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Functional Connectivity of the Brain In Older Women With Urgency Urinary Incontinence

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Abstract

Background—The brain's role in continence is critical but poorly understood. Although regions activated during bladder stimulation have been identified, little is known about the interaction *between* regions. In this secondary analysis we evaluate resting state and effective connectivity in older women treated for urgency urinary incontinence (UUI).

Method—54 women 60 years old with UUI and 10 continent women underwent fMRI scanning during provocation of urinary urgency, both before and after therapy. Response was defined by >50% reduction in leaks on bladder diary. Regions of interest (RoIs) were selected *a priori*: right insula, medial prefrontal cortex, and dorsal anterior cingulate cortex. Generalized psychophysiological interaction (gPPI) was used to calculate 'effective connectivity' between RoIs during urgency. We performed a one-way ANOVA pre-treatment between groups (continent/responders/non-responders), as well as a two-way mixed ANOVA between group and time (responders/non-responders; pre-/post-therapy) using false discovery rate (FDR) correction. Principal component analysis was used to assess the variance within RoIs. Exploratory voxel-wise connectivity analyses were conducted between each RoI and the rest of the brain.

Results—RoI-RoI connectivity analysis showed connectivity differences between controls, responders and non-responders, although statistical significance was lost after extensive correction. Principal component analysis confirmed appropriate RoI selection.

Voxel-wise analyses showed that connectivity in responders became more like that of controls after therapy (cluster-wise correction $p < 0.05$). In non-responders, no consistent changes were seen.

Conclusion—These data support the postulate that responders and non-responders to therapy may represent different subsets of UUI, one with more of a central etiology, and one without.

Keywords

Functional connectivity; urgency urinary incontinence; fMRI; bladder; pelvic floor muscle therapy

Introduction

Urgency urinary incontinence (UI) is prevalent, morbid, and costly, especially among older adults. Its effects range from significant reduction in quality of life, to considerable public health burden. While current understanding of UI is based largely on bladder behavior, it has become apparent that higher bladder control mechanisms are important. Accordingly, we initiated an investigation of the brain-bladder connection.

Building on our previous studies^{1,2}, we conducted a new functional magnetic resonance imaging (fMRI) study to assess the role of the brain in UI³. We evaluated older women with UI receiving biofeedback-assisted pelvic floor muscle therapy (PFMT), which focuses on identifying the correct muscles via biofeedback, urge suppression strategies, and behavioral techniques to avoid panic reactions to urgency⁴ and compared them to a similar continent group ('controls') who did not receive treatment using three different approaches. Firstly, we examined *functional* MRI BOLD (Blood Oxygenation Level Dependent) activation differences associated with continence status and response to therapy, focusing on three main regions of interest (RoIs) identified consistently in previous studies: medial prefrontal cortex (mPFC – executive control); supplementary motor area/dorsal anterior cingulate cortex (SMA/dACC – motor control); and right insula (visceral sensation).^{1,5} Women who responded well to therapy had different baseline brain activation patterns associated with urgency³ and these patterns tended to 'normalize' (i.e. look more like the control group's reaction to bladder stimulation) among responders, but not among non-responders. Next we analyzed *structural* brain differences. Results suggested that at baseline, those who responded well to PFMT initially had more overt structural damage which somewhat improved; we hypothesized that this may be a subset in which the main contributor to the UI was brain-based.⁶

Here we present the third approach, a hypothesis-driven secondary analysis of this data, focusing on the *functional connectivity* of *a priori* selected brain areas during rest and urinary urgency, specifically differences between continent women and those with UI, and changes attributed to the effects of PFMT. We assessed 'resting state' and 'effective' task-based connectivity.

'Resting state functional connectivity' assesses the inter-connectivity of individual brain regions and pre-defined networks when not assigned a task. Differences in resting state connectivity between groups might help explain differences in continence status and responsiveness to treatment.

'Effective connectivity', which is measured using generalized psychophysiological interaction (gPPI), evaluates the connectivity between *a priori* defined RoIs during a task, in this case, suppression of voiding in response to repeated small-bolus infusion of fluid into the bladder. This analysis highlights differential connectivity between RoIs during the 'urgency' and 'non-urgency' states, and therefore suggests components of active mechanisms that may be related to continence. In addition, we conducted an *exploratory* voxel-wise analysis to determine connections between each individual RoI and the rest of the brain.

We investigated and compared continent controls (the ‘normal’ reaction to the urgency stimulating protocol), responders, and non-responders to therapy, and assessed how these baseline differences changed post-treatment. We expected this analysis to reveal how connections between these *a priori* RoIs differ according to continence status and treatment effect, providing more evidence to refine our working model of continence and allowing us to generate additional hypotheses concerning how these regions interact with the rest of the brain.

