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Brain responses to mechanical rectal stimuli in patients with faecal incontinence: an fMRI study

Short Title: Brain responses in faecal incontinence – an fMRI study

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- NM contributed to study design, data collection, analysis and interpretation, and preparation of manuscript.
- SH contributed to data analysis and interpretation and assisted in drafting and reviewing the manuscript.
- KSN contributed to study design and review of the manuscript.
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Abstract

Aim

Continence is dependent on anorectal/brain interactions. Consequently, aberrations of the brain-gut axis may be important in the pathophysiology of faecal incontinence (FI) in certain patients. The aim of this study was to assess the feasibility of recording brain responses to rectal mechanical stimuli in patients with FI using functional Magnetic Resonance Imaging (fMRI).

Method

A prospective, cohort pilot study was performed to assess brain responses during rectal stimulation in 14 patients (4 male, mean [SD] age 62 [15] years). Blood oxygen level-dependent (BOLD) signals were measured by fMRI during rest and mechanical distension, involving random repetitions of isobaric phasic rectal distensions at fixed (15 & 45 mmHg) and variable (10% above sensory perception threshold) pressures.

Results

Increases in BOLD signals in response to high-pressure rectal distension (45mmHg) and maximum toleration were observed in the cingulate gyrus, thalamus, insular cortex, inferior frontal gyrus, cerebellum, caudate nucleus, supramarginal gyrus, putamen and amygdala. Additionally, activation of the supplementary motor cortex and caudate nucleus with inconsistent activity in the frontal lobe was observed.

Conclusions

This study has demonstrated the feasibility of recording brain responses to rectal mechanical stimulation using fMRI in patients with FI, revealing activity in widespread areas of the brain involved in visceral sensory processing. The observed activity in the supplementary motor cortex and caudate nucleus, with relative paucity of activity in the frontal lobes, warrants investigation in future studies to determine whether aberrations in cerebral processing of rectal stimuli play a role in the pathogenesis of FI.

What does this paper add to the literature?

It is feasible to record brain responses to rectal distension using fMRI in patients with FI. Central aberration in the processing of visceral afferent signals is a potential pathophysiological mechanism for the development of FI. Specifically, activity in the supplementary motor cortex, caudate nucleus, and frontal lobes warrants further investigation.

Introduction

Faecal incontinence (FI) is a debilitating condition that affects 10% of adults in the community.[1] The traditionally held view that FI occurs solely due to anal sphincter dysfunction secondary to neurological[2] or anatomical disruption[3] has been superseded by the contemporary understanding that 'extra-sphincteric' mechanisms may also be important in the development of FI. Notably, abnormalities of the rectal reservoir, leading to problems with the storage[4] and / or evacuation of faeces[5] may result in FI. The maintenance of continence is dependent on a complex interaction between somatic, enteric and autonomic nervous systems and smooth and striated muscles under higher cerebral control,[6] although these areas have not received equal attention by researchers. Abnormal communications between the rectum and the brain (i.e. the brain-gut axis) may play an important role in the pathophysiology of FI in certain patients. Indeed, abnormalities of peripheral and central nerve conduction[7] and higher cerebral and cognitive function have been shown to lead to FI.[8] [9]

Functional magnetic resonance imaging (fMRI) is the gold standard to evaluate brain function, although there have been no previous fMRI studies in patients with FI. Therefore, the aim of this study was to assess the feasibility of recording brain responses to rectal mechanical stimuli using fMRI in patients with FI to determine whether a meaningful fMRI response occurs after rectal distension in such patients.

Materials & Methods

Subjects

Patients with FI to solid or liquid stool, irrespective of type (i.e. urge, passive or mixed), occurring more frequently than once per week were eligible for inclusion. Exclusion criteria included: (a) cognitive impairment/dementia, (b) psychiatric disorders (c) pregnancy, (d) neurological disorders (e) current treatment with medications with a central effect e.g. antiepileptics/neuroleptics and (f) contraindications to a MRI scan (e.g. implanted electric and electronic devices etc.) Accordingly, 14 subjects (4 male) with a mean age of 62 (SD 15) years participated and underwent assessment of the severity of incontinence (Vaizey incontinence score[10]). All 10 women were multiparous. Significant past history in women included: traumatic vaginal delivery (n=10), rectal prolapse (n=1) and in men included: previous anorectal surgery (n=2) and diabetic neuropathy (n=2). All participants provided written informed consent. Ethical approval was obtained from the University of Sydney (2014/494) and Sydney Local Health District, Concord Hospital (2012-158).

Baseline Anorectal Physiological Investigation

Assessment of anorectal function was performed using station pull-through manometry (including assessment of maximum anal resting and squeeze pressures and rectal sensation) and endoanal ultrasound (EAUS), as previously described.[11] [12] Anal canal pressures were compared to published normal values,[13] and rectal hypersensitivity and hyposensitivity were diagnosed when rectal sensory threshold volumes were below[14] or elevated above[15], the normal range, respectively.

Rectal distension and determination of individual rectal perception thresholds

Rectal distensions were performed using a barostat (Distender series II; G&J Electronics, Inc, Toronto, Canada) in accordance with accepted recommendations.[16] One lumen of a closed-tip double lumen catheter was attached to the output port of the barostat for insufflation and the other to the pressure transducer port to monitor pressure directly within the barostat bag. An ultra-thin polyolefin bag with a maximum capacity of 600 mL was secured over a distance of 10 cm at the distal end of the catheter. The barostat was placed outside the MRI scanner room and connected to the anorectal catheter via a 5 m long polyvinyl extension tubing with an internal diameter of 4.76 mm and an external diameter of 7.94 mm. Subjects were instructed to open their bowels prior to attendance but enemas were not administered to avoid potential stimulatory effects on the rectum. The anorectal catheter was inserted into the rectum, ensuring that it came to rest with the tip at 15 cm from the anal verge to ensure that the distending bag was located entirely above the anal canal and only stimulated the rectum during insufflation. It was then taped securely to prevent movement during fMRI acquisition. The balloon was insufflated with 100 mL of air after insertion and then deflated.[17] Subsequently, individual perception threshold pressures for three rectal sensations (First Constant Sensation Pressure [FSP], Desire to Defaecate Pressure [DDP] and Maximum Tolerated Pressure [MTP])[18 19] were determined using ascending methods of limits during phasic isobaric distension (4 mmHg increments every 30 seconds followed by 30 seconds at 0 mmHg) up to a maximum of 48 mmHg or until maximum toleration was reached.

