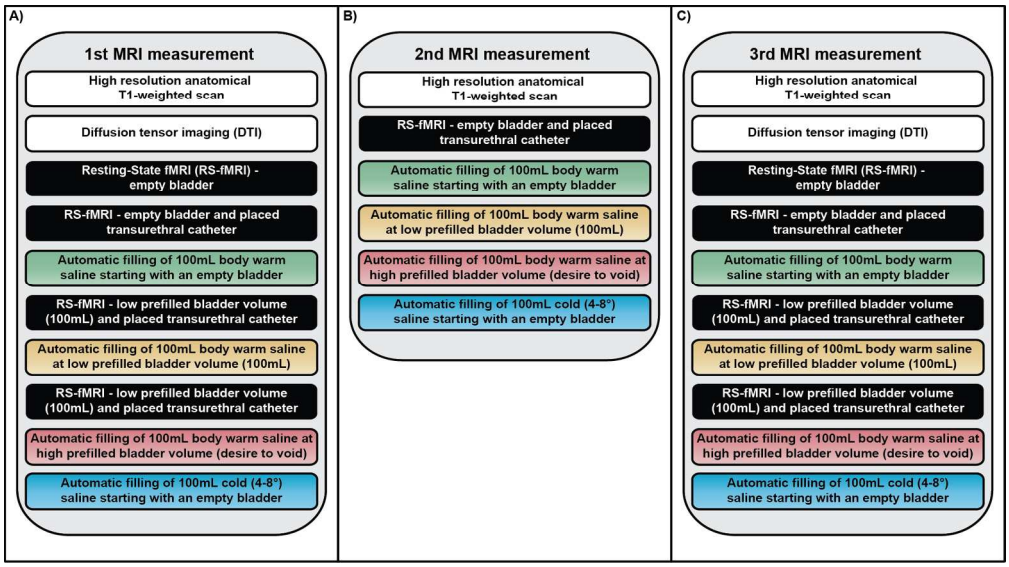

Figure 2 – Schematic protocol of operational sequences of magnetic resonance imaging (MRI) measurements including functional MRI (fMRI): A) 1st MRI measurement, B) 2nd MRI measurement, and C) 3rd MRI measurement.
171x95mm (300 x 300 DPI)

