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Test-retest repeatability of patterns of brain activation provoked by bladder filling

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Abstract

Objective: To assess short-term repeatability of an fMRI protocol widely used to assess brain control of the bladder. fMRI offers the potential to discern incontinence phenotypes as well as the mechanisms mediating therapeutic response. If so, this could enable more targeted efforts to enhance therapy. Such data, however, require excellent test-retest repeatability.

Methods: 59 older women (age 60 years) with urgency incontinence underwent two fMRI scans within 5-10 minutes with a concurrent bladder infusion/withdrawal protocol. Activity in three brain regions relevant to bladder control was compared using paired t-tests and intra-class correlation.

Results: There were no statistically significant differences in brain activity between the two consecutive scans in the regions of interest. Intra-class correlation was 0.19 in the right insula, 0.32 in the dorsal anterior cingulate cortex/supplementary motor area and 0.44 in the medial pre-frontal cortex. Such correlations are considered fair or poor, but are comparable to those from studies of other repeated fMRI tasks.

Conclusions: This is the first evaluation of the repeatability of a bladder fMRI protocol. The technique used provides a framework for comparing different fMRI protocols applied to brain-bladder research. Despite universal patient response to the stimulus, brain response had limited repeatability within individuals. Improvement of the investigational protocol should magnify brain response and reduce variability. These results suggest that although analysis of fMRI data among groups of subjects yields valuable insight into bladder control, fMRI is not yet appropriate for evaluation of the brain's role in continence on an individual level.

Keywords

fMRI; Urinary incontinence; urge; test retest reliability; reproducibility of results; women; neuroimaging

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Disclosure

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Introduction

In the past twenty years, the brain's role in bladder control has been studied with a range of imaging modalities¹⁻⁵. Study populations have included young, old, healthy, and incontinent participants as well as some with neurological disorders⁶⁻¹¹. Our group has used fMRI to focus on the role of the brain in urgency urinary incontinence (UUI), the most common type of urinary incontinence in older women. Other researchers have used a variety of bladder maneuvers to stimulate bladder-related brain activity, including entire bladder fills, multiple pelvic floor squeezes, and imagined or real voiding; during these the brain has been scanned to detect any change in activity^{6, 12-14}. In our studies, stimulation has comprised repeated short infusions and withdrawals of water into the bladder, typically performed when the bladder is filled to near-capacity⁴. These are intended to elicit urgency or very strong desire to void. Based on this^{4, 9, 15} and others' work^{1, 6, 16, 17}, a tentative model of brain-bladder control has been developed¹⁸ in which cortical neural circuits control the long-loop voiding reflex via its rostral terminus in the pons and midbrain. These circuits include key regions: the insula (particularly the right), medial pre-frontal cortex (mPFC), and dorsal anterior cingulate cortex and supplementary motor area (dACC/SMA).

In this body of work, it is striking that the blood oxygen level dependent (BOLD) signals recorded by fMRI appear quite variable, resulting in rather weak levels of significance and limited replication. This is also true when fMRI has been used to investigate other physiological functions¹⁹. However, the origin of the variability has not been identified and repeatability of bladder-related BOLD signals has not been tested. Moreover, no measure of repeatability has been identified which would enable one bladder stimulation protocol to be compared with another.

Assessing and maximizing reproducibility of fMRI is crucial to advancing our understanding of the brain's role in bladder control. Its reproducibility is suggested by its consistent identification of the same brain centers as key to bladder control, despite differing protocols, investigators, and participants. Yet, fMRI studies currently require use of a large number of subjects and analyses based on group means. In turn, this impedes identification of UUI "phenotypes" among individuals as well as identification of factors that correlate with therapeutic response. This information could be helpful not only in predicting response but also in identifying mechanisms that mediate it and could be used to enhance therapeutic efficacy still further.