Methods

Study Design

This analysis utilized a dataset comprising community dwelling, functionally intact women over 60 years of age with UUI > five times per week³ who had undergone brain fMRI before and after PFMT.³ Data from a similar cohort of continent women who had undergone similar baseline evaluation, served as controls. Baseline assessment included clinical and incontinence history, videourodynamic testing, and 3-day bladder diary with 1-day pad diary. All participants underwent an hour-long MRI scanning procedure following baseline assessment. For the UUI group, the MRI visit was repeated approximately three months later, after 3 sessions of biofeedback-assisted PFMT following Burgio’s protocol⁷. Incontinent women were stratified into ‘responders’ (> 50% reduction in leakage episodes on diary) and ‘non-responders’ (< 50% reduction) to PFMT.^{3,8,9} All participants gave written informed consent prior to all procedures. The University of Pittsburgh IRB approved the study.

MRI Protocol

For each MRI visit, participants underwent structural and functional scans on a Siemens 3T Trio TIM scanner with a 12-channel head coil at rest (empty bladder), and during the filling task at both low and high bladder volume (technical data in appendix).

Each participant had a five minute ‘resting state’ fMRI scan with their bladder drained < 20 minutes prior. Subsequently, fMRI scanning was carried out during repeated infusion and withdrawal of a small bolus of fluid at low volume (empty bladder) and high volume (filled via catheter to ‘strong desire to void’). This task¹⁰ consisted of four repeats of this sequence: 12 second pause; 22 ml water infused over 12 seconds (Infuse); 12 second pause; and 20 ml withdrawn (Withdraw) over 12 seconds..

Regions of Interest (RoIs)

Three *a priori* RoIs (sphere; r=18mm) were selected based on our previous studies and working model of continence control:¹¹ insula (MNI [38 16 6]); dorsal anterior cingulate/supplementary motor area (dACC/SMA, MNI [4 14 42]); and medial prefrontal cortex (mPFC, MNI [4 50 14]).

All connectivity analyses were carried out between each pair of these three RoIs (hypothesis-driven analysis) or using each of the RoIs as a seed region for connectivity with

the rest of the brain (exploratory analysis). The validity of these regions is tested using principal component analysis (PCA) of spatial distribution.

Resting state functional connectivity analysis

Resting state functional connectivity was assessed both between our *a priori* RoIs and within well-established brain networks, on an empty bladder (results in appendix).

Exploratory voxel-wise resting state connectivity, assessing temporal correlation of brain activity between the seed RoI and every other voxel in the brain, is reported on grouped with the exploratory voxel-wise effective connectivity.

Effective Connectivity (Generalized Psychophysiological Interaction - gPPI)

Connectivity between RoIs was explored using gPPI (Appendix). Brain activity in the three regions during the infusion and withdrawal conditions (the ‘task’) was modeled and temporally correlated with activity in: (1) each of the other two RoIs, giving a regression coefficient or ‘connectivity value’ for each RoI pair (RoI-RoI Effective Connectivity); and (2) every other voxel in the brain, giving a spatial map of connectivity (Exploratory Voxel-wise Effective Connectivity). This approach assesses the temporal correlation of brain activity during the task; highly correlated areas suggest a ‘connection’ during the bladder filling task, which does not exist at rest, and suggest that the two brain areas potentially ‘communicate’ during bladder control.

Statistical Analysis

RoI-RoI Effective Connectivity: We extracted ‘mean connectivity’ from the gPPI connectivity map, between each seed RoI and the other two RoIs at low and high bladder volume. For each of these measures, we performed a one-way independent ANOVA to compare baseline differences between healthy controls, responders, and non-responders. We then performed a 2-way mixed ANOVA with pre- and post-treatment as the repeated measures, and the responder/non-responder group as the independent measure. We tested for significant interactions: group differences in the change between pre- and post-treatment; time effects (pre-post differences adjusting for group); and group effects (responder and non-responder differences adjusting for time). We performed multiple comparisons correction using the false discovery rate (FDR) method¹².

Exploratory Voxel-wise Connectivity: We utilized voxel-wise resting state or gPPI connectivity maps for each RoI, at low and high bladder volume. We used a one-way ANOVA at baseline to examine group differences (including controls) and a paired t-test to examine treatment effects independent of group. All results were corrected using a cluster-level inference method that controls the family wise error (FWE) rate ($p < 0.05$).