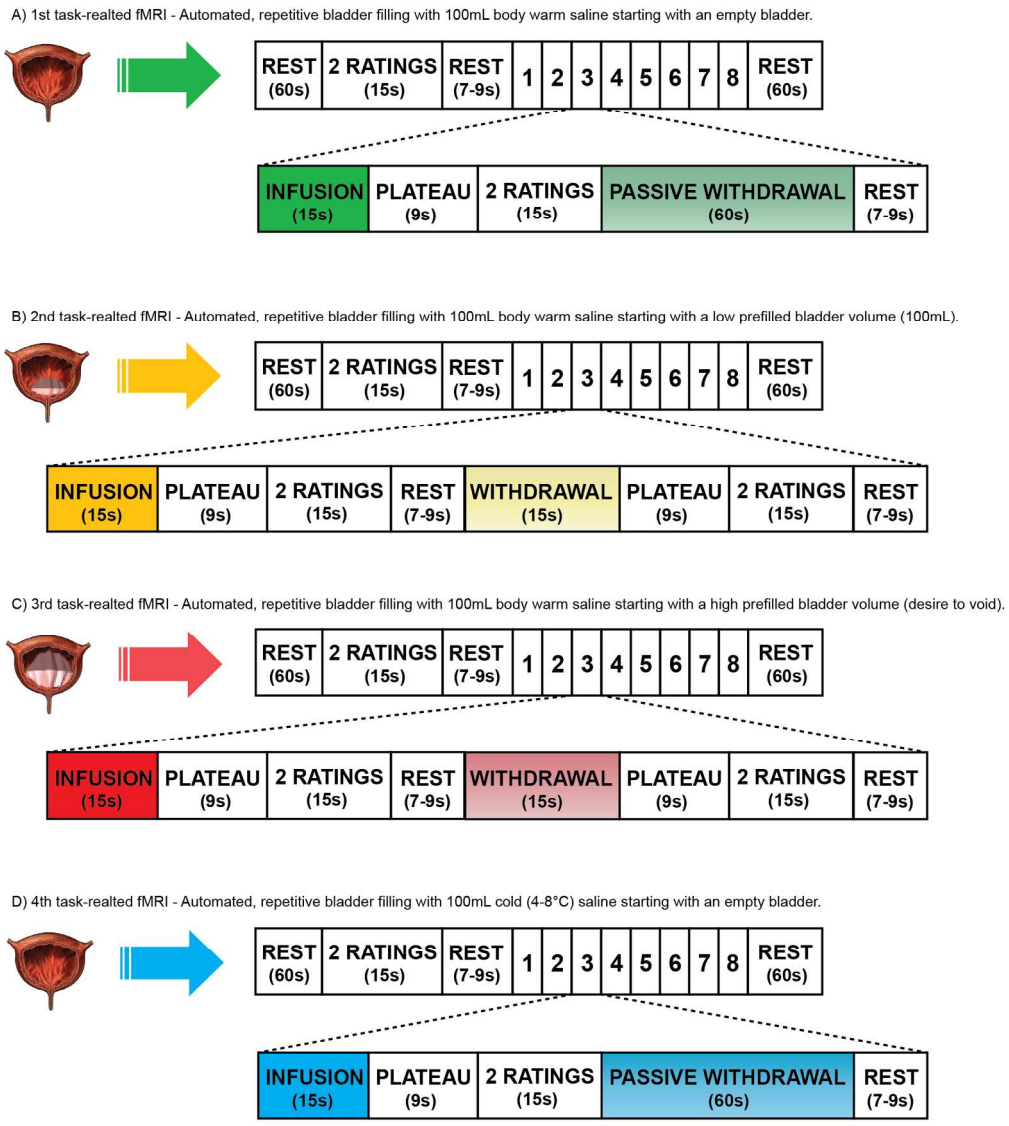

Figure 3 – Schematic diagram of the scan paradigm of four different task-related functional magnetic resonance imaging (fMRI) at visits 2, 3, and 4. All task-related fMRIs identically start with a "baseline" rest (60s, no specific stimulus or task is performed), a "baseline" rating of desire to void and level of pain, a short rest jittered between 7 to 9s in which blood-level-oxygen-dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition, and conclude with a "last" rest (60s, no specific stimulus or task is performed).
All task-related fMRIs consist of 8 repetitive blocks, each with either 5 (1st and 4th fMRI) or 8 (2nd and 3rd fMRI) conditions.

A) Conditions of the 1st task-related fMRI: 1) automated infusion of 100mL body warm saline, 2) plateau phase (bladder distention after infusion is perceived), 3) rating of desire to void and level of pain, 4) passive withdrawal to empty the bladder completely, and 5) short rest jittered between 7 to 9s. This task-related fMRI starts with an empty bladder and will be performed in patients with non-neurogenic LUTS in visit 2, 3, and 4, while in healthy controls only at visit 3 (2nd MRI measurement).
B and C) Conditions of the 2nd and 3rd task-related fMRI: 1) automated infusion of 100 mL warm saline, 2)

plateau phase (bladder distention after infusion is perceived), 3) rating of desire to void and level of pain, 4) short rest jittered between 7 to 9s in which blood-level-oxygen-dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition, 5) automated withdrawal of 100mL, 6) plateau phase (bladder distention after withdrawal is perceived), 7) rating of desire to void and level of pain, and 8) short rest jittered between 7 to 9s in which blood-level-oxygen-dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition. The 2nd task-related fMRI (B) starts with a low prefilled bladder volume (100mL) and will be performed only in healthy controls at visit 2 and 3 (1st and 2nd MRI measurement). The 3rd task-related fMRI (C) starts with a high prefilled bladder volume (persistent desire to void) and will be performed in all subjects (patients with non-neurogenic LUTS and healthy controls) during visit 2 and 3 (1st and 2nd MRI measurement). Additionally, this task-related fMRI will be carried out in patients with non-neurogenic LUTS at visit 4 (3rd MRI measurement).
D) Conditions of the 4th task-related fMRI task: 1) automated infusion of 100mL cold (4-8°C) saline, 2) plateau phase (bladder distention after infusion is perceived), 3) rating of desire to void and level of pain, 4) passive withdrawal to empty the bladder completely, and 5) short rest jittered between 7 to 9s. This task-related fMRI starts with an empty bladder and will be performed in all subjects (patient
167x192mm (300 x 300 DPI)

Correction

Walter M, Michels L, Kollias S, *et al*. Protocol for a prospective neuroimaging study investigating the supraspinal control of lower urinary tract function in healthy controls and patients with non-neurogenic lower urinary tract symptoms. *BMJ Open* 2014;4:e004357.

