Investigation of Brain Mechanisms Involved in the Urinary Continence Mechanism Associated With Aging

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University of Pittsburgh STUDY20080217
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Study Protocol

Screening

Screening procedures include: a) telephone interview and b) Visit 1 to study research suite - Continence Research Unit at UPMC Montefiore Hospital. If the subject is interested and qualifies, then the other visits are scheduled.

Telephone Interview

Use IRB approved telephone script to determine eligibility (5-20mins; study nurse/coordinator)

Visit 1a

1 hour; study nurse; telephone

1. Obtain informed consent and document.
2. Redcap Questionnaires:
    - Basic demographics
    - Current incontinence
    - Incontinence history
    - Obstetrics
    - Daily routine
    - Medical and surgical history
    - MRI screen
3. Make appointment for V1b

Visit 1b

1 hour; study nurse; continence research unit MUH NE547

1. Recap consent and sign document.
2. Uroflow and dip (whenever participant is ready to void – give water and continue if unable)
    - Measure flow rate and volume of normal void
    - Test for infection (leukocytes/nitrites) using a dipstick
    - Post-void residual measurement by ultrasound
    - *Urinalysis (conditional): If the dip procedure indicates or suggests infection, a catheterized specimen will be sent for urinalysis and culture. If positive, participant will be advised to see PCP for treatment.*
3. Redcap Questionnaires (participant completes on Redcap on own):
    - Sensation of urge
    - PHQ-9
    - Beck
    - SF-36
4. MoCA (paper)
5. Physical examination: Short physical/neurological examination. If participant report significant prolapse, participant may also have a gynecological exam.
6. Voiding diary: Explain 3 day voiding diary and one day pad diary. Give diary, specimen pan and 12 weighed pads in Ziploc bags.
7. Schedule visit 2 with enough time to complete diary
8. Pay (Vincent) and parking ticket.

Visit 2

About 3-4 hours; study nurse; MUH NE547 and PUH MRRC

1. Uroflow and dip (suggested infection will end visit)
    - Measure flow rate and volume of normal void
    - Test for infection (leukocytes/nitrites) using a dipstick
    - Post-void residual measurement by ultrasound
2. Give single dose prophylactic antibiotic **nitrofurantoin 100 mg** (or ciprofloxacin 250 mg, trimethoprim/ sulfamethoxazole DS 160/800).
3. Escort to MRRC
4. MRI screen (MRRC)
5. Change into gown
6. Two 8Fr catheters inserted per urethra and secured with tape.
7. Transfer to MRI scan room
8. Connect catheters to tube connections to Goby Laborie urodynamics system
9. Structural scans (20 mins)
10. Functional MRI scans with coordinated bladder filling on empty bladder x2 (ePrime; 5 minutes)*
11. Fill bladder until button signal *
12. Functional MRI scans with coordinated bladder filling on full bladder x2 (ePrime;5 minutes)*
13. Remove catheters
14. Empty bladder into specimen pan (measure)
15. Post void residual ultrasound
16. Pay
17. End of visit

* If participant signals that she is uncomfortable we will pause, or stop the whole procedure if necessary.

Unanticipated disease: If the above procedure suggests a serious disease (e.g., brain tumor or brain abnormality), the subject will be informed and permission asked to inform either the appropriate physician or

*This fMRI paradigm is based on the well tolerated paradigm used in over 100 subjects in PRO09030387, PRO11020351, PRO10030358 and PRO08020109.

Analysis Plan

Specific Aim 1 – Determine *normal* CNS control of bladder function and associated changes with age. In this aim, we will characterize brain responses to filling in continent, asymptomatic young and old individuals

> *Hypothesis 1: Normal brain response to bladder filling can be characterized in continent young adults.*
>
> *Hypothesis 2: Differences in such brain responses will be found in* continent, asymptomatic *older adults.* Because they will have been found in *continent* and *asymptomatic* elderly, these differences will be considered 'normal' in the elderly, and their identification will enable us to differentiate them from the abnormalities that cause/contribute to – or compensate for – UUI in older adults.

Specific Aim 2 – **Develop a comprehensive model of brain changes associated with age and *UUI*.** We will identify brain abnormalities that are associated with UUI in older adults and, in conjunction with the current working model, try to determine which are likely causal/contributory and which are compensatory.

Hypothesis 3: Abnormalities of brain response likely to cause/contribute to UUI in the elderly--or to compensate for it—can be identified and characterized.

