PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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
AUTHORS	Walter, Matthias; Michels, Lars; Kollias, Spyros; van Kerrebroeck, Philip; Kessler, Thomas; Mehnert, Ulrich

VERSION 1 - REVIEW

REVIEWER	Ryuji Sakakibara Neurology, Sakura Medical Center, Toho University, Japan
REVIEW RETURNED	16-Feb-2014

GENERAL COMMENTS	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 bmjopen-2013-004357 Looks good. However, protocol can be published ? If yes, please change the decision yes.

REVIEWER	Yuko Komesu University of New Mexico Albuquerque, New Mexico USA
REVIEW RETURNED	02-Mar-2014

GENERAL COMMENTS	The investigators propose a protocol to investigate the supraspinal mechanisms which control lower urinary tract function utilizing fMRI analyses of Multiples Sclerosis subjects with and without lower urinary tract symptoms, patients with idiopathic (non-neurogenic) lower urinary tract symptoms and health controls without lower urinary tract symptoms. Comments: fMRI— 1. Description regarding the design matrix to be used for analysis of brain activation is not described. 2. The regions of interest identified for functional connectivity and DTI analyses are large and more specific areas will be required to adequately perform the analysis. 3. Details for DTI analysis are incomplete—these include the methods of analyses for fractional anisotropy, probabilistic tractography, or the method of seed point determination for the latter 4. Greater detail regarding performance of the fMRI bladder filling tasks would be helpful. For example, will the repetitive filling be performed using an infusion pump? Will the infusion occur over a

	pre-specified time interval? 5. Since the subjects will be rating their urinary urgency using a displayed VAS and a hand held response system, how will the investigators adjust their fMRI analysis to account for the neural activity required by the subjects to perform these ratings? 6. It seems as though the fMRI task modeled after Griffith's work, but further detail regarding the methodology would be appreciated.. Data Analysis Section 1. The analyses of clinical findings were described in general terms and it would have been helpful for the investigators to supply information regarding how they plan to assess the relationship between structural or functional abnormalities in the brain with clinical symptoms. Clinical issues— 1. It would seem that the investigators are evaluating 4 different groups of patients which may complicate the analysis and interpretation of their findings. Perhaps the investigators could consider decreasing this number in order to most clearly delineate between group differences on fMRI 2. The investigators have chosen to have healthy controls with /= 3 urgency episodes/week. This potentially includes patients with relatively mild OAB who may not vary greatly from the healthy controls in their fMRI findings. Including patients with more severe urgency or urgency incontinence could help the investigators better delineate fMRI differences between patients and controls. 3. Those with LUTS are defined as subjects with >/= 3 urgency episodes/week and those without LUTS as subjects with 4. It would seem that MS patients with demyelination within the spine (e.g. transverse myelitis) should be excluded from the evaluation as this is an additional cause of neurogenic bladder and could confound the supraspinal fMRI findings. 5. It would be helpful if the investigators would make certain that subjects were age-matched, particularly for the DTI analysis 6. Greater detail regarding the power analysis and determination of the sample size would have been helpful 7. It would have been helpful for the investigators to describe their rationale for the timing of the fMRIs. For example, why did the investigators decide to do a 2nd fMRI "1-4 weeks" after the baseline fMRI and prior to treatment? How did the investigators decide to perform the 3rd fMRI "5-7 weeks" after treatment initiation? 8. The OAB treatment that patients are to undergo was not clearly defined. Heterogeneity in the treatments could complicate interpretation of the post-treatment fMRI changes. 9. The investigators stated that patients "might" return for the 3rd fMRI following treatment. Pre-specification of the number of patients required for post-treatment fMRI follow-up (based on a power analysis for sample size calculation) would have been preferable. Human Protections Issues 1. Do the investigators plan to give prophylactic antibiotics for this procedure? Will post-procedure UTI data be collected as a potential AE? Summary: The proposed study is described very generally and as such, it would be difficult for future investigators to replicate the study design and analysis The proposed study would be a valuable addition to the literature

	regarding the supraspinal mechanisms of bladder control. However, further descriptions regarding the methodology and specificity regarding analyses of the data would be extremely helpful to better understand the potential results and their interpretation for this study.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Comments to the Author

Looks good.