Rectal Distension Paradigm

The distension paradigm involved 'fixed' and variable rectal pressures (supraliminal); the latter tailored to the individual to allow for inter-subject variation in perceptual sensitivity on account of anticipated differences in rectal sensitivity and / or compliance.[15] These variable

(supraliminal) distensions were set at 10% above each individual's previously determined rectal sensory threshold and were each delivered three times in random order (see Figure 1) for a duration of 30 seconds. Two 'fixed' pressure distensions, one at 15 mmHg (low) and the other at 45 mmHg (high)[20] were also repeated three times in random order (see Figure 1).

fMRI Data Acquisition

MRI data acquisition was performed using a 3T scanner. A structural T1 weighted sequence was acquired with a Repetition Time (TR)=6.5s; Echo Time (TE)=2.4ms; flip angle=12°; matrix=210×210; Field of View (FOV)=230×230mm and slice thickness=1.1mm. Functional T2* weighted images were collected in an axial plane, using a spin echo EPI sequence with the following parameters TR=3s; TE=32ms; FOV=240×240mm; matrix=64×64; 150 whole brain acquisitions. Each volume contained 32 interleaved 4 mm slices. Image acquisition and delivery of rectal stimuli were synchronized.

Data analysis

Data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00. XYZ coordinates were based on the Montreal Neurological Institute (MNI) system.[21] Whole brain analyses were performed. Prior to formal statistical processing, all data was subjected to pre-processing (e.g. motion correction to account for translational motion). In order to decrease the effects of anatomical variability, the data were spatially smoothed using a 5 mm full width half maximum Gaussian kernel. All T1-images had non-brain structures removed using the brain extraction tool of the software (BET).[22] Time-series statistical analysis was carried out using FILM (FMRIB Improved Linear Model) with autocorrelation correction.[23] Z statistical images were thresholded using clusters determined by Z>2.3 and a corrected

cluster significance threshold of P= 0.05.[24] Contrasts included comparison of responses of absolute stimulus (i.e. rectal distension to 45mmHg, 15mmHg, FSP, DDP and MTP) versus rest (0 mmHg) and relative stimulus (e.g. 45mmHg compared to 15mmHg). Given the small sample size, subgroup analysis could not be reliably performed. Results were tabulated with standard space coordinates (x y z), in mm, determined for each region of brain. The number of voxels (volumetric element of brain as detected by fMRI) in each area (cluster regions) was determined. Cluster sizes indicate the number of voxels within each cluster and are only provided for local maxima (i.e. regions with the highest statistical value but not necessarily the highest size). Results are also illustrated using statistical maps by overlaying the mean area activated for the group on a standard space template of the brain and is color rendered to signify its corresponding z value (which is a normalized p value). The lower the Z score, the greater the probability of a false positive result.

Results

Participant characteristics

The baseline clinical and physiological characteristics of the participants are shown in Table 1. Five reported passive incontinence, 2 urge incontinence and the remaining 7 mixed (passive and urge) incontinence. All patients experienced moderate/severe FI with a median Vaizey score of 16.5 (range, 13-24). In terms of anorectal physiology, median anal resting pressures were low. More specifically, 12 (86%) and 4 patients (29%) had reduced resting and squeeze pressures, respectively. Six patients had evidence of rectal hyposensitivity and two patients rectal hypersensitivity. Sonographic evidence of anal sphincter disruption was

noted in 10 patients (71%). Notably, the vast majority of subjects (13/14) perceived fixed rectal distension at 45 mmHg as painful.

Altered BOLD signal in response to 'fixed' pressure rectal distension

When compared to BOLD signals at rest (i.e. 0 mmHg), repeated application of high pressure (45mmHg) mechanical stimuli resulted in activation (i.e. increased BOLD signals) of two main clusters of the brain: the left cingulate gyrus [voxel size 23517, P<0.001] and left supramarginal gyrus [1516, P <0.001]. Other statistically significant areas of the brain with increased BOLD activity are listed in Table 2 and illustrated in Figures 2 & 3.

Areas of the brain activated when comparing high pressure (45 mmHg) to low pressure (15 mmHg) included the right insular cortex [voxel size: 15296, coordinates: 38,18, -4, P<0.001, Z= 4.61], left cerebellum [size: 5341, coordinates: -28, -60, -36, P<0.001, Z= 4.17], left supramarginal gyrus [size: 2019, coordinates: -60, -36, 34, P<0.001, Z= 4.49], right supramarginal gyrus [size: 1676, coordinates: 60, -28, 24, P<0.001, Z= 4.48] and right lateral occipital cortex [size: 1396, coordinates: 12, -60, 56, P<0.001, Z= 3.70].

Given the statistical threshold employed, no brain regions showed significant BOLD activation in response to low pressure (15 mmHg) relative to the resting phase. Similarly, no consistent deactivation (i.e., reduced BOLD signal) was noted for the group using random effect analysis for either the high or low pressure compared to rest. Controlling for patient's age, gender or HAD score did not considerably modify the brain regions activated (see Figure 4).

Altered BOLD signal in response to 'variable' pressure rectal distension

Brain regions activated in response to MTP versus rest included the left cingulate gyrus [voxel size 2125, P<0.001], right inferior frontal gyrus [1212, P= 0.015], cerebellum [1376, P=0.008] and right supramarginal gyrus [978, P=0.039]. All other statistically significant areas are listed in Table 3 and illustrated in Figure 5. No significant activation was observed in response to distension pressures in the range of FSP or DDP (versus 0 mmHg), consistent with the lack of activation to low pressure (15 mmHg) distensions during the fixed pressure part of the paradigm (see above).