Principal Component Spatial Distribution.

We assessed the origins of the identified ‘connections’ within RoIs using principal component analysis. We transformed PCA loadings into Z-scores, with a threshold of 2 standard deviations above the mean. We calculated, for each voxel, the likelihood that the loading was greater than 2 standard deviations from the mean. This resulted in one

likelihood map per RoI, showing the distribution of connections, allowing us to evaluate the shape and size of our chosen RoI.

Results

Ten of 11 continent women and 54 of 60 incontinent women had the necessary fMRI data for analysis. Table 1 shows demographic and clinical variables. ‘Main Effects’ fMRI activation has already been reported³.

RoI-RoI Effective Connectivity (Table 2)

Unlike the remainder of our results, the significant relationships seen in RoI-RoI effective connectivity ($p < 0.05$) were no longer significant after extensive correction for age and multiple comparisons. However, uncorrected results at $p < 0.05$ are highlighted in table 2 and these trends are displayed graphically in Fig 1 to enable visualization of the networks. We summarize them here. In this gPPI analysis ‘connectivity difference’ describes the difference between connectivity during the ‘infuse’ task and the ‘withdraw’ task.

At high bladder volume:

- dACC-insula connectivity: non-responders had higher *connectivity difference* than responders at both baseline and post-treatment;
- mPFC-dACC connectivity: responders had higher *connectivity difference* than the non-responders at both baseline and post-treatment.

At low bladder volume:

- insula-mPFC connectivity: a group by time interaction effect showed responders had increased *connectivity difference* post-treatment while non-responders decreased.

Exploratory Voxel-wise Connectivity

Our *exploratory* voxel-wise analyses, both resting state and gPPI, are presented in Table 3 and described here. Only results that passed multiple comparisons correction (cluster wise inference using FWE correction) are reported.

In the resting state, we report any significant differences in temporal correlation of the seed region and another brain region between groups (responder/non-responder) or time (pre-/post-intervention):

Resting state (Figure 2)

- dACC to caudate/thalamus connectivity: significant group by time interaction – connectivity decreases following treatment in responders (towards controls) and increases in non-responders (away from controls).
- Insula to cerebellum/culmen connectivity: significant group by time interaction – connectivity decreases in responders following therapy (away from controls) and increases in non-responders (towards controls).

In the task states, we found significant changes in the ‘connectivity difference’, the difference between connectivity during the ‘infuse’ task and the ‘withdraw’ task, with group or time.

Low volume task (Figure 3)

- Insula to primary visual cortex connectivity: significant decreases in connectivity in both responders and non-responders (towards controls)

High volume task (Figure 1)

- dACC to primary visual cortex connectivity: significant group by time interaction – connectivity difference decreases in responders (towards controls) and increases in non-responders (away from controls).
- Insula to motor/sensory cortex connectivity: significant group by time interaction – connectivity difference decreases in responders (towards controls) and increases in non-responders (away from controls).
- mPFC to middle cingulate connectivity: responders had greater connectivity difference than both controls and non-responders at baseline
- insula to primary visual cortex connectivity: responders had greater connectivity difference than non-responders at baseline. Controls showed lower infuse relative to withdraw connectivity (negative connectivity difference).

Principal Component Spatial Distribution

Figure 1 shows brain sections associated with the three RoIs indicating the spatial distribution of the origin of connections within the spheres. In the dACC/SMA, the majority of the variance from the first principal component was from a region closer to the dACC than the SMA. Within the other two RoIs, the anterior areas were more strongly represented.

Discussion

Our aim was to further elucidate how regions known to be integral to continence interact with each other and the rest of the brain, and how these interactions change with continence status and in response to treatment (PFMT). These associations allow us to suggest possible brain mechanisms associated with UII that should be further investigated.

Although these results are associations and do not prove causal effect, findings are congruent with our prior data^{3,6} and supportive of our hypothesis that there are two subsets of UII: one in which the predominant contributor is within the brain and responds to PFMT; and another in which the predominant contributor is elsewhere and does not respond to biofeedback therapy which targets the brain.

Principal component of spatial distribution

The principal component of spatial distribution of connections within the original RoIs suggests that these were appropriately selected, if slightly off center. This suggests that our

analysis most closely resembles the connectivity of the dACC (rather than SMA), anterior insula, and anterior mPFC.

