Brain activation in response to bladder filling and simultaneous stimulation of the dorsal clitoral nerve-an fMRI study in healthy women

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Abstract: AIMS: Using functional magnetic resonance imaging (fMRI) we investigated the cortical and subcortical representations during bladder filling and the effect of simultaneous stimulation of the dorsal clitoral nerve on these cortical and subcortical structures. METHODS: After approval of the local ethics committee, 8 healthy females were included. Prior to scanning, subjects were catheterized and the bladder was filled until first desire to void occurred. In a block design protocol we performed repetitive manual bladder filling (FILLING) and emptying of additional 80 ml saline, alternating with rest conditions (REST) of constant bladder volume. The protocol was repeated with simultaneous stimulation of the dorsal clitoral nerve during the filling periods (COMBINED). Activation maps were calculated by means for 3 different contrasts: 1) FILLING>REST, 2) COMBINED>REST and 3) FILLING>COMBINED. RESULTS: A group analysis of contrast 1) showed activation of the right prefrontal and orbitofrontal cortices, the insula bilaterally, the left precuneus, the parietal operculum bilaterally, the cerebellum bilaterally (q(FDR)< or =0.001), the right anterior cingulate gyrus (q(FDR)< or =0.005) and the right anterior mid pons (q(FDR)< or =0.05). Contrast 2) showed activation in the right frontal area, the left insula, the parietal operculum bilaterally and the left cerebellum (q(FDR)< or =0.001). Deactivations were found in the middle frontal gyrus bilaterally and the post- and paracentral gyri bilaterally. Contrast 3) revealed stronger activation during FILLING in the bilateral frontal and prefrontal areas, the right anterior cingulated gyrus, and the right putamen (q(FDR)< or =0.05). Only the right insula showed stronger activation during the COMBINED condition. CONCLUSION: Simultaneous dorsal clitoral nerve stimulation during bladder filling reduced the activation of certain cortical areas suggesting a neuromodulatory effect of this stimulation on supraspinal centres involved in lower urinary tract control.

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Brain activation in response to bladder filling and simultaneous stimulation of the dorsal clitoral nerve—An fMRI study in healthy women

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Aims: **Using functional magnetic resonance imaging (fMRI) we investigated the cortical and subcortical representations during bladder filling and the effect of simultaneous stimulation of the dorsal clitoral nerve on these cortical and subcortical structures.**

Methods: **After approval of the local ethics committee, 8 healthy females were included. Prior to scanning, subjects were catheterized and the bladder was filled until first desire to void occurred. In a block design protocol we performed repetitive manual bladder filling (FILLING) and emptying of additional 80 ml saline, alternating with rest conditions (REST) of constant bladder volume. The protocol was repeated with simultaneous stimulation of the dorsal clitoral nerve during the filling periods (COMBINED). Activation maps were calculated by means for 3 different contrasts: 1) FILLING>REST, 2) COMBINED>REST and 3) FILLING>COMBINED.**

Results: **A group analysis of contrast 1) showed activation of the right prefrontal and orbitofrontal cortices, the insula bilaterally, the left precuneus, the parietal operculum bilaterally, the cerebellum bilaterally (q(FDR)≤0.001), the right anterior cingulate gyrus (q(FDR)≤0.005) and the right anterior mid pons (q(FDR)≤0.05).**

Contrast 2) showed activation in the right frontal area, the left insula, the parietal operculum bilaterally and the left cerebellum (q(FDR)≤0.001). Deactivations were found in the middle frontal gyrus bilaterally and the post- and paracentral gyri bilaterally.

Contrast 3) revealed stronger activation during FILLING in the bilateral frontal and prefrontal areas, the right anterior cingulated gyrus, and the right putamen (q(FDR)≤0.05). Only the right insula showed stronger activation during the COMBINED condition.

Conclusion: **Simultaneous dorsal clitoral nerve stimulation during bladder filling reduced the activation of certain cortical areas suggesting a neuromodulatory effect of this stimulation on supraspinal centres involved in lower urinary tract control.**

Keywords: fMRI; Bladder; Micturition control; Neuromodulation; Dorsal clitoral nerve; Urge to void