Comparison of BOLD signal response during 'fixed' and 'variable' pressure rectal distension

As shown in Tables 2 and 3, there was activation in the Supplementary Motor Cortex (SMC) and the putamen during fixed distension to 45mmHg, although such activation was not evident in response to distension to MTP (variable pressure). By contrast, a significant response was observed in both caudate nuclei in response to variable pressure distension to MTP but this was not evident during distension to a fixed distension pressure of 45 mmHg. Figure 6 shows the areas activated in response to 45mmHg (pain) versus those in response to maximum toleration (i.e. MTP) and revealed that the areas of brain with increased in BOLD signal were anatomically distinct when comparing brain responses of 45mmHg distension with that of MTP distension.

Discussion

This pilot study demonstrated the feasibility of recording brain activity following rectal distension in patients with FI. A meaningful response was noted with activation of widespread areas of the brain involved in visceral sensory processing. Additional activity in the supplementary motor cortex and caudate nucleus and a relative paucity of activity in the frontal lobes was noted which is in contrast to other published studies of healthy subjects using a similar experimental design. Consequently, further studies with a direct comparison to a control group are needed to determine if aberrations in cerebral processing of rectal stimuli play a role in the pathogenesis of FI in some patients.

All patients included in the study had moderate / severe FI on objective assessment using the Vaizey incontinence score, although there was heterogeneity in the associated aetiopathophysiological factors. The pathophysiology of fecal incontinence is complex and multifactorial. Normal rectal function is important in the maintenance of continence, particularly as the perception of 'graded' rectal distension provides early awareness of impending defaecation[19] and heightened and reduced awareness of rectal distension (rectal hyper[4]- and hypo-sensitivity[5], respectively) can affect normal continence mechanisms. Indeed, there was evidence of abnormal rectal sensation in 43% of patients in the present study.

Brain responses to distending stimuli in healthy cohorts and patients with functional gastrointestinal disorders suggest that three central networks are responsible for processing visceral signals:[25, 26] (i) the homeostatic visceral network (insula, mid-cingulate gyrus, thalamus and orbitofrontal cortex), which is involved in pain perception and interoception of gut signals; (ii) the emotional visceral network (anterior insula, hippocampus, amygdala,

anterior cingulate gyrus, dorsal pons and medial prefrontal cortex) generates appropriate affective responses to the perception of that stimulus; and (iii) the cortico-modulatory network (medial and lateral prefrontal cortices) regulates the overall cognitive modulation of visceral sensory perception by decreasing activity in limbic regions such as the amygdala and anterior insula. The primary and secondary somatosensory cortices also receive signals from the rectum.[27] In this study, there was consistent activation in the regions described to be part of the homeostatic and emotional afferent networks, namely the cingulate gyrus, thalamus, insula, hippocampus and amygdala. However, no reliable activation was observed in the regions previously described to be part of the cortico-modulatory network, as most of the frontal lobe activation observed was in the lateral prefrontal cortex in response to high-pressure (45mmHg) rectal distension. However, the role of the other areas that were shown to be activated in this study (e.g. supramarginal gyrus, occipital cortex, temporal lobe and lingual gyrus) is less certain.

Previous reviews[28 29], including a meta-analysis[30] of the published literature, have established the definition of what is considered a 'normal brain response' to rectal distension in over 150 healthy volunteers. With these results in mind, the present study identified two areas of brain that were activated in response to rectal distension that have not been consistently reported in healthy volunteers; namely the supplementary motor cortex (SMC) and the caudate nucleus. Whilst merely speculative at this stage, these areas may be of particular importance in patients with FI and warrant further investigation in future controlled studies. The SMC controls movement and has been shown to be involved in evaluating 'one's actions'.[31] However, a previous study of 13 healthy subjects demonstrated that contractions of the external anal sphincter resulted in activation of the SMC.[32] Consequently, SMC activation in the present study may actually represent 'reflex' anal sphincter contraction in response to rectal distension.[32, 33] Interestingly, a recent study investigating patients with urinary urge

incontinence demonstrated increased activity in the SMC, possibly reflecting a compensatory mechanism when the 'normal' motor cortex fails to maintain continence.[34] Its activation in patients with FI may also represent early engagement to assist with the maintenance of continence as part of a compensatory mechanism. However, it should also be noted that activation of supplementary motor cortex has been seen in previous studies of healthy volunteers in response to visceral and somatic pain. However, it may not have been sufficiently strong to show significance in the aforementioned meta-analysis[30] on account of interstudy variability in subject characteristics and experimental parameters that brain imaging studies are susceptible to.

The caudate nucleus was activated in patients with FI. Generally, the caudate nucleus is thought to be involved in cognitive integration of sensation.[35] Whilst activation has not generally been noted in studies of healthy volunteers[28, 29], one previous study of 13 healthy volunteers did show activation of the caudate nucleus in response to rectal distension at levels of discomfort.[32] Consequently, further studies are required to determine whether activation of this area of brain is specific to patients with FI. In the present study, the lack of significant frontal lobe activation to rectal distension (especially at MTP) and that of the medial prefrontal lobe cortex during high pressure rectal distension in the present study may also provide some explanation for the pathophysiology of FI. The frontal lobes have been long known to be involved in inhibiting socially inappropriate behavior and learning from experiences. Historical observations in patients with frontal lobe lesions or resections noted significant issues with bladder and bowel control.[36-38] Indeed, it has been proposed that frontal lobe dysfunction results in loss of the gradual filling sensation of the organ (bladder or rectum) with preservation of sensation at extreme distension (maximum toleration) only.[37]

Abnormal central processing of rectal sensation during distension appears important in the development of FI. [15] A recent study revealed that cortical evoked potentials had significantly longer latency in patients with FI compared to controls, although it was unable to determine if this aberrant communication between the gut and the brain is at the level of the rectum, peripheral nerves or the CNS. [39] Interestingly, somatosensory evoked cortical potential amplitudes were reduced following crush or compression injury to the inferior rectal nerve in an animal model of neurogenic FI, leading the authors to conclude that alteration in cortical awareness may be the result of processing modification at a central and not peripheral level.[40]