RoI-RoI Effective connectivity

Although RoI-RoI comparisons did not survive extensive correction for multiple comparisons in gPPI analysis, table 2 and figure 1 reveal trends at $p < 0.05$ (uncorrected). In the low volume task, insula-mPFC connectivity after therapy changes differently in responders to therapy (away from ‘normal’) compared to non-responders. Such differences might represent a compensatory reaction in responders, since the changes correlate with treatment improvement, but in the *opposite* direction to ‘normalization’. At high volume, changes tend more towards ‘normal’ in responders than non-responders. Since this is the urgency simulation task, we suggest that high volume normalization could reflect normalization in response to successful therapy. It is unclear why this changes in the opposite way to the change seen at low volume, but may represent part of a more complex mechanism invoked to re-recruit areas involved in this circuit that we have yet to fully understand.

Exploratory voxel-wise results

Initial analysis of the connections between the three RoIs and the rest of the brain included a consistency check of the direction of connectivity changes. Significant connections of each correlated cluster displayed patterns consistent with nearby regions (e.g. clusters identified as significant around the caudate all showed changes in similar directions), lending confidence to our interpretation.

Resting state—During resting state, much of the basal ganglia exhibited significant differences in connectivity to the dACC between treatment response groups both before and after therapy. Involved are the caudate (motor processing, process learning, inhibitory control of actions), putamen (movement regulation) and thalamus (motor/sensory relay, regulation of sleep/consciousness); each has been identified as involved in continence in previous studies^{13–16}. This suggests that motor-processing mechanisms may vary depending on continence status and may be altered by therapy.

Task at low and high volume—Use of three seed RoIs to examine connections to the rest of the brain suggests that the occipital areas play a significant role in continence, specifically the lingual area, responsible for both visualization and analysis of the logical order of events. This has been seen in our previous studies, but not emphasized, due to the lack of *a priori* selection. These areas, which form connections with the insula and dACC, may form a coping mechanism for urgency, especially since responders tend to ‘normalize’ these connections after therapy. As non-responders remain in the normal range, their lack of response may suggest why these strategies are not useful –connections were not ‘abnormal’ initially.

The connection between the dACC and visual cortex is present even at low volumes, though it diminishes after therapy, possibly indicating a change in perception of the test.

At baseline, responders exhibit significant differences in connectivity from non-responders. These differences are between the mPFC seed region and the precuneus, cingulum and post-central gyrus. This finding is consistent with our working model. While our previous work focused on the individual areas involved, this analysis shows connectivity *between* these areas, which is dependent on continence status. These associations are consistent with this connectivity being a potential contributor to or cause of the disease.

The insula seed region exhibits significant differences in connectivity with the motor/sensory cortex, depending on treatment response, both before and after therapy. This also supports our original hypothesis of related connectivity between the insula and motor area.

Previously, we have postulated that there are two types of UUI⁶, both of which have some degree of underlying bladder dysfunction, but one in which abnormalities of brain control play a major role and one in which the brain's role is less important. Since the effect of PFMT is presumably mediated through brain mechanisms, then individuals with the first type of UUI—in which baseline brain connectivity is most abnormal—might be more likely to respond to PFMT, and develop connectivity closer to that seen in continent controls. By contrast, among those in whom the brain plays a less prominent role, connectivity might appear more normal at baseline and would be little affected by PFMT. Our data are consistent with both hypotheses. It is likely that symptom severity is a combination of underlying bladder dysfunction and the ability of the brain to compensate for it in the above ways.

Existing Literature on Connectivity in UUI

There have been few other investigations into the functional connectivity of brain areas involved in continence control. Ketai et al¹⁷ found that the interoceptive network was involved differently in continent and incontinent people; specifically, the connection with attention networks. These results complement our own resting state findings (see appendix). Our findings are also congruent with those described by Nardos et al¹⁸ who identified seven regions having significantly different connectivity during resting state functional connectivity analysis, including the same three found here (posterior cingulate, post-central gyrus, and caudate). Nardos also¹⁹ identified other regions that corroborate our results, although their resting state connectivity protocol included comparison with a full bladder and therefore is not directly comparable to our own.

Limitations: An 'unnatural' catheter-based infuse/withdraw protocol had to be used to make repeated measures of the brain's response to urgency and thereby obtain an acceptable fMRI signal-to-noise ratio. This protocol, which other research groups have subsequently implemented,^{15,20} reproducibly simulates urgency, since 'natural' urgency is neither reliable nor reproducible in a scanning environment. We focused on the brain's role, positing biologically plausible explanations for both a brain *cause of* and *reaction to* bladder behavior, but must emphasize that these are *contributors* which act alongside other (e.g. bladder) abnormalities as a cumulative cause of UUI.