Variability of BOLD signal in a single individual may arise (1) because the second measurement differs systematically from the first (a habituation or sensitization effect); (2) because the signal is weak (a defect of the protocol), so that it is swamped by random noise and cannot be measured accurately; or (3) because of intrinsic physiological variability, so that different measurements correspond to different physiological states with different patterns of brain activity. Type three is especially likely in bladder control, where brain responses depend on subjective sensations and on social and emotional circumstances. Variability may occur over the short term (a few minutes) or the longer term (a few weeks or more). Moreover all of these potential contributions to the single-subject variability

contribute also to between-subject variability, so tending to mask any real differences between participants or groups of participants.

Over the past few years we have performed a large number of fMRI measurements, approved by the local Institutional Review Board (IRB), in older women with UUI who have consented to allow their data to be used for further analyses. The present study is a secondary analysis of the measurements in these women. Our aim was to test repeatability of our fMRI bladder stimulation protocol, specifically targeting the areas of the brain identified in the model, and to provide a benchmark for repeatability that can be used in the future to compare the reliability of different protocols.

Methods

Recruitment

In the course of a number of studies from 2008-2013 examining the role of the brain in UUI^{9, 15, 20}, we recruited 96 community-dwelling women aged 60 years and over with UUI to undergo a bladder-filling and brain-scanning protocol. Participants had to report more than five UUI episodes per week. We excluded participants with: metal or electronic implant incompatible with fMRI; impaired mobility or cognition sufficient to preclude study procedures; neurological abnormalities that are clinically-apparent, obvious on structural MRI or from history; prolapse beyond the hymen; interstitial cystitis; spinal cord injury; history of pelvic radiation or advanced uterine/bladder cancer; multiple sclerosis; urethral obstruction; post void residual >200 ml; medical instability or expected medication change; conditions requiring intravenous antibacterial prophylaxis; incontinence treatment within 2 months of baseline; fecal incontinence, and symptomatic colitis/IBS. Further exclusions were made on technical grounds on a scan-by-scan basis (see following section).

Protocol design

The goal of the bladder-stimulation protocol was to repeatedly generate a strong sensation of urgency (without inducing detrusor overactivity, DO) and obtain the corresponding pattern of brain activation (BOLD signal). Participants emptied the bladder and then were positioned in a Siemens 3T Trio scanner with a push-button to allow them to signal a strong desire to void or urgency. Two 8 F urethral catheters were inserted, one for infusion and withdrawal of sterile water and one for bladder pressure measurement. The catheters were connected by water-filled tubes to pressure transducers and urodynamic equipment (Laborie Avanti) in the adjacent control room. A structural MRI was then recorded (duration about 30 min, allowing <100 ml urine to accumulate in the bladder; we refer to this situation as a near-empty bladder; see Fig.1). It was followed by functional MRI scanning (BOLD signal) while sterile water was repeatedly infused into and withdrawn from the bladder by a syringe pump (Harvard PHD 22/2000), driven by the MRI scanner. Intravesical pressure was measured to exclude DO. The pressure at the pump was measured for later synchronization of urodynamic measurements with the fMRI BOLD signal.

For scanning we used an echo-planar imaging protocol with flip angle = 85°; echo time TE = 27 ms; one scan per 2 s. Data were obtained from 40 brain slices with thickness 3.2 mm,

3.3 × 3.3 mm resolution, and field of view 210 × 210 mm, covering approximately the whole brain. As shown in Fig.1, the basic pattern of bladder stimulation comprised: pause (resting scan for 12 s); infusion (22 ml water infused in 12 s = 110 ml/min); pause (resting scan to allow sensation to subside for 12 s); withdrawal (20 ml water withdrawn in 12 s). Four such cycles, immediately repeated, formed one measurement 'block'. After 2 measurement blocks with near-empty bladder (not used for this analysis, but to allow for a 'practice run' for the participant), the bladder was filled with a hand-operated syringe at approximately 50 ml/minute, until the participant signaled strong desire to void. Further blocks were then performed. After each measurement block, participants were asked about their comfort and sensation and another block was recorded unless DO occurred or the participant stopped the study because of intense sensation. The BOLD signals acquired in the last block without DO (but with urgency or very strong desire to void; block B) and the preceding block (block A) were considered for the repeatability analysis. Only one block A/block B pair was included per participant. Exclusion conditions included:

- no block A to pair with block B (only one successful urgency block recorded);
- more than 50 ml infused between block A and block B (to standardize bladder volume/sensation between repeat blocks);
- blocks more than 5 minutes apart (to minimize natural filling between blocks A and B);
- DO or leakage in either or both blocks (to ensure measurement was made during storage phase i.e. < 5 cmH₂O pressure change, even though sensation was strong);
- no button press to signal urgency/strong desire to void during either or both blocks.

Each participant's measurements thus included 2 iterations of the bladder stimulation protocol (block A and block B), performed a few minutes apart, enabling calculations concerning short-term variability of brain activity in key regions.

Data analysis

SPM5 (Statistical Parametric Mapping, Wellcome Trust, UK) was used to analyze the fMRI BOLD data. Preprocessing included alignment of successive scans within each block, to correct for head motion; affine transformation to fit to a standard brain (ICBM152), and smoothing with an 8 mm Gaussian filter. As in our previous work, first-level (single subject) analysis was performed for every voxel by calculating brain activation in response to the stimulation protocol as the difference between the average of the BOLD signal during the 4 infusions and 4 withdrawals, allowing for the delayed hemodynamic response to neural activity, and with a low-frequency cutoff at 128 s. Activation defined in this way is typically positive; negative values are taken to represent deactivation.

Second-level (group) analysis was performed in 2 ways: Firstly, we used the REX²¹ MatLAB package to extract the mean value of the voxels within each of 3 predetermined regions of interest (ROIs). Each ROI was a sphere of 18 mm radius, centered at MNI coordinates [x, y, z] taken from the literature: R insula [38, 16, 6]; midcingulate (or dorsal

anterior cingulate/supplementary motor area dACC/SMA) [4, 14, 42]; and medial prefrontal cortex mPFC [4, 50, 14]. These mean values were used to analyze repeatability at ROI level as described in the following section. Secondly, we used SPM to perform group-level paired t-tests in the whole brain, to detect *a posteriori* any brain region (not necessarily a predetermined ROI) where activation in blocks A and B differed systematically.

Tests of repeatability at ROI level

For each ROI we performed: paired t-tests (to assess the difference between mean activation in block A and block B); F-tests (to assess the difference in variance between the two blocks); and Bland-Altman plots to assess any other systematic differences between signal in block A and block B (see Introduction, case 1).

If no significant systematic differences were found we calculated the intra-class correlation coefficient (ICC) for each key region of interest, as suggested by Sheu et al,²² using Fisher's original formula²³. The ICC (a generalization of the familiar Pearson correlation coefficient)^{22, 24} attaches a numerical value (range 0 – 1) to the overall repeatability of successive blocks of measurements in the whole group of participants. The higher the ICC, the greater is the short-term repeatability (and the smaller the variability) of the mean BOLD signal in the given ROI. ICC values have been interpreted as follows: 0-0.2 indicates *poor* repeatability; 0.3-0.4 indicates *fair* repeatability; 0.5-0.6 indicates moderate repeatability; 0.7-0.8 indicates *strong* repeatability; and >0.8 indicates *excellent* repeatability.²⁵

Results

Of 96 women who reported five or more UUI episodes per week and had two consecutive scans (< 5 minutes apart), 32 did not signal urgency in both blocks, 16 had > 50 ml infused between blocks, and 5 had DO or leakage; some were excluded by more than one criterion. Thus, 59 women met all inclusion criteria, five of whom used anticholinergic medication (for > 6 months prior to the study with incomplete resolution of symptoms). Included subjects' median age was 69 (60-90), and the mean (and standard deviation [SD]) of the volume infused between the two scans was 9.6 (14) ml.