Figure 3 legend was inadvertently transposed with a section of the main text. The correct figure 3 legend is as follows:

> Figure 3: Schematic diagram of the scan paradigm of four different task-related functional MRIs (fMRIs) at visits 2, 3 and 4. All task-related fMRIs identically start with a 'baseline' rest (60 s, no specific stimulus or task is performed), a 'baseline' rating of desire to void and level of pain, a short rest jittered between 7 and 9 s in which blood-oxygen-level dependent (BOLD) activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition and conclude with a 'last' rest (60 s, no specific stimulus or task is performed). All task-related fMRIs consist of eight repetitive blocks, each with either five (first and fourth fMRIs) or eight (second and third fMRIs) conditions. (A) Conditions of the first task-related fMRI: (1) automated infusion of 100 mL body warm saline, (2) plateau phase (bladder distention after infusion is perceived), (3) rating of desire to void and level of pain, (4) passive withdrawal to empty the bladder completely and (5) short rest jittered between 7 and 9 s. This task-related fMRI starts with an empty bladder and will be performed in patients with non-neurogenic LUTS in visits 2, 3 and 4, while in healthy controls only at visit 3 (second MRI measurement). (B and C) Conditions of the second and third task-related fMRIs: (1) automated infusion of 100 mL warm saline, (2) plateau phase (bladder distention after infusion is perceived), (3) rating of desire to void and level of pain, (4) short rest jittered between 7 and 9 s in which BOLD activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition, (5) automated withdrawal of 100 mL, (6) plateau phase (bladder distention after withdrawal is perceived), (7) rating of desire to void and level of pain and (8) short rest jittered between 7 and 9 s in which BOLD activation resulting from motor activity during the previous rating will return to baseline to avoid contamination of the following condition. The second task-related fMRI (B) starts with a low prefilled bladder volume (100 mL) and will be performed only in healthy controls at visits 2 and 3 (first and second MRI measurements). The third task-related fMRI (C) starts with a high prefilled bladder volume (persistent desire to void) and will be performed in all participants (patients with non-neurogenic LUTS and healthy controls) during visits 2 and 3 (first and second MRI measurements). Additionally, this task-related fMRI will be carried out in patients with non-neurogenic LUTS at visit 4 (third MRI measurement). (D) Conditions of the fourth task-related fMRI task: (1) automated infusion of 100 mL cold (4–8°C) saline, (2) plateau phase (bladder distention after infusion is perceived), (3) rating of desire to void and level of pain, (4) passive withdrawal to empty the bladder completely and (5) short rest jittered between 7 and 9 s. This task-related fMRI starts with an empty bladder and will be performed in all participants (patient with non-neurogenic LUTS and healthy controls) during visit 2 and 3 (first and second MRI measurement). Additionally, this task-related fMRI will be executed in patients with non-neurogenic LUTS at visit 4 (third MRI measurement).

In addition, on page 5 'Study outcome measures'/'Primary' the first section '(A)' should read:

> BOLD signal intensity changes during task-related fMRI in relation to the specific condition, that is, infusion or to a contrast, that is, low versus full bladder volume, during two (healthy controls) or three (patients with non-neurogenic LUTS) visits. Investigation of these changes will focus on supraspinal regions of interest (ROI) that are known from the existing literature, for example, pons, insula, anterior cingulate cortex, thalamus, hypothalamus, supplementary motor area and prefrontal cortex. However, the precise selection of ROIs will be based on the coordinates of the peak activations during task-related fMRI taken from the Montreal Neurological Institute (MNI) space.

Finally, in table 1 the expansion of the acronym 'UTI' should have been included – 'UTI, urinary tract infection'.

BMJ Open 2014;**4**:e004357. doi:10.1136/bmjopen-2013-004357corr1