Conclusion

These analyses highlight how brain areas interact, and how interactions differ between disease states and change with therapy. When coupled with our previously published structural and functional findings, our results suggest that there may be at least two mechanisms contributing to UUI: one in which a breakdown in central control plays the greater role and is thus responsive to a behaviorally targeted therapy like PFMT and another in which central control is still largely intact and for which a brain/behavior-targeted approach is less useful. We neither recommend nor envisage this fMRI technique becoming a clinical tool, however, if these results are confirmed by others, the findings may lead to new approaches to the pathogenesis, diagnosis and treatment of UUI, and also advance understanding of the brain-bladder mechanism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

dACC	dorsal anterior cingulate cortex
FWE	Familywise error
gPPI	generalized psychophysiological interaction
mPFC	medial pre-frontal cortex
MNI	Montreal Neurological Institute
PFMT	(biofeedback-assisted) pelvic floor muscle therapy
RoIs	regions of interest
SMA	Supplementary motor area
UUI	Urgency Urinary Incontinence

References

1. Griffiths DJ, Tadic SD, Schaefer W, Resnick NM. Cerebral control of the lower urinary tract: how age-related changes might predispose to urge incontinence. *Neuroimage*. 2009;47(3):981–986. [PubMed: 19427909]
2. Griffiths D, Derbyshire S, Stenger A, Resnick N. Brain control of normal and overactive bladder. *The Journal of Urology*. 2005;174(5):1862–1867. [PubMed: 16217325]
3. Griffiths D, Clarkson B, Tadic SD, Resnick NM. Brain Mechanisms Underlying Urge Incontinence and its Response to Pelvic Floor Muscle Training. *J Urol*. 2015;194(3):708–715. [PubMed: 25828973]
4. Burgio KL, Goode PS, Locher JL, et al. Behavioral training with and without biofeedback in the treatment of urge incontinence in older women: a randomized controlled trial. *JAMA*. 2002;288(18):2293–2299. [PubMed: 12425706]

5. Tadic SD, Griffiths D, Schaefer W, Resnick NM. Abnormal connections in the supraspinal bladder control network in women with urge urinary incontinence. *Neuroimage*. 2008;39(4):1647–1653. [PubMed: 18089297]
6. Clarkson B, Griffiths D, Resnick NM. Do brain structural abnormalities differentiate separate forms of urgency urinary incontinence? *Neurourology and Urodynamics*. 2018;[epub ahead of print].
7. Burgio KL, Locher JL, Goode PS, et al. Behavioral vs drug treatment for urge urinary incontinence in older women: a randomized controlled trial. *JAMA*. 1998;280(23):1995–2000. [PubMed: 9863850]
8. Resnick NM, Perera S, Tadic S, et al. What Predicts and what Mediates the response of urge urinary incontinence to biofeedback? *Neurourology and Urodynamics*. 2013;32(5):408–415. [PubMed: 23168606]
9. Tadic SD, Griffiths D, Schaefer W, Murrin A, Clarkson B, Resnick NM. Brain activity underlying impaired continence control in older women with overactive bladder. *Neurourology & Urodynamics*. 2012;31(5):652–658. [PubMed: 22473921]
10. Clarkson BD, Tyagi S, Griffiths DJ, Resnick NM. Test-retest repeatability of patterns of brain activation provoked by bladder filling. *Neurourol Urodyn*. 2016.
11. Griffiths D Neural control of micturition in humans: a working model. *Nat Rev Urol*. 2015;12(12):695–705. [PubMed: 26620610]
12. Benjamini Y, Y H. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B (Methodological)*. 1995;57(1):289–300.
13. Tadic SD, Griffiths D, Murrin A, Schaefer W, Aizenstein HJ, Resnick NM. Brain activity during bladder filling is related to white matter structural changes in older women with urinary incontinence. *Neuroimage*. 2010;51(4):1294–1302. [PubMed: 20302947]
14. Fowler CJ. Integrated control of lower urinary tract--clinical perspective. *Br J Pharmacol*. 2006;147 Suppl 2:S14–24. [PubMed: 16465178]
15. Shy M, Fung S, Boone TB, Karmonik C, Fletcher SG, Khavari R. Functional Magnetic Resonance Imaging During Urodynamic Testing Identifies Brain Structures Initiating Micturition. *The Journal of Urology*. 2014;192(4):1149–1154. [PubMed: 24769029]
16. Mehnert U, Boy S, Svensson J, et al. Brain activation in response to bladder filling and simultaneous stimulation of the dorsal clitoral nerve - An fMRI study in healthy women. *Neuroimage*. 2008;41(3):682–689. [PubMed: 18434200]
17. Ketai LH, Komesu YM, Dodd AB, Rogers RG, Ling JM, Mayer AR. Urgency urinary incontinence and the interoceptive network: a functional magnetic resonance imaging study. *Am J Obstet Gynecol*. 2016;215(4):449.e441–449.e417. [PubMed: 27173081]
18. Nardos R, Gregory WT, Krisky C, et al. Examining mechanisms of brain control of bladder function with resting state functional connectivity MRI. *Neurourology & Urodynamics*. 2014;33(5):493–501. [PubMed: 23908139]
19. Nardos R, Karstens L, Carpenter S, et al. Abnormal functional connectivity in women with urgency urinary incontinence: Can we predict disease presence and severity in individual women using Rs-fcMRI. *Neurourology and Urodynamics*. 2016;35(5):564–573. [PubMed: 25933352]
20. Leitner L, Walter M, Freund P, et al. Protocol for a prospective magnetic resonance imaging study on supraspinal lower urinary tract control in healthy subjects and spinal cord injury patients undergoing intradetrusor onabotulinumtoxinA injections for treating neurogenic detrusor overactivity. *BMC Urol*. 2014;14:68–74. [PubMed: 25132340]
21. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage*. 2009;44(1):83–98. [PubMed: 18501637]