Reviewer: 2

Comments to the Author

fMRI –
1. “Description regarding the design matrix to be used for analysis of brain activation is not described.”

We agree with this comment. However, we did not provide a predefined design matrix of the general linear model (GLM) in SPM for the following reasons: In this protocol paper, we rather intend to propose a general concept of an investigation using neuro-imaging techniques. Providing a fixed GLM design matrix, would limit our options for subsequent fMRI analyses. We adjusted the manuscript on page 9, lines 3-29 (section Data analysis) to explain how images from task-related fMRI will be analyzed, i.e. preproccesing, 1st and 2nd level analysis.

2. “The regions of interest identified for functional connectivity and DTI analyses are large and more specific areas will be required to adequately perform the analysis.”

We agree with this comment. We only named a limited number of regions of interest (ROI), e.g. pons, insula, anterior cingulate cortex, thalamus, hypothalamus, supplementary motor area, and prefrontal cortex, which in context to the existing literature (Fowler, Griffiths et al. 2008; Fowler and Griffiths 2010) in the field of neuro-imaging in urology are known and commonly seen when stimulating the LUT. 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.
We changed the manuscript text accordingly on page 7, lines 55-60 and page 8, lines 3-8 (in section Primary Study outcome measures), and on page 9, lines 41-43 (in section Data analysis).

3. “Details for DTI analysis are incomplete, these include the methods of analyses for fractional anisotropy, probabilistic tractography, or the method of seed point determination for the latter.”

Thank your for your comment. We now provide a brief description about the potential DTI analyses on page 9, lines 33-46 (in section “Data analysis”).

4. “Greater detail regarding performance of the fMRI bladder filling tasks would be helpful. For example, will the repetitive filling be performed using an infusion pump? Will the infusion occur over a

pre-specified time interval?

Thank you for this comment. All task-related fMRI scans will be performed using an automated MR-compatible and MR-synchronised pump system. Opposite to an unpredictable and possibly imprecise manual handling, this pump system will allow us to perform infusion and withdrawal of specific volumes over a predefined period of time. The abstract was adapted accordingly on page 2 in lines 27-29 (in section Methods & analysis) and within the manuscript on page 6 in lines 50-54 (in section Investigations).
The infusion and withdrawal will occur at and over a pre-specified time as displayed in the original figure 3. However, to avoid any misinterpretation, we adapted figure 3. The conditions “active infusion” and “active withdrawal” both last 24 seconds each. The active infusion consists of an effective infusion phase (15sec) of 100 mL saline followed by a steady state “plateau” phase (9sec). Likewise, the active withdrawal comprises of an effective withdrawal phase (15sec) of 100 mL saline followed by a steady state “plateau” phase (9sec). Therefore, we adapted the entire figure 3 and the legend to figure 3.

5. “Since the subjects will be rating their urinary urgency using a displayed VAS and a hand held response system, how will the investigators adjust their fMRI analysis to account for the neural activity required by the subject to perform these ratings?”

This is an excellent comment. We discussed this issue before planning the fMRI tasks. We incorporated rest periods with a randomized jitter (see task 2 and 3 in figure 3) following each rating period. During the rest periods blood-level-oxygen-dependent (BOLD) activation resulting from motor activity during the previous rating period will return to baseline and thus prevent contamination of the following infusion or withdrawal condition,
The legend to figure 3 has been adapted to explain your remark in more detail.

6. “It seems as though the fMRI tasks modelled after Griffith’s work, but further detail regarding the methodology would be appreciated.”

We appreciated your comment. The work of Griffith and his colleagues clearly inspired us. Our main aim was to extend the “classical” fMRI bladder paradigm by additional structural and functional MRI techniques including RS-fMRI to examine bladder processing in full detail. Hence, we will examine if bladder processing is already altered on the structural level (T1 weighted MRI and DTI), 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 (Raichle, MacLeod et al. 2001) of the brain. This conceptual aspect is now elaborated in the current version of the paper on page 4 in lines 46-58 (in section Introduction), on page 6 in lines 43-49 (in section Investigations) and on page 8 in lines 35-40 (in section Secondary study outcomes measures).
In addition, the methodology of the task-related fMRIs are explained on pages 6-7 (in section Investigations) and within the legend to figure 3.