This study has some limitations. Firstly, this study was a pilot study to determine whether a meaningful fMRI response could be measured following rectal distension in patients with FI. It did not include a control group of healthy volunteers and thus comparison with such a control group is required to determine any differences in activity and so begin to understand if aberrant central processes play a role in FI. Secondly, the patients were heterogeneous in terms of their clinical and pathophysiological characteristics, as the intention was to examine a representative sample reflective of patients with FI seen in clinical practice. Further studies are required to study more homogenous subgroups of patients with FI. Thirdly, it was not possible to exclude the confounding impact of central responses to anal canal stimulation due to the presence of the anorectal catheter used to deliver rectal stimulation, although attempts were made to minimize this by firmly securing the catheter in place to prevent undue anal stimulation. Finally, the sample size of patients in this study was relatively small, as is usually the case with neuroimaging studies given the logistical and financial challenges associated with conducting such studies. Consequently, it was not possible to perform sub-analyses based on underlying pathophysiology of FI.

In conclusion, this study has demonstrated the feasibility of recording brain responses to rectal mechanical stimulation using fMRI in patients with FI, revealing activity in widespread areas of the brain involved in visceral sensory processing. The observed activity in the supplementary motor cortex and caudate nucleus with relative paucity of activity in the frontal lobes warrants further investigation in future studies to determine whether aberrations in cerebral processing of rectal stimuli play a role in the pathogenesis of FI.

References

1. Ng K-S, Sivakumaran Y, Nassar N, Gladman MA. Fecal Incontinence: community prevalence and associated factors—a systematic review. Dis Colon Rectum. 2015;58(12):1194-209.
2. Snooks SJ, Swash M, Setchell M, Henry MM. Injury to innervation of pelvic floor sphincter musculature in childbirth. Lancet. 1984;324(8402):546-50.
3. Law PJ, Kamm M, Bartram C. A comparison between electromyography and anal endosonography in mapping external anal sphincter defects. Dis Colon Rectum. 1990;33(5):370-3.
4. Chan CLH, Lunniss PJ, Wang D, Williams NS, Scott SM. Rectal sensorimotor dysfunction in patients with urge faecal incontinence: evidence from prolonged manometric studies. Gut. 2005;54(9):1263-72.
5. Gladman MA, Scott SM, Williams NS, Lunniss PJ. Clinical and physiological findings, and possible aetiological factors of rectal hyposensitivity. Br J Surg. 2003;90(7):860-6.
6. Bharucha AE. Recent Advances in Functional Anorectal Disorders. Curr Gastroenterol Rep. 2011;13(4):316-22.
7. Snooks SJ, Swash M, Henry MM. Abnormalities in central and peripheral nerve conduction in patients with anorectal incontinence. J R Soc Med. 1985;78(4):294-300.
8. Ihnát P, Kozáková R, Rudinská LI, Peteja M, Vávra P, Zonča P. Fecal incontinence among nursing home residents: Is it still a problem? Arch Gerontol Geriatr. 2016;65:79-84.
9. Kushner DS. Changes in cognition and continence as predictors of rehabilitation outcomes in individuals with severe traumatic brain injury. J Rehabil Res Dev. 2014;51(7):1057.
10. Vaizey CJ, Carapeti E, Cahill JA, Kamm MA. Prospective comparison of faecal incontinence grading systems. Gut. 1999;44(1):77-80.
11. Eckardt VF, Jung B, Fischer B, Lierse W. Anal endosonography in healthy subjects and patients with idiopathic fecal incontinence. Dis Colon Rectum. 1994;37(3):235-42.
12. Scott SM, Gladman MA. Manometric, Sensorimotor, and Neurophysiologic Evaluation of Anorectal Function. Gastroenterol Clin North Am. 2008;37(3):511-38.
13. Jameson JS, Chia YW, Kamm MA, Speakman CT, Chye YH, Henry MM. Effect of age, sex and parity on anorectal function. Br J Surg. 1994;81(11):1689-92.

14. Chan CL, Lunniss PJ, Wang D, Williams NS, Scott SM. Rectal sensorimotor dysfunction in patients with urge faecal incontinence: evidence from prolonged manometric studies. Gut. 2005;54(9):1263-72.
15. Gladman MA, Dvorkin LS, Lunniss PJ, Williams NS, Scott SM. Rectal Hyposensitivity: A Disorder of the Rectal Wall or the Afferent Pathway? An Assessment Using the Barostat. Am J Gastroenterol. 2005;100(1):106-14.
16. Whitehead WE, Delvaux M. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. The Working Team of Glaxo-Wellcome Research, UK. Dig Dis Sci. 1997;42(2):223-41.
17. Hammer HF, Phillips SF, Camilleri M, Hanson RB. Rectal tone, distensibility, and perception: reproducibility and response to different distensions. Am J Physiol Gastrointest Liver Physiol. 1998;274(3):584-90.
18. Rao SSC, Azpiroz F, Diamant N, Enck P, Tougas G, Wald A. Minimum standards of anorectal manometry. Neurogastroenterol Motil. 2002;14(5):553-9.
19. Farthing MJ, Lennard-jones JE. Sensibility of the rectum to distension and the anorectal distension reflex in ulcerative colitis. Gut. 1978;19(1):64-9.
20. Larsson MBO, Tillisch K, Craig AD, Engström M, Labus J, Naliboff B, et al. Brain responses to visceral stimuli reflect visceral sensitivity thresholds in patients with irritable bowel syndrome. Gastroenterology. 2012;142(3):463-72.
21. Mazziotta JC, Toga AW, Evans A, Fox P, Lancaster J. A probabilistic atlas of the human brain: theory and rationale for its development the international consortium for brain mapping (ICBM). NeuroImage. 1995;2:89-101.
22. Smith SM. Fast robust automated brain extraction. Hum Brain Mapp. 2002;17(3):143-55.
23. Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate linear modeling of FMRI data. Neuroimage. 2001;14(6):1370-86.
24. Worsley KJ. Statistical analysis of activation images. Functional MRI: An introduction to methods. 2001;14:251-70.
25. Johnson LR, Gerwin TA. Gastrointestinal physiology: Mosby Elsevier Philadelphia; 2007.
26. Al Omran Y, Aziz Q. Functional brain imaging in gastroenterology: to new beginnings. Nat Rev Gastroenterol Hepatol. 2014;11(9):565-76.
27. Hobday DI, Aziz Q, Thacker N, Hollander I, Jackson A, Thompson DG. A study of the cortical processing of ano-rectal sensation using functional MRI. Brain. 2001;124(Pt 2):361-8.
28. Sheehan J, Gaman A, Vangel M, Kuo B. Pooled analysis of brain activity in irritable bowel syndrome and controls during rectal balloon distension. Neurogastroenterol Motil. 2011;23(4):336-e158.
29. Rapps N, Van Oudenhove L, Enck P, Aziz Q. Brain imaging of visceral functions in healthy volunteers and IBS patients. Journal of psychosomatic research. 2008;64(6):599-604.
30. Tillisch K, Mayer EA, Labus JS. Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. Gastroenterology. 2011;140(1):91-100.
31. Bonini F, Burle B, Liégeois-Chauvel C, Régis J, Chauvel P, Vidal F. Action monitoring and medial frontal cortex: leading role of supplementary motor area. Science. 2014;343(6173):888-91.
32. Bittorf B, Ringler R, Forster C, Hohenberger W, Matzel KE. Cerebral representation of the anorectum using functional magnetic resonance imaging. Br J Surg. 2006;93(10):1251-7.