Figure 1.

Visualization of the interconnectivity during urgency (gPPI high volume task). *A priori* chosen RoIs (mPFC, dACC and Insula) are shown depicted by the principal component spatial distribution of the origin of connections within the 18mm radius sphere. Connections between these are trends which do not reach significance (grey plots). Other results show the significant clusters showing connectivity with the associated RoI (plots with black outlines). Violin plots are displayed to show the distribution of connectivity parameters within the cluster. The 25, 50 and 75th centile markers are shown as black lines within the plots. Values indicate the difference in the regression terms between infuse $\times$ RoI minus withdraw $\times$ RoI, which represent the differential connectivity of the RoI and the significant region during infuse compared to withdraw (positive values indicate connectivity greater during infuse relative to withdraw). Note that while we have plotted controls, they serve only as a reference as this analysis did *not* utilize that group. Significant interactions are denoted by

blue arrows; areas where a group difference was found are shown with a purple arrow (graphs are shaded grey if not significant but trending).

Figure 2.

Significant group (responders/non-responders) by time (pre-/post-treatment) differences during resting state using the dACC and insula seeds. The color bar indicates the F-statistic of the interaction term for the dACC-caudate/thalamus (left, top) and insula-cerebellum culmen connectivity (left, bottom). Violin plots are displayed to show the distribution of connectivity parameters within the cluster. The 25, 50 and 75th centile markers are shown as black lines within the plots. Note that while we have plotted controls, it serves only as a reference as this analysis did *not* utilize that group. Values indicate the connectivity between the RoI and the significant region at rest.

Figure 3.

Significant time (pre vs post-treatment) differences (independent of group) during the low volume infusion task using the insula seed. The color bar indicates the T-statistic of the paired t-test for the insula-primary visual cortex connectivity.). Violin plots are displayed to show the distribution of connectivity parameters within the cluster. The 25, 50 and 75th centile markers are shown as black lines within the plots. Note that while we have plotted controls, they serve only as a reference as this analysis did *not* utilize that time point. Values indicate the difference in the regression terms between $\text{infuse} \times \text{RoI (Ins)}$ minus $\text{withdraw} \times \text{RoI}$, which represent the differential connectivity of the RoI and the significant region during infuse compared to withdraw (positive values indicate connectivity greater during infuse relative to withdraw).

Table 1,

demographics of participants (*control vs UI significantly different by t-test $p < 0.05$; no other significant differences using Z test of proportions). All were independent in all IADLs and scored $>28/30$ on MMSE. Those on anticholinergics had taken the same drug and dose consistently for 6 months prior to, and throughout the study.

	All UI (n=54)		Responders (n=26)		Non Responders (n=28)		Controls (n=10)
Age (years)	70.6		67.3		73.6		63.3*
Anticholinergic use	7 (13%)		1 (4%)		6 (21%)		0
Depression (history)	18 (33%)		7 (27%)		11 (39%)		2 (20%)
Diabetes	12 (22%)		6 (23%)		6 (21%)		0
Stroke	8 (15%)		4 (15%)		4 (14%)		1 (10%)
Hysterectomy	28 (52%)		14 (54%)		14 (50%)		2 (20%)
UI episodes /24h	Pre	Post	Pre	Post	Pre	Post	
	3.4	1.9	3.3	0.7	3.5	3.0	

Table 2,

1-way ANOVA comparing connectivity measures between controls, responders, and non-responders. A 2-way mixed ANOVA investigates (excludes controls) the interaction between group (responders/non-responders) and time (pre-/post-treatment), as well as the group differences (independent of time) and time differences (independent of group). Both comparisons control for age. It is important to note that *none* of these results pass a multiple comparisons correction (FDR), however we indicate trends at $p < 0.05$ in grey.