Data Analysis Section

1. “The analyses of clinical findings were described in general terms and it would have been helpful for the investigators to supply information regarding how they plan to assess the relationship between structural and functional abnormalities in the brain with clinical.”

Thank you for your comment regarding this important issue.
Using a multimodal imaging protocol, we will test if functional connectivity (assessed by task- and resting-state fMRI) will parallel structural connectivity (as examined by DTI), as already examined in

several developmental and clinical studies in other fields in neuroscience (Supekar, Menon et al. 2008; Greicius, Supekar et al. 2009; Lynch, Uddin et al. 2013; Koskinen, Hakulinen et al. 2014), but not yet in the context of the supraspinal control of the LUT.
We know from the literature, that structural changes are associated with changes in clinical scores or symptoms. For example, in contrast to healthy controls, brain atrophy is progressing within the first year in subjects who sustained an acute spinal cord injury (SCI). The degree of atrophy in these SCI patients correlates with the clinical outcome, e.g. the spinal cord independence measure (SCIM). (Freund, Weiskopf et al. 2013)
To address the relationship between structural and functional changes with clinical parameters, e.g. from urodynamic investigation along with scores from questionnaires, we will perform regression analyses between imaging parameters (global and local (f)MRI, T1, and DTI) and urodynamic scores. We now describe this strategy in the manuscript on page 8-9 in the section “Data analysis”.

Clinical issues

1. “It would seem that the investigators are evaluating 4 different groups of patients which may complicate the analysis and interpretation of their findings. Perhaps the investigators could consider decreasing this number in order to most clearly delineate between group differences on fMRI“

We agree with the reviewer and considered the recommendation by only using the groups: (1) patients with non-neurogenic LUTS and (2) healthy subjects. The entire manuscript was adapted accordingly.

2. “The investigators have chosen to have healthy controls with /= 3 urgency episodes/week. This potentially includes patients with relatively mild OAB who may not vary greatly from the healthy controls in their fMRI findings. Including patients with more severe urgency or urgency incontinence could help the investigators better delineate fMRI differences between patients and controls.“

Thank you for this important comment. Healthy subjects, as mentioned in section „study population & recruitment“ will be assessed by using the medical history, 3-day bladder diary, urodynamic parameter, standardized questionnaires. To avoid the pitfall of mixing up patients with relatively mild LUTS with healthy controls, we adapted the inclusion criteria for healthy subjects. As of now, we will only recruit healthy subjects without any episode of urinary urgency per week. In contrast to this, only non-neurogenic patients with LUTS with 2 or more urinary urgency episodes of per week will be included. Table 1 was adapted accordingly.

3. “Those with LUTS are defined as subjects with >/= 3 urgency episodes/week and those without LUTS as subjects with“

We assume the reviewer refers to less than 3 urgency episodes per week. We hope to have answered that within the previous statement.

4. “It would seem that MS patients with demyelination within the spine (e.g. transverse myelitis) should be excluded from the evaluation as this is an additional cause of neurogenic bladder and could confound the supraspinal fMRI findings.“

We understand the reviewers concern and refrain from MS patients.

5. “It would be helpful if the investigators would make certain that subjects were age-matched, particularly for the DTI analysis“

Thank your for this remark. In fact, we will use age- and gender-matched healthy controls for comparison to the patients for all applied (f)MRI and DTI imaging analyses. The manuscript has been adapted accordingly on page 5, in lines 29-31 in section Methods and analysis, subsection Study population & recruitment.

6. “Greater detail regarding the power analysis and determination of the sample size would have been helpful“

Thank you for this recommendation. We performed a power analysis using G*Power (www.gpower.hhu.de). On pages 5-6 in the section “Methods and analysis” we now added a subsection on “Determination of sample size”.
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7. “It would have been helpful for the investigators to describe their rationale for the timing of the fMRIs. For example, why did the investigators decide to do a 2nd fMRI “1-4 weeks” after the baseline fMRI and prior to treatment? How did the investigators decide to perform the 3rd fMRI “5-7 weeks” after treatment initiation?“