Comparing block A and block B in each region of interest, there were no significant differences in the *magnitude* of activation using a paired t-test. The *variance* of activation in Block B was significantly greater in the right insula. (see Table 1). but this result depended on a single observation(see footnote to Table 1). More importantly, Bland-Altman analysis showed no systematic difference in activity in the repeated blocks A and B for any of the 3 regions of interest (Fig. 2 A, B and C).

In the absence of systematic difference between blocks A and B, the repeatability of the measurements on a ROI basis could be expressed by the intra-class correlation coefficient (ICC). As shown in Table 2, the ICC varied from 0.19, poor (for Right insula) to 0.32 and 0.44, fair (for dACC/SMA and mPFC respectively). Stratifying the analysis by 3 levels of leakage frequency had no impact on the results.

A t-test for systematic differences between blocks A and B anywhere in the whole brain confirmed the absence of significant difference in the anterior part of the brain (see Fig. 3), specifically in the locations of the 3 ROIs. In the posterior brain however it suggested less activation in block B than block A. The regions involved included the medial frontal and post central gyri and posterior cingulate cortex ($P < 0.001$ at cluster level, thresholded at $P < 0.05$, corrected for multiple comparisons, see table 3). Activation extended as far as the midcingulate dACC/SMA, whose center is indicated by the cross-hairs in Fig. 3.

Discussion

Focusing on the 3 regions of interest identified *a priori* as important in bladder control,^{9, 15} brain activation evoked by bladder stimulation shows no systematic change when repeated a few minutes later (Table 1). Therefore it is not increased by the small amount of bladder filling that occurs between blocks A and B. Nor does it show significant decrease in block B, which would indicate habituation to the stimulus. In the right insula, one of the key ROIs, however, there is significantly increased variance in block B (caused by one outlier). If real, this might suggest a wider range of responses reflecting both habituation and increased sensation.

Expanding to the whole brain instead, other regions are revealed where activation tends to decline from block A to block B, implying habituation. They form a large area, significant at $P < 0.001$ at cluster level, corrected for multiple comparisons. This area includes parts of the occipital lobe and the posterior- and mid-cingulate cortex, and extends as far as the dACC/SMA where a few activated voxels suggest a small amount of habituation that was too weak to be detected in the ROI analysis (Table 1).

Absence of significant *systematic* change between blocks A and B rules it out as a main contributor to poor repeatability (see Introduction, case 1). Nevertheless there is considerable *random* variability in the measurements, as indicated by values of ICC coefficient that range from 0.19 to 0.44, corresponding to poor to fair repeatability. Although these values are low they fall within the range reported for other block design fMRI studies (0.17-0.75 [mean=0.50]),¹⁹ including ones believed to be most reliable, such as finger tapping (0.23-0.74; mean=0.53) and those more similar to the present task (e.g., anticipatory anxiety -0.06-0.66; mean 0.34).

Our finding of a low ICC suggests that there may be considerable opportunity to improve the methods currently used to stimulate the bladder and provoke brain activity. Bladder control involves a number of neural circuits⁹ that subserve subjective aspects such as sensation, emotion and social behavior. Consequently, unlike voluntary muscle movement, bladder behavior – continence and voiding – is subjective, depends on many factors, and can involve layers of processing that differ from time to time and between participants. For example, initial panic/in only one of two blocks may influence results. This line of reasoning would suggest that much of the variability is physiological in nature. In that case (Introduction, case 3) repeatability might be improved by better standardizing the circumstances of the scanning and by separately examining subgroups with similar fMRI responses, an approach that has already proved valuable⁹. By contrast if low ICC reflects a weak signal masked by

measurement noise (Introduction, case 2), then repeatability could be improved by redesigning the stimulus protocol to yield larger brain responses.