Measure	Statistical Test	1-way ANOVA (Baseline)		2-way Mixed ANOVA (Group by Time)					
	Comparison	Control vs. Resp. vs. Non-Responders		Group by Time Interaction		Time Effect (Pre vs. Post)		Group Effect (Pre vs. Post)	
	Association	F-statistic (2,61)	P-value	F-statistic (1,52)	P-value	F-statistic (1,52)	P-value	F-statistic (1,52)	P-value
High gPPI	dACC - Ins	3.49	0.037	0.94	0.337	0.03	0.858	8.63	0.005
	dACC - mPFC	0.95	0.394	0.27	0.606	0.81	0.372	1.73	0.195
	Ins - dACC	0.04	0.961	0.04	0.841	2.52	0.119	0.02	0.892
	Ins - mPFC	0.97	0.385	0.11	0.746	1.30	0.259	2.47	0.122
	mPFC - dACC	1.71	0.189	0.24	0.630	0.20	0.657	4.59	0.037
	mPFC - Ins	0.34	0.712	0.03	0.869	1.06	0.307	2.05	0.158
Low gPPI	dACC - Ins	0.21	0.809	0.40	0.532	0.21	0.647	0.57	0.454
	dACC - mPFC	0.34	0.716	1.24	0.271	1.31	0.258	0.53	0.470
	Ins - dACC	0.78	0.462	0.62	0.436	1.11	0.296	0.22	0.642
	Ins - mPFC	2.47	0.093	5.24	0.026	1.08	0.304	0.51	0.480
	mPFC - dACC	1.08	0.345	1.13	0.293	0.01	0.920	0.60	0.442
	mPFC - Ins	0.00	0.996	0.04	0.844	0.68	0.414	0.04	0.841

Table 3.

Results of an *exploratory* voxel-wise analysis showing only *significant* results (as determined by a cluster-wise inference FWE multiple comparisons correction). The columns indicate the analysis performed, the task (rest and high/low infusion), the connectivity seed (dACC, Ins (insula), or mPFC), the hemisphere, the region that was significant (determined via the automatic anatomical labeling or AAL atlas), the Brodmann area (if any) associated with that region, the network that the region falls into (determined by the Smith networks²¹ threshold at $Z > 3$ and minimum cluster size of 50 voxels), the cluster size (in number of voxels), the maximum T-statistic, and the location (x, y, z) in MNI space of the max.

Analysis	Task	Seed	Side	Region	BA	Networks	# Voxels	Tmax	x, y, z
Group By Time Differences	Rest	dACC	L	Caudate	12		163	4.2	-18, 18, 8
			R			LECN	86	3.7	18, -8, 20
			L	Pallidum			55	4.1	-10, 2, 2
			L	Putamen	35		178	4.4	-18, 16, 6
			R		37		104	3.9	28, -4, 10
			L	Thalamus			83	4.3	-10, -4, 4
			R			LECN	63	3.6	2, -12, 6
		Ins	R	Cerebellum/ Culmen	37	vDMN	61	4.0	28, -34, -32
			L	Cerebellum Tonsil			659	5.5	-28, -50, -48
			R				245	4.4	20, -62, -46
			L	Cerebellum Tonsil			352	4.6	-14, -52, -50
			R				110	4.4	18, -50, -46
	High	dACC	R	Calcarine	18, 19		75	3.1	26, -92, 4
			L	Cerebellum /Declive	19, 37		156	3.8	-28, -52, -20
			L	Fusiform	19, 37		311	4.3	-30, -54, -12
			R		18, 19		124	4.0	24, -74, -6
			L	Lingual	18		461	4.4	-20, -70, -6
			R		18		345	4.5	22, -74, -4
			L	Inferior Occipital	18		85	3.5	-22, -86, -10
			R		19		58	3.2	34, -78, -8
			L	Middle Occipital	18		69	3.1	-22, -88, 6
			R		18, 19		218	3.7	40, -86, 4
			R	Superior Occipital	18, 19	vDMN	71	3.5	30, -74, 20
		Ins	L	Middle Cingulate		ASN	154	4.1	-16, -32, 44
			R				172	4.0	18, -30, 46
			R	Paracentral Lobule	4	vDMN	80	3.6	16, -38, 48
			L	Inferior Parietal	40	ASN	387	5.5	-26, -46, 50
			L	Superior Parietal	2, 7	ASN	139	5.2	-26, -46, 52
			L	Post-central	3		683	5.4	-30, -36, 56
			R		3		99	3.4	34, -26, 44
			L	Pre-central	4, 6		292	4.9	-34, -12, 50