Hypothesis 4: Information from these four groups of subjects, combined with findings from our previous study—which identified brain *changes* with successful therapy—***should finally enable us to determine which brain changes in older adults with UUI are* incidental *to aging (but unrelated to UUI) and thus should be ignored; which are* causal/contributory *to UUI and should be addressed; and which are* compensatory *and should be augmented.***

First level analysis (functional)

Infusion-withdrawal contrast: For the infusion withdrawal protocol (the 'task'), we will perform a mass-univariate regression that models the mean of the voxel-wise time series, six motion parameters (from motion correction), and the onset and duration of the infusion and withdrawal task convolved with canonical hemodynamic response function. The model uses a series of temporal cosines to model low frequency noise (1/128 Hz) and an autoregressive [AR(1)] filter to model aliased biorhythms and unmodelled activity/noise. The primary contrast is withdrawal minus infusion. This contrast map will be used in subsequent group analyses.

Functional Connectivity: Generalized psychophysiological interaction (gPPI) analysis[54] estimates functional connectivity for each ROI as in our previous publication.[50] Briefly, an Eigenvariate time series will be extracted and a second regression analysis performed, modeling the infusion/withdrawal blocks *and* the *a priori* ROI time series (mPFC, SMA, Insula; see Fig. 2) and its interactions with 'infuse' and 'withdraw' blocks. The 'infuse–withdrawal' contrast will be calculated to investigate the differential connectivity of the ROI and the entire brain between infuse and withdraw. This contrast map will be used in subsequent group analyses.

Resting state analysis: We will extract the eigenvariate of three a priori selected ROIs and calculate voxel-wise connectivity maps for each region. We will also use ReHo, which evaluates regional changes in connectedness that are thought to correspond to local activation (temporal and spatial). These connectivity maps will be used in subsequent group analyses. We will evaluate connectivity in full and empty bladders.

BST activity: Task-related activity within the BST will be assessed using a ROI mask[79-81] hand-drawn using a template based on the Atlas of the Human Brain[82], plates 18 through 24 (Talairach y = -2.7 to +2.7mm; central, medial, lateral and ventral divisions). To acquire estimates of activity, we will use a random effects analysis with an averaged contrast map of all participants. A t-test will identify clusters in each ROI displaying significant BOLD signal changes (false discovery rate, FDR of 0.05) and cluster threshold of 20 voxels. Individual BOLD signal change in ROIs will be described by the first eigenvariate of the adjusted contrast in the cluster.

Covariates: We will add age, co-morbidities, adrenergic drugs and potentially fMRI reactivity as covariates in a multiple regression model in SPM to control for these factors. If standard model assumptions are violated, we will use nonparametric counterparts of the proposed approach based on the Statistical non-Parametric Mapping (SnPM) toolbox (http://warwick.ac.uk/snpm).

Corrections: All hypothesis-testing analyses will be targeted at clusters within the *a priori* ROIs, using a small-volume correction to further reduce the impact of the multiple comparisons inherent in examining many voxels. At a later hypothesis-generating stage, we shall search for trends to significance in additional brain regions, based on uncorrected probability values (e.g. $p < 0.01$ or 0.001) in accordance with common practice.

Structural connectivity and tractography

DSI: data will be collected using a 20-min, 271-direction sequence using a twice-refocused spin-echo EPI sequence and multiple *q* values (TR = 4250 ms, TE = 150 ms, voxel size = 2.4 X 2.4 X 2.4 mm, FoV = 230 X 230 mm, b-max = 4,000 s/mm^2), motion and eddy current corrected, and reconstructed using DSI Studio software and a Q-space diffeomorphic reconstruction (QSDR) approach.[83] Tractography will be performed in

individuals using a seed-based approach. *STwm*: the seed will be a fornix ROI dilated to the mid-point of the head of the caudate encompassing the STwm and terminal vein[84]; left and right STwm fibers will be separated from the fornix. *MFB*: a dorsal brainstem seed will be used to generate tractography; fibers that course through the mid-brain tegmentum towards BST/PVN will be selected (Fig. 4C). Measures of structural integrity (generalized fractional anisotropy, GFA, and normalized quantitative anisotropy, NQA), which describe the diffusion of water molecules around neuronal compartments (e.g., myelin, neurofilaments and microtubules) will be extracted from each individual.[85] Larger values are interpreted as greater structural integrity. NQA is superior to DTI-derived FA; it is less sensitive to partial volume effects of crossing fibers and free water and provides better tractography resolution and fewer false fibers than FA tractography[86]. These measures provide mechanistic indices of demyelination (i.e., increased diffusion *across* the axon) and/or axonal degeneration (e.g., reflecting decreased diffusion along the *length* of the axon due to debris). [87,88] "Neighboring DWI correlation"[89] will be used to exclude outlying images with low data quality using a standard 3 median absolute deviation standard. *FLAIR*: White matter hyperintensities will be assessed using in-house software[90] to analyze the FLAIR image and will be available for necessary corrections.