Our study has limitations. It is a secondary analysis of fMRI scans obtained from several studies in which brain regions involved in UUI were investigated. Consequently, only short-term repeatability could be assessed. Further, statistical power was determined by the numbers who survived the exclusion criteria, and thus was outside our control. As part of the design of the primary studies, the measurement protocol included repeated measurement blocks in a situation resembling urgency, standardization of which is difficult, which is why we excluded those with DO and those who did not signal with infusion in both blocks. The blocks were repeated mainly with the aim of maximizing bladder sensation and the brain's responses, because with each measurement block the volume in the bladder increased by a few ml, due to both natural and artificial filling. In fact there was no significantly higher or lower activation in the second block except for less activation in the posterior and midcingulate brain. Thus most of the variability in fMRI results for the 3 regions of interest was apparently random in nature. Although 5 women were taking anticholinergic medication, inclusion criteria mandated that they should have been taking it for over six months and still experience urgency and leakage. We do not expect this to have an effect on immediate test-retest measurement.

Conclusion

Among individuals, short-term repeatability of the activation evoked by our fMRI bladder stimulation protocol is low. A measure of repeatability, the intra-class correlation coefficient (ICC), ranges from 0.19 to 0.44 in the 3 ROIs. Since a value of 1 signifies perfect repeatability, there is much room for improvement.

In some posterior and midcingulate parts of the brain, and possibly the right insula, repeatability may be limited by habituation to repeated stimuli. In the 3 predetermined bladder-control regions, however, habituation is not significant and repeatability may be limited by random variation. If the origin of this variation proves to be mainly measurement noise, repeatability could be improved by intensifying the bladder stimulation so as to magnify brain responses. To the extent that the random variation reflects actual physiological changes in the pattern of brain activity, other means of dealing with the variation will be necessary.

This study has emphasized the limitations of fMRI to assess bladder control *in individuals*. Nonetheless, *group-level* fMRI remains the best technique currently available and it has yielded important insights into brain control of the bladder. An approach mindful of the limits of technology and natural variability will ensure the future of this field.

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Fig. 1. Schematic diagram of the protocol used to stimulate the bladder and generate fMRI BOLD signals for analysis. 'Empty bladder' corresponds to natural filling during 30 minutes of structural scanning. SDV-Strong desire to void

Figure 2 A-C.

Bland Altman analyses of activation in each of 3 regions of interest. Horizontal lines show mean (black) and mean $\pm$ 2 standard deviations (grey).

Figure 3.

Whole-brain paired t-test for the difference between block A and block B. Activation is significantly less in block B than Block A in a region of the post central and medial frontal gyri and posterior cingulate (significant $P < 0.001$ at cluster level, corrected for multiple comparisons). The cross-hairs indicate the center of the dACC/SMA region of interest.

Table 1.

Mean and standard deviation (SD) of the activation in each region of interest, for block A and block B, and the results of paired T-tests for the difference between blocks A and B, and F-tests for the ratio of their variances.

<i>Brain region</i>	Mean (SD)		p-value	
	Block A	Block B	Paired t-test	F-test
Right Insula	0.157 (0.312)	0.010 (0.492)	0.406	0.0004[*]
dACC/SMA	0.162 (0.349)	0.094 (0.366)	0.212	0.360
mPFC	-0.108 (0.441)	-0.119 (0.535)	0.876	0.073

^{*}
p = 0.33 if the possible outlier at bottom left of Fig. 2C is omitted.

Table 2.

Intra-class correlation coefficient (ICC) for 3 regions of interest.

<i>Brain region</i>	<i>ICC</i>
Right insula	0.19
dACC/SMA	0.32
mPFC	0.44

Table 3,

cluster-level statistics for the whole brain paired t-test of block B vs block A, significant at $p > 0.05$. K_E = number of significant voxels

Area	Coordinates (MNI)	$P_{\text{corrected}}$	K_E	$P_{\text{uncorrected}}$
Postcentral Gyrus	-18, -26, 48	5.02×10^{-7}	29284	2.05×10^{-8}
Medial frontal gyrus	2, -4, 56			
Posterior cingulate	20, -60, 18			