Analysis	Task	Seed	Side	Region	BA	Networks	# Voxels	Tmax	x, y, z
			L	Precuneus		ASN	102	3.5	-14, -46, 44
			L	Supplemental Motor	6		179	4.2	-16, -6, 64
			R		4, 6		350	4.1	12, -16, 54
Baseline Group Differences	High	Ins	R	Calcarine	18, 19		320	8.5	22, -78, 8
			R	Fusiform	18, 19		185	8.9	24, -62, -8
			R	Lingual	18, 19		510	12.3	20, -72, -6
			R	Inferior Occipital	18, 19		80	7.0	32, -88, 0
			R	Middle Occipital	18		117	7.6	34, -88, 2
		mPFC	R	Middle Cingulate	23	ASN	99	6.5	4, -28, 48
			L	Paracentral Lobule	4	vDMN	91	7.9	-10, -26, 54
			R		4	vDMN	68	6.1	8, -30, 52
			L	Post-central	3		203	10.0	-22, -34, 58
			R	Supplemental Motor	4	vDMN	85	6.5	2, -24, 64
Pre - Post	Low	dACC	R	Calcarine	17, 18		269	9.9	8, -84, 10
			L	Cuneus	18	dDMN	99	11.7	4, -86, 26
			R		18		191	13.1	6, -86, 26

Table 4,

1-way ANOVA comparing connectivity measures between controls, responders, and non-responders. A 2-way mixed ANOVA investigates (excludes controls) the interaction between group (responders/non-responders) and time (pre-/post-treatment), as well as the group differences (independent of time) and time differences (independent of group). It is important to note that *none* of these results pass a multiple comparisons correction (FDR), however we indicate results with some effect in red ($p < 0.05$).

Measure	Statistical Test	1-way ANOVA (Baseline)		2-way Mixed ANOVA (Group by Time)					
	Comparison	Healthy vs. Resp. vs. Non-Resp.		Group by Time Interaction		Time Effect (Pre vs. Post)		Group Effect (Pre vs. Post)	
	Association	F-statistic (2,61)	P-value	F-statistic (1,52)	P-value	F-statistic (1,52)	P-value	F-statistic (1,52)	P-value
Rest ROI	dACC - Ins	0.56	0.577	0.01	0.943	1.16	0.287	1.49	0.227
	dACC - mPFC	0.59	0.560	0.08	0.776	0.00	0.989	1.43	0.237
	Ins - mPFC	0.39	0.680	1.59	0.212	2.35	0.131	0.00	0.975
Rest Intra-Network	dDMN	0.56	0.574	0.32	0.577	0.55	0.461	0.91	0.345
	vDMN	1.55	0.221	0.83	0.368	0.02	0.877	1.34	0.252
	ASN	2.57	0.085	3.13	0.081	1.15	0.288	0.20	0.659
	LECN	0.72	0.492	0.09	0.772	0.01	0.919	0.03	0.871
	RECN	1.95	0.151	1.00	0.322	1.19	0.280	0.12	0.726
Rest Inter-Network	dDMN - vDMN	2.40	0.099	0.12	0.727	0.29	0.594	4.78	0.033
	dDMN - ASN	0.38	0.684	0.28	0.598	1.41	0.240	0.51	0.479
	dDMN - LECN	0.15	0.863	0.09	0.770	4.41	0.041	0.15	0.697
	dDMN - RECN	0.54	0.586	0.00	0.965	0.09	0.768	0.78	0.383
	vDMN - ASN	1.68	0.196	0.68	0.412	0.15	0.698	3.61	0.063
	vDMN - LECN	1.75	0.183	0.24	0.624	3.94	0.052	2.96	0.091
	vDMN - RECN	0.42	0.658	0.00	0.961	0.33	0.571	0.47	0.497
	ASN - LECN	2.81	0.068	0.01	0.912	4.23	0.045	3.97	0.052
	ASN - RECN	0.36	0.700	0.00	0.981	0.23	0.638	0.68	0.412
	LECN - RECN	0.01	0.991	0.13	0.717	0.45	0.505	0.14	0.707