33. Moisset X, Bouhassira D, Ducreux D, Glutron D, Coffin B, Sabaté JM. Anatomical connections between brain areas activated during rectal distension in healthy volunteers: a visceral pain network. Eur J Pain. 2010;14(2):142-8.
34. Tadic SD, Griffiths D, Schaefer W, Murrin A, Clarkson B, Resnick NM. Brain activity underlying impaired continence control in older women with overactive bladder. Neurourol Urodyn. 2012;31(5):652-8.
35. Grahn JA, Parkinson JA, Owen AM. The cognitive functions of the caudate nucleus. Prog Neurobiol. 2008;86(3):141-55.
36. Andrew J, Nathan PW. Lesions of the anterior frontal lobes and disturbances of micturition and defaecation. Brain. 1964;87(2):233-62.
37. Nathan PW. Lesions of the anterior frontal lobe and micturition disorders. . Brain and nerve. 1964;16:225-30.
38. Ueki K. Disturbances of micturition observed in some patients with brain tumor. Neurol Med Chir. 1960;2(1-2):25-33.
39. Haas S, Brock C, Krogh K, Gram M, Lundby L, Drewes AM, et al. Abnormal neuronal response to rectal and anal stimuli in patients with idiopathic fecal incontinence. Neurogastroenterology & Motility. 2015;27(7):954-62.
40. Peirce C, Healy CF, O'Herlihy C, O'Connell PR, Jones JFX. Reduced somatosensory cortical activation in experimental models of neuropathic fecal incontinence. Dis Colon Rectum. 2009;52(8):1417-22.

Table 1 Participant clinical and physiological characteristics

	Median	**Range**
Age (years)	65	20-91
Vaizey score	16.5	13-24
HAD score for anxiety	7.5	0-15
HAD score for depression	4	1-14
Anal resting pressure (mmHg)	20	12-76
Anal maximum squeeze pressure (mmHg)	51	18-130

Rectal sensory threshold volumes (mls)			
	FCS	40	10-180
	DD	115	40-240
	MT	213	40-360
Rectal sensory threshold pressures (mmHg)			
	FCS	12	4-36
	DD	24	8-44
	MT	28	12-48

FCS; First constant sensation, DD; Desire to defeacate, MT; Maximum toleration.

Table 2 Brain regions with increased BOLD activity at high fixed rectal pressure (45 mmHg) vs. rest

Cluster Region	**Side**	**Z score**	**X**	**Y**	**Z**
Cingulate gyrus	Right	3.16	12	16	34
	Left	5.15	-4	14	34
Lateral prefrontal Lobe	Left	4.71	-36	44	12

Cluster Region	Side	Z score	X	Y	Z
Inferior Frontal gyrus	Right	4.54	52	14	10
Paracingulate gyrus	Left	3.84	-48	16	4
	Right	4.44	4	36	34
Pars Opercularis	Right	4.41	56	16	6
Temporal lobe	Right	4.38	52	14	-2
Supramarginal gyrus	Left	4.16	-62	-28	24
Parietal Operculum cortex	Left	4.15	-62	-28	18
Insular cortex	Left	3.77	-38	6	6
	Right	3.80	36	6	4
Thalamus	Left	3.20	-14	-18	6
	Right	3.86	22	-24	6
Cerebellum	Left	3.23	-24	-62	-38
Putamen	Right	3.20	32	0	0
	Left	2.57	-28	-6	0
Lingual gyrus	Right	3.15	2	-76	2
SMC	Right	2.55	0	-10	60
	Left	3.16	-6	-8	58
Amygdala	Left	3.15	22	-6	-20

Table 3 Clusters within the brain where there was an increase in BOLD signal in response to a rectal mechanical stimulation at Maximum Tolerated Pressure (MTP) versus rest.

Cluster Region	Side	Z score	X	Y	Z
Caudate nucleus	Right	3.82	16	-2	22
	Left	3.65	-16	-6	20
Cingulate gyrus	Left	3.78	-4	-32	24
Inferior Frontal gyrus	Right	3.44	60	14	10
Frontal Operculum	Right	3.45	32	16	12
Frontal Orbital cortex	Right	3.23	52	28	-20
Insular cortex	Right	3.21	40	12	-16
Thalamus	Right	2.77	14	-4	14
	Left	2.78	-14	-4	14
Supramarginal gyrus	Right	3.72	60	-34	34
Parietal Operculum	Right	3.09	56	-34	30

Figure 1 **Schematic representation of the rectal distension paradigm.** The stimulation paradigm involved distension at variable pressures, set at 10% above each individual's first constant sensation (A); desire to defecate (B) and maximum toleration (C), as well as distension at fixed pressures of 15 and 45 mmHg. The bars represent 30 seconds stimulation each interrupted by 30 seconds rest (at 0 mmHg).

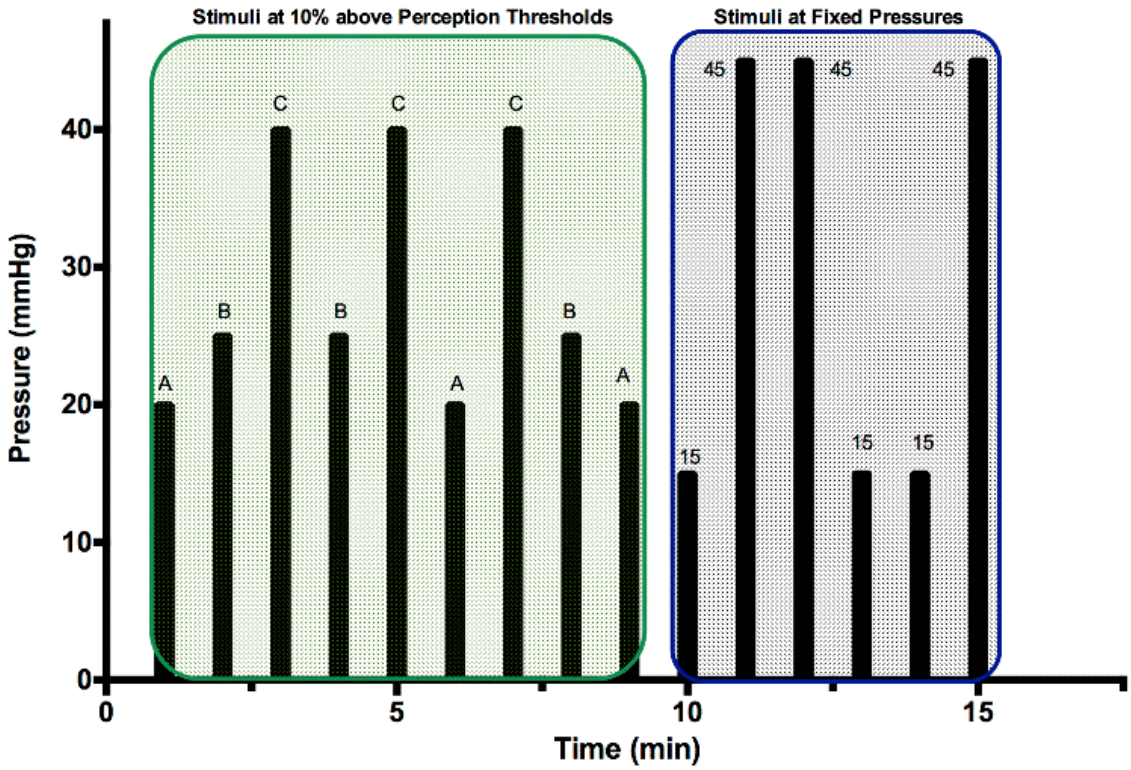

Figure 2 **Threshold activation brain maps (rendered on standard space template) in response to high-pressure rectal distension (45mmHg vs. rest)** showing widespread areas of the brain with increase in BOLD signal as listed in Table 1. The intensity of the color is representative of the Z score as shown in the horizontal bar in the bottom right.

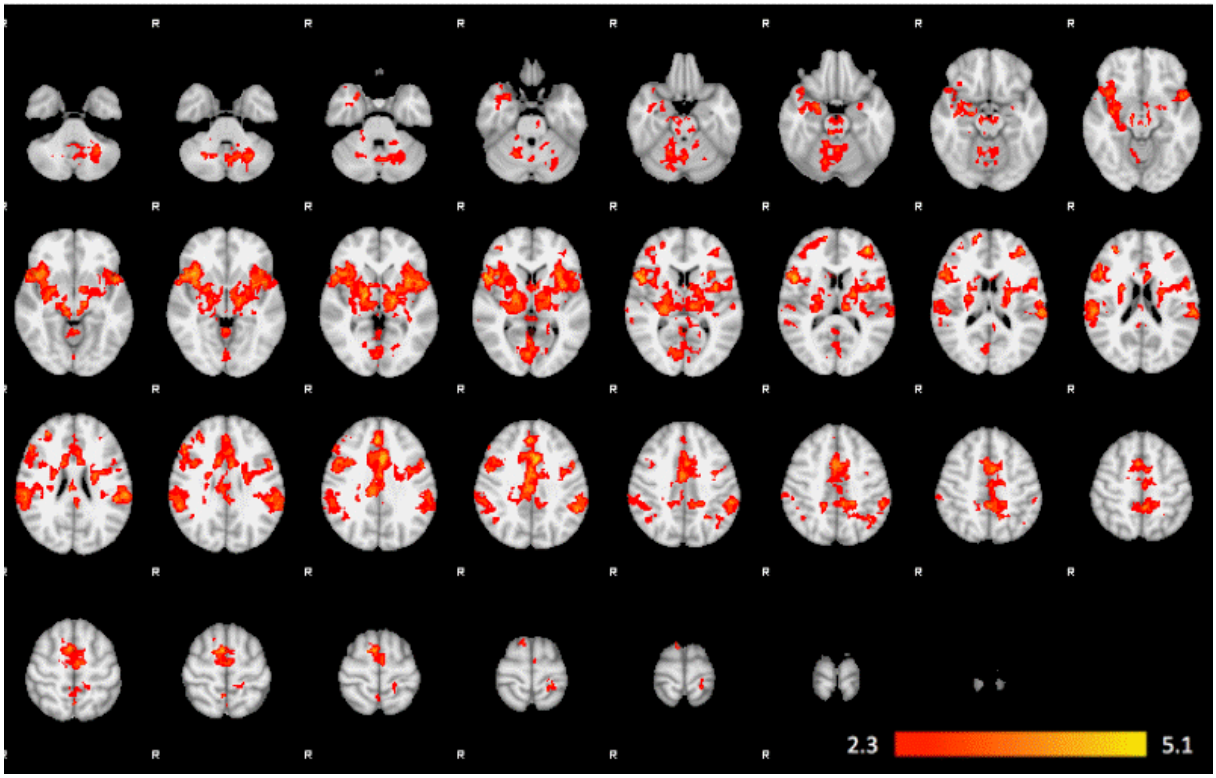

Figure 3 **3D rendered brain map image,** showing clusters of activity (P<0.05, Z>2.3) as seen in a patient with fecal incontinence in response to rectal distension at 45mmHg. The areas as highlighted in red include the supplementary motor cortex (S), the cingulate & paracingulate gyri (C, P), and the thalamus (T).

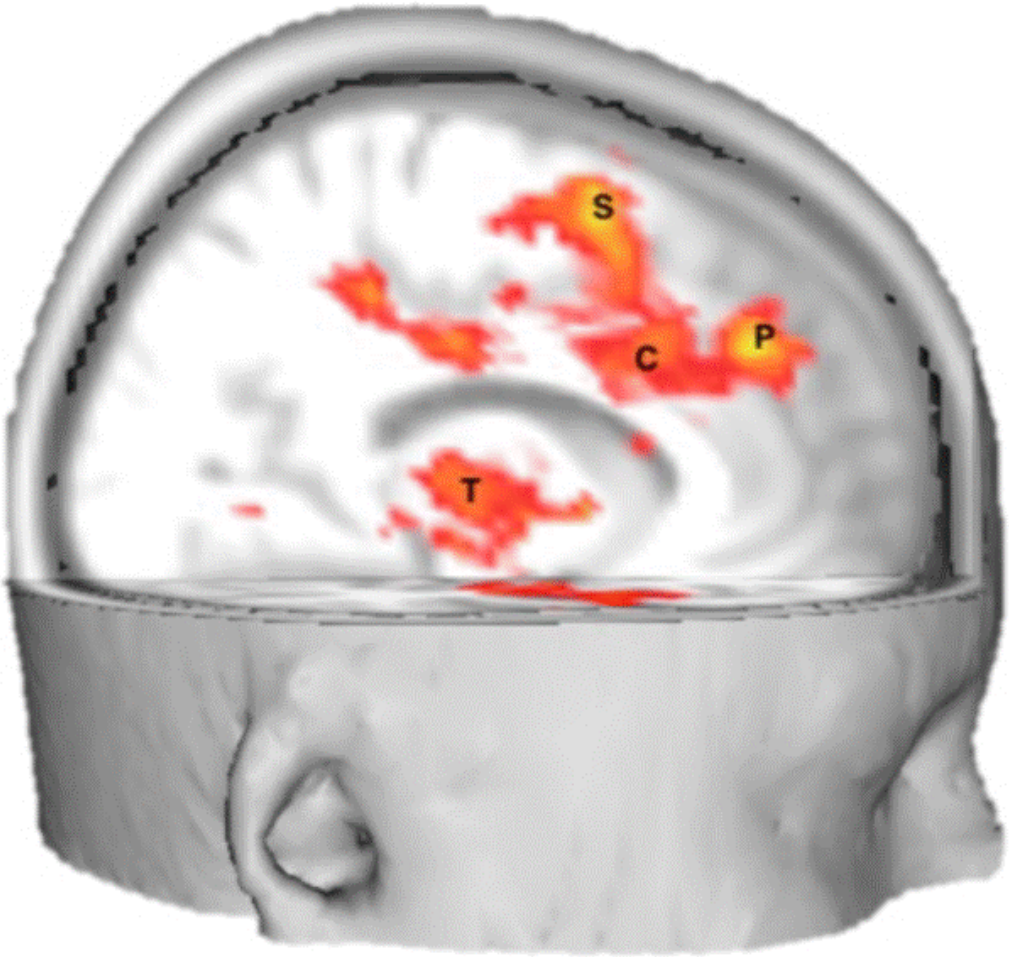

Figure 4 **Threshold activation brain maps (rendered on standard space template) in response to high-pressure (45 mm Hg) rectal distension controlled for hospital anxiety and depression scale and age.** The areas of brain with increased BOLD signal are similar in distribution to those activated in Figure 2, suggesting that age and psychological factors of the patients had minimal influence on the overall results.

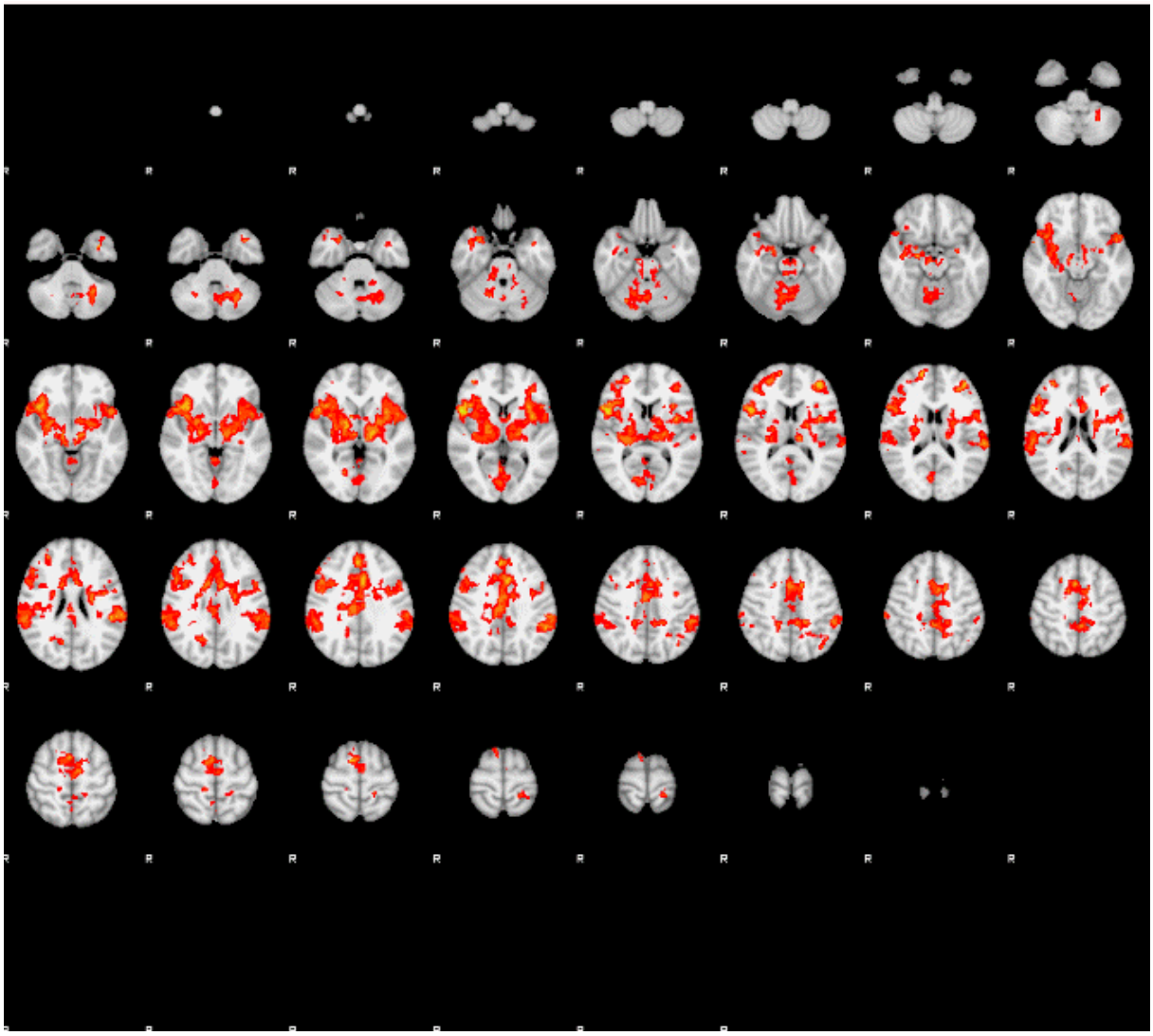

Figure 5 **Threshold activation images (rendered on standard space template) showing regions of brain with increased BOLD signal in response to rectal pressure distension in the range of maximum tolerated pressure.** The intensity of the color is reflective of the Z score as shown in the horizontal bar. Main areas illustrated include the thalamus, caudate nucleus, insular cortex and the supramarginal and cingulate gyri

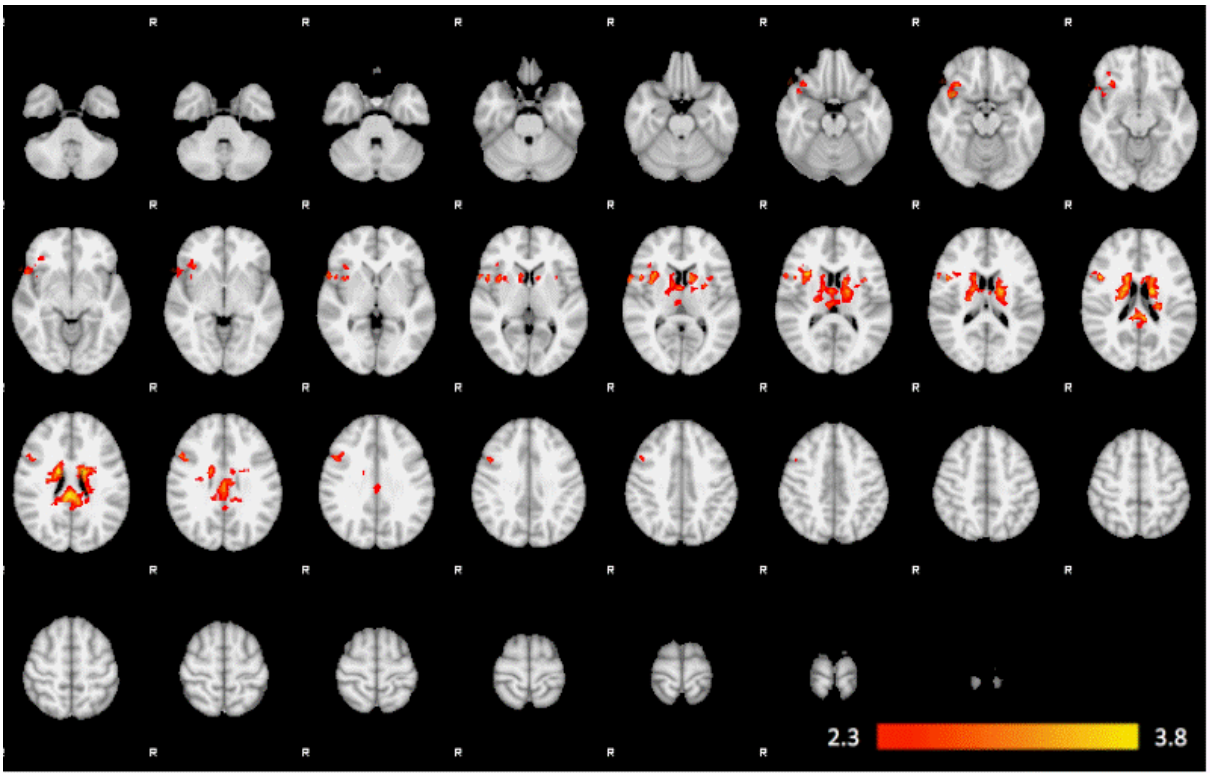

Figure 6 **Multiplanar standard space template of the brain overlaid with areas of the brain with statistically significant BOLD signal (P<0.05, Z>2.3) in response to painful rectal stimulus (red, 45mmHg) and sense of rectal urgency (MTP, green).** The figure illustrates different regions of brains that may process graded intensity of peripheral visceral signal (maximum urgency vs. pain).