Introduction

Perception and processing of bladder filling sensations within the spinal cord and higher centres are crucial for the maintenance of continence. With increasing filling levels, bladder distension becomes stronger until it reaches consciousness and the wish to empty the bladder arises. Healthy subjects are without difficulty able to defer the act of voiding until appropriate circumstances are available. However, when desire to void becomes too strong or urgent, subjects may try to defer voiding by manoeuvres like manual genital squeezing (Kondo et al., 1982). Voluntary pelvic floor contractions can as well decrease the intensity of the desire to void and ameliorate urgency/frequency symptoms by activating an inhibitory reflex arc (Burgio et al., 1998). Genital stimulation, which includes stimulation of a branch of the pudendal nerve, could be successfully used in an urodynamical experiment to suppress urgency and delay sensations of bladder filling in patients suffering from overactive bladder syndrome (OAB) (Oliver et al., 2003; Shah, 1999a,b). During the storage phase of the bladder a sympathetic and a somatic reflex arc act as guarding reflexes that promote continence by activating the internal and external urethral sphincter (de Groat and Steers, 1990; Garry et al., 1959). However, control of continence and the functioning of the guarding reflex do not rely on spinal control only but also on the input from supraspinal brain structures (Morrison, 1987).

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Recent progresses in neuroimaging using positron emission tomography (PET) (Athwal et al., 2001; Blok et al., 1998, 1997b; Matsuura et al., 2002) and functional magnetic resonance imaging (fMRI) (Griffiths et al., 2005; Kuhtz-Buschbeck et al., 2005; Seseke et al., 2006; Zhang et al., 2005) demonstrated a network of cortical and subcortical structures involved in the control of lower urinary tract function.

The aim of our study is to demonstrate the modulatory effect of the stimulation of pudendal nerve branches – by manipulating the dorsal genital nerves – on the activation pattern of the supraspinal network, using fMRI. Our specific hypothesis is that stimulation of the dorsal clitoral nerve would decrease desire to void and therefore, decrease activation in the supraspinal centres involved in lower urinary tract (LUT) control (especially the limbic and orbitofrontal centres).

Subjects and methods

Subjects

After approval from the local ethics committee, a group of healthy female volunteers was recruited. The inclusion criteria were as follows: healthy female subjects without any medical illness, especially no neurological or urological disorders. The exclusion criteria were: pregnancy, urinary tract infection, any medical illness, any intake of prescription drugs or drugs influencing cerebral blood flow, any incompatibility with MRI safety rules (e.g. ferromagnetic materials in or on the body).

All participants had to give their written informed consent before entering the study. Current urinary tract infection and pregnancy were ruled out using dipstick tests prior to the investigation.

Imaging

The magnet resonance (MR) scanning was performed using a Philips Achieva 3.0 Tesla MR scanner (Philips Medical Systems, Best, The Netherlands) with an 8-element head coil. Functional BOLD sensitive images were acquired using a single-shot gradient echo EPI pulse sequence (TE/TR = 35/3000 ms, flip angle = 82°, FOV = 220 × 220 mm^2, matrix = 128 × 128, slices = 39, slice thickness = 3 mm). Sensitivity encoding (SENSE) with a reduction factor of two was used to minimize the influence of susceptibility artefacts and to maximize the possible number of slices acquired within one TR.

High-resolution anatomical images were acquired before the functional experiments, using a 3D T1-weighted gradient echo sequence (TE/TR = 2.3/20 ms, FOV = 220 × 220 mm^2, matrix = 256 × 256, slices = 180, slice thickness = 0.75 mm). All images were acquired in an oblique axial orientation covering the entire head.

Pneumatic stimulation device

Stimulation of the dorsal clitoral nerve (DCN) was performed using small plastic membranes attached bilaterally on the clitoris, delivering a light superficial pressure generated by a pneumatically driven stimulator (Elbert et al., 1998). A standardized, non-painful stimulation protocol was used (pulse frequency = 2 Hz, pulse length = 100 ms, 2 bars overpressure at the membranes). The membranes were fixed in position with tape and a foam pad placed on top of the membranes in combination with a net pant.

The desired stimulation paradigms were delivered to the device from a PC running the control software Presentation (Neurobehavioral Systems Inc., Albany, CA, USA). The control PC was placed outside the scanner room, and connected to the MR scanner, synchronizing the stimulation paradigm with the actual scanning using trigger pulses from the scanner.

Experimental protocol

Prior to scanning, subjects were catheterized with a 14 Fr Foley catheter and the clitoral stimulation membranes were attached. A test stimulation was performed to ensure subjects could feel the stimulation directly at their clitoris. The bladder was filled with saline to a level where the subjects reported a feeling that would lead them to pass urine at the next convenient moment, but voiding could be easily delayed. This sensation is called first desire to void, as defined by the Standardisation Sub-Committee of the International Continence Society (Abrams et al., 2002).

Functional imaging was performed in a block design of alternating rest and active conditions, always starting with a rest condition. Two different sessions were performed using different active conditions (see Fig. 1 for sequence and timing of the experiment):

1. FILLING condition: Bladder filling (15 s) and draining (15 s) with 80 ml saline, alternating with REST conditions (each 30 s) in between. This procedure was repeated 5 times for a total imaging time of 7:30 min. Filling and draining was performed using a syringe connected to the catheter.
2. COMBINED condition: Repetition of the first session, but now with simultaneous stimulation of the DCN during the bladder filling and draining periods.

Our basic experimental design was similar to that of Griffiths et al. (2005).

After the experiment valence and arousal were assessed using a standardized visual rating scale (the pencil-and-paper version of the Self-Assessment Manikin (SAM)), (Bradley and Lang, 1994) for both sessions to evaluate possible emotional influence on brain

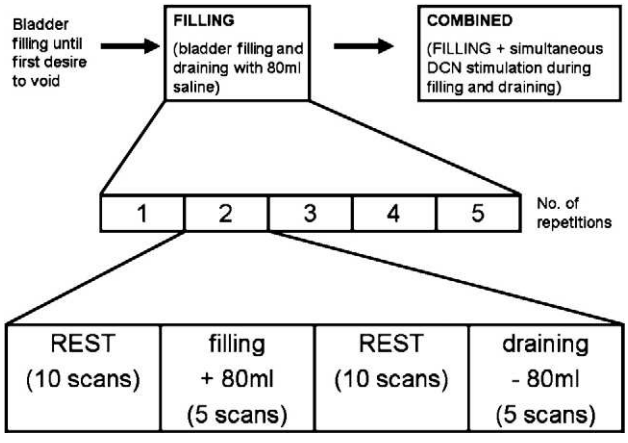

Fig. 1. Schematic diagram of the scanning protocol used in this study, including the sequence, timing and number of repetitions. 10 scans = 30 s, and 5 scans = 15 s. DCN = dorsal clitoral nerve.

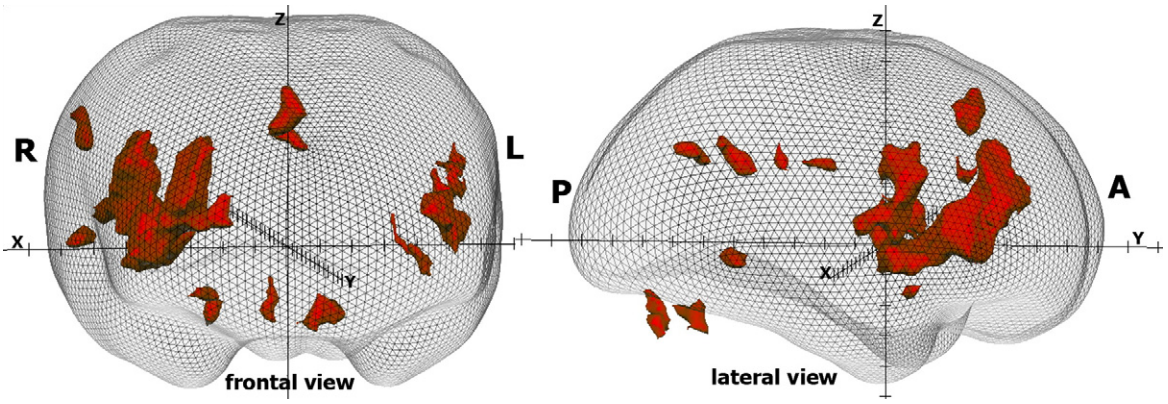

Fig. 2. Frontal and lateral view on a glass brain, showing activations in brain regions during the FILLING condition at q(FDR)=0.001 (p=0.000021) and a cluster size of ≥100 anatomical voxels. For detailed description and coordinates of activated regions consider Table 1. Talairach axes (X, Y, Z) are displayed for easier orientation. Red cluster indicate stronger activation during FILLING compared to REST. R = right hemisphere, L = left hemisphere, P = posterior, and A = anterior.

activation especially associated with sexual arousal during clitoral stimulation.

Post-processing and evaluation

All images were transferred to an off-line workstation running Brainvoyager QX (Brain Innovation B.V., Maastricht, The Netherlands) for further processing. The functional data were pre-processed with Brainvoyager's default parameters for motion correction, spatial smoothing (kernel FWHM=4 mm), linear trend removal, and temporal high-pass filtering (limit set to 3 cycles). The 3D anatomical images were interpolated to iso-voxel size ($1\times1\times1$ mm^3) and co-registered with the functional data. All data were then transformed into the Talairach space (Talairach and Tournoux, 1988).

Table 1
Listing of all brain regions found to be activated/deactivated in the 3 different contrasts (a–c)

a) FILLING>REST condition, q(FDR) 0.001						b) COMBINED>REST condition, q(FDR) 0.001						c) FILLING>COMBINED condition, q(FDR) 0.05					
Region	BA	Talairach coordinates			Z-score	Region	BA	Talairach coordinates			Z-score	Region	BA	Talairach coordinates			Z-score
		X	Y	Z				X	Y	Z				X	Y	Z	
Inferior frontal gyrus	45	47	12	15	7.61	Middle frontal gyrus	10	35	41	26	6.21	Inferior frontal gyrus	46	49	29	12	4.77
	46	42	35	13	7.80		10	0	51	5	−6.10		45	−48	19	19	4.95
	47	27	12	−13	5.53		9	−34	30	33	5.23	Insula	13	56	−34	20	−4.52
	9	−50	20	20	4.87	Insula	13	−35	−51	31	7.30	Anterior cingulate gyrus	24	7	19	24	4.80
Middle frontal gyrus	46	−41	37	15	5.79	Posterior cingulate gyrus	23	3	−58	17	−6.04		32	8	37	24	4.62
Medial frontal gyrus	8	5	29	44	6.27	Precentral gyrus	44	49	7	11	6.15	Putamen (lentiform nucleus)		28	3	7	4.80
Insula	13	43	10	2	6.57	Postcentral gyrus	2	45	−23	46	−6.12						
	13	−39	14	−2	5.13		3	26	−29	61	−7.99						
Precuneus	31	−8	−64	29	5.14		4	−32	−29	56	−8.27						
Middle temporal gyrus	21	58	−42	0	5.76	Paracentral gyrus	5	2	−36	55	−8.84						
Supramarginal gyrus	40	58	−41	33	6.75	Inferior parietal lobe	40	56	−37	24	8.10						
Precentral gyrus	44	−51	1	7	5.77		40	51	−48	45	6.28						
Postcentral gyrus	40	−54	−28	22	5.31		40	−55	−29	23	6.84						
Inferior parietal lobe	40	−58	−42	22	5.89		40	−51	−41	36	5.93						
Cerebellum		13	−73	−25	5.10	Supramarginal gyrus	40	−35	−51	31	5.88						
		−5	−75	−23	5.09	Superior temporal gyrus	22	−52	−4	6.8	6.75						
		−20	−65	−26	5.55	Lingual Gyrus	18	−10	−53	3	−5.78						
						Cerebellum		−25	−66	−26	7.23						

Positive and negative values in the X-coordinate indicate activation in the right and left hemisphere respectively. Positive Z-scores indicate activation, and negative Z-scores indicate deactivation. BA = Brodmann area.

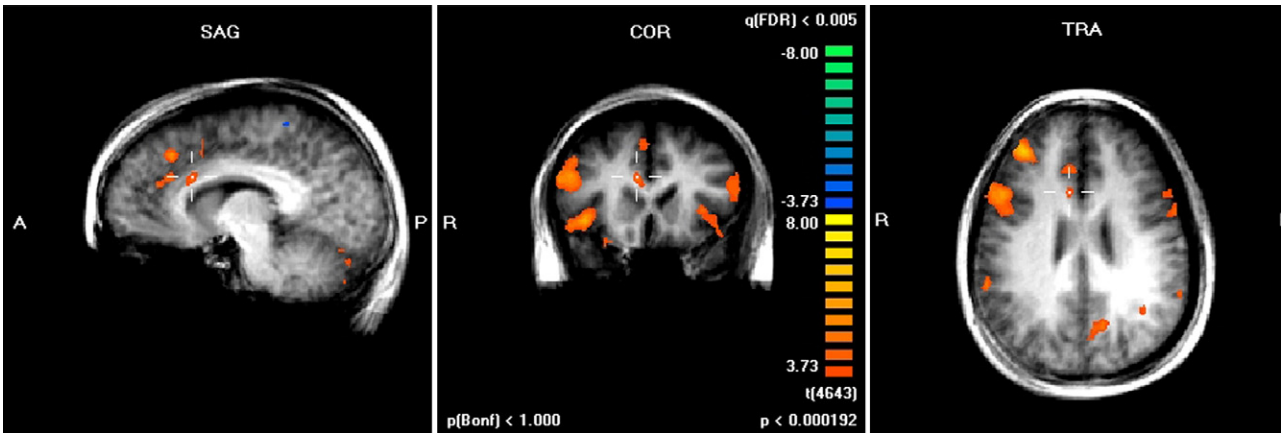

Fig. 3. Display of activation of the anterior cingulate gyrus during the FILLING condition in the right hemisphere, using q(FDR)=0.005 (p=0.000192) and a cluster size of ≥100 anatomical voxels. Talairach coordinates: X=8, Y=17, and Z=27. A = anterior, P = posterior, and R = right hemisphere.

The data from all eight subjects were combined and analysed as a group using the general linear model. Statistical maps were calculated for three different contrasts: 1) FILLING>REST, 2) COMBINED>REST and 3) FILLING>COMBINED.

Only the second half of each REST condition was used for evaluation in order to avoid influence of remaining activation from the previous condition. The resulting activation maps were overlaid on the anatomical image volumes and corrected for multiple comparisons using a false discovery rate q(FDR) of 0.001 for contrasts 1) and 2) and a less restrictive q(FDR) of 0.05 for contrast 3).

All activated clusters were evaluated for their size (in number of anatomical voxels), and their Talairach coordinates at the centre of gravity, using a region-growing tool. Only clusters with a size≥100 voxels were considered. In cases where the spreading of the activated clusters made obvious separate clusters float together the clusters were manually separated by restricting the region-growing limit to a suitable level.

Results

8 healthy female subjects (mean age 24.3 years, range 21–27 years) gave their written informed consent and could be included in this study. All subjects tolerated the experimental protocol well and 8 complete data sets could be obtained. Report by all subjects confirmed that the maximum sensation of bladder filling typically occurred during saline infusion and the minimum during the REST phase.

Six of the 8 subjects completed the score for assessment of emotional context. SAM ratings (±SD) for FILLING were mean 3.6 (±1.2) for valence and 4.8 (±1.5) for arousal. Ratings for COMBINED were mean 3.5 (±1.2) for valence and 4.8 (±2.0) for arousal. These values correspond to a slightly unpleasant sensation and moderate arousal during both experiments.

Central representations of increasing bladder filling levels (FILLING>REST)

The group analysis of all subjects at q(FDR)<0.001 (p=0.000021) revealed activation in the bilateral inferior and left middle frontal gyri, including parts of the prefrontal and orbitofrontal cortices (Brodmann areas 9, 46, 47), the bilateral insula, the left precuneus, the left parietal operculum (Brodmann area 40) and the bilateral cerebellum (Fig. 2, Table 1). At less restrictive but still significant thresholds activation of the right anterior cingulate gyrus (q(FDR)= 0.005) and the right ventral pons (q(FDR)=0.05) could be observed (Figs. 3 and 4). Overall a strong right sided predominance could be observed.

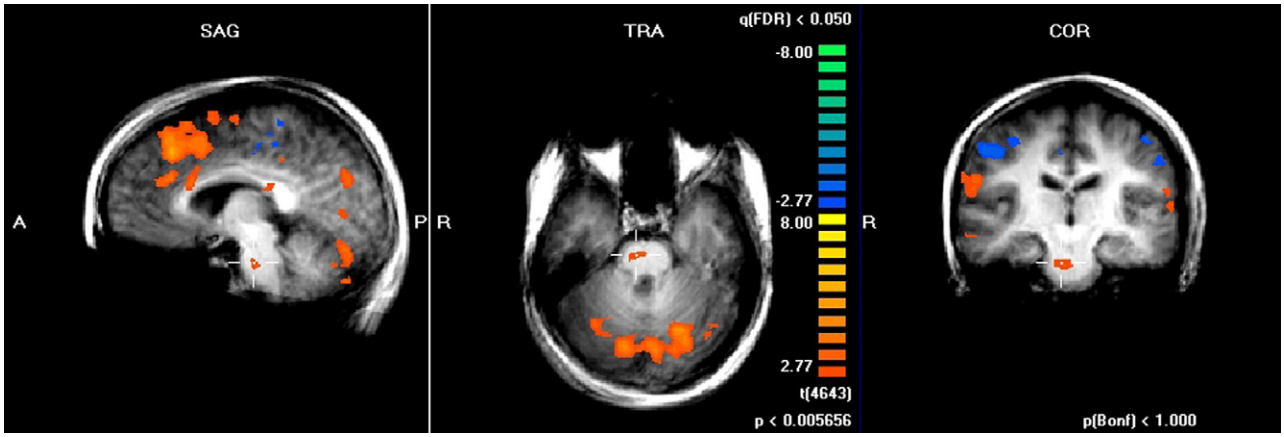

Fig. 4. Display of activation of the right sided ventral pontine region during the FILLING condition, using q(FDR)=0.05 (p=0.0056) and a cluster size of ≥100 anatomical voxels. Talairach coordinates: X=5, Y=−20, and Z=−24. A = anterior, P = posterior, and R = right hemisphere.