Our intention for the 2nd fMRI (3rd visit) within 1 to 4 weeks is to evaluate the reliability of subject activations from visit to visit within and between groups using RS-fMRI (baseline with an empty bladder and a placed transurethral catheter) and task-related fMRI, and to compare between groups. To quantify the reliability of activations we will use the intra-class correlation coefficient (Caceres, Hall et al. 2009). This purpose of the reliability analysis (including a timeline) has been implemented in the manuscript on page 3, lines 37-41 and on page 5, lines 7-14 (in section Introduction).
The proposed time gap of 5 to 7 weeks until the 3rd MRI measurement (Figure 1) is required for the manifestation for potential clinical improvements, i.e. the efficacy of the treatment (e.g. antimuscarinergics or botulinum toxin). (Agency for Healthcare Research and Quality 2009; Gormley, Lightner et al. 2012) The manuscript was adjusted to display this topic on page 7 in line 15-20 in section Investigation.

8. “The OAB treatment that patients are to undergo was not clearly defined. Heterogeneity in the treatments could complicate interpretation of the post-treatment fMRI changes.“

Since the nature of this protocol paper is to show a general idea of possibilities (i.e. different treatment options, e.g. antimuscarinergic or botulinum toxin), we did not specify the treatment. However, every treatment option used in this study will be labelled specifically to group patients. In this way, we will avoid patient’s contamination in regard of treatment success and post-treatment imaging.

9. “The investigators stated that patients “might” return for the 3rd fMRI following treatment. Pre-specification of the number of patients required for post-treatment fMRI follow-up (based on a power analysis for sample size calculation) would have been preferable.“

As a matter of fact, patients will return for a 3rd fMRI. Therefore, we changed the manuscript accordingly on page 6, lines 32-34 (in section Investigations) to:
“Patients with non-neurogenic LUTS will return for a 4th visit (3rd fMRI measurement), either after receiving treatment for LUTS or without treatment acting as a direct control group within the patient cohort with non-neurogenic LUTS (Figure 1).”

Human Protections Issues
1. “Do the investigators plan to give prophylactic antibiotics for this procedure? Will post-procedure UTI data be collected as a potential AE?“

On page 6 under Safety (last five lines), we stated “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.“
No prophylactic antibiotics will be given, but post-procedure UTI will be recorded as an AE according to the ICH and GCP guidelines and treated accordingly.

As corresponding author, I state that all co-authors have read the revision of the manuscript and agreed being submitted to you.

We hope after revising this article according to the comments of the reviewers, you will consider our manuscript for publication.

Yours sincerely,
Ulrich Mehnert

References:

Agency for Healthcare Research and Quality (2009). Treatment of Overactive Bladder in Women.
Caceres, A., D. L. Hall, et al. (2009). "Measuring fMRI reliability with the intra-class correlation coefficient." NeuroImage 45(3): 758-768.
Fowler, C. J., D. Griffiths, et al. (2008). "The neural control of micturition." Nature reviews. Neuroscience 9(6): 453-466.
Fowler, C. J. and D. J. Griffiths (2010). "A decade of functional brain imaging applied to bladder control." Neurourology and urodynamics 29(1): 49-55.
Freund, P., N. Weiskopf, et al. (2013). "MRI investigation of the sensorimotor cortex and the corticospinal tract after acute spinal cord injury: a prospective longitudinal study." Lancet Neurol 12(9): 873-881.
Gormley, E. A., D. J. Lightner, et al. (2012). "Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline." The Journal of urology 188(6 Suppl): 2455-2463.
Greicius, M. D., K. Supekar, et al. (2009). "Resting-state functional connectivity reflects structural connectivity in the default mode network." Cereb Cortex 19(1): 72-78.
Koskinen, E. A., U. Hakulinen, et al. (2014). "Clinical correlates of cerebral diffusion tensor imaging findings in chronic traumatic spinal cord injury." Spinal Cord 52(3): 202-208.
Lynch, C. J., L. Q. Uddin, et al. (2013). "Default mode network in childhood autism: posteromedial cortex heterogeneity and relationship with social deficits." Biol Psychiatry 74(3): 212-219.
Raichle, M. E., A. M. MacLeod, et al. (2001). "A default mode of brain function." Proc Natl Acad Sci U S A 98(2): 676-682.
Supekar, K., V. Menon, et al. (2008). "Network analysis of intrinsic functional brain connectivity in Alzheimer's disease." PLoS Comput Biol 4(6): e1000100.