Approach to Hypothesis 1: Normal brain response to bladder filling can be characterized in young

Having completed the basic analysis detailed in C.2.2.5.2 for the young, continent group, we will be able to identify the main brain areas involved in continence control in this group at the $p<0.01$ cluster corrected level, which we can qualitatively assess in conjunction with our working model. In addition, we will use the functional connectivity analyses to further understand the connections within the control system. The goal will be to confirm the veracity of our working model in this population and potentially add detail.

Secondary analysis will include identification of significantly active brain area clusters with >16 contiguous voxels, cluster corrected, not already included in the *a priori* analysis, and use of these as seed clusters to perform further functional connectivity calculations (methods above).

Secondary qualitative comparison will involve evaluating the activation elucidated by the contrasts and the interaction between brain areas to assess the plausibility of each candidate as part of the working model.

Hypothesis 2: Changes in continent bladder control occur with aging and can be detected

The analysis for hypothesis 1 will be repeated in the ‘old continent’ group and we will fit a series of second-level models using SPM’s multiple regression procedure with infusion-withdrawal contrast t-value (= activation level) as the dependent variable and age group as the factor of interest. A series of t-tests will compare functional connectivity at rest and during the task state between the two age groups and allow us to assess changes in successful bladder control with age. Changes seen in the older group might reflect successful compensation for age-related changes in the continence mechanism, or incipient pathological changes; we will examine these in the context of our previous studies and the working model to address hypothesis 4.

When comparing age groups, special attention will be paid to differences in structural integrity of the brain; this will reveal changes related to aging but unrelated to UUI since these groups are continent. Global white matter damage volumes will be compared between the two groups, and significance of differences in structural damage in each of the identified tracts between age groups will be assessed by Mann-Whitney tests as in our previous work[92]. Location and volume of grey matter atrophy will also be compared between the two groups. These comparisons will allow us to assess structural brain changes which are unrelated to UUI, or those which can be easily compensated for by the functional changes.

Approach to Hypothesis 3: Abnormalities associated with UUI can be identified in young and old

We will identify the main brain areas involved in the continence mechanism in both the old and young 'wet' groups at the $p<0.01$ cluster corrected level. We will use the functional connectivity analyses to evaluate the connections within the 'faulty' control system and compare between the age groups, allowing us to evaluate the differences in the control mechanism according to age. Differences between the young and old wet groups will suggest the variation in contribution of the brain changes to incontinence.

Secondary analysis will include identification of significantly active brain area clusters with >16 contiguous voxels, cluster corrected, not already included in the *a priori* analysis, and use of these as seed clusters to perform further functional connectivity calculations (methods above). Additionally, qualitative comparison will involve evaluating the activation elucidated by the contrasts and the interaction between brain areas to understand how each group differs in light of the working model, leading to hypothesis 4.

Hypothesis 4: Data from all 4 groups will suggest incidental, pathological, compensatory changes

We will use results from hypotheses 2-3 (*continent* old vs young, and *UUI* old vs young) and examine further contrasts of age-matched group pairs (i.e. *old* UUI vs dry and *young* UUI vs dry) at the $p<0.01$ cluster corrected level to elucidate and potentially determine the contributors to age-related UUI. We will evaluate all results in light of the working model and our prior studies. We will consider the location and the direction of the functional brain changes and how these fit with the circuits identified in the model and results from other research groups; we will also pair the functional results with the structural data. Comparison of young and old continent individuals from hypothesis 2 will allow us to discount the benign age-related changes. We will use our prior studies and knowledge of the working model to propose which remaining changes are likely compensatory and which are likely causal. In turn, this should highlight changes to suppress, enhance, or ignore in the quest for a brain-based therapy. We will also use the same comparison pairs to evaluate changes in structural (grey and white matter) abnormalities such as location and volume assessed by Mann-Whitney tests. These comparisons will allow us to assess structural brain changes that are unrelated to bladder control, and those that may be causal in light of their location and our current knowledge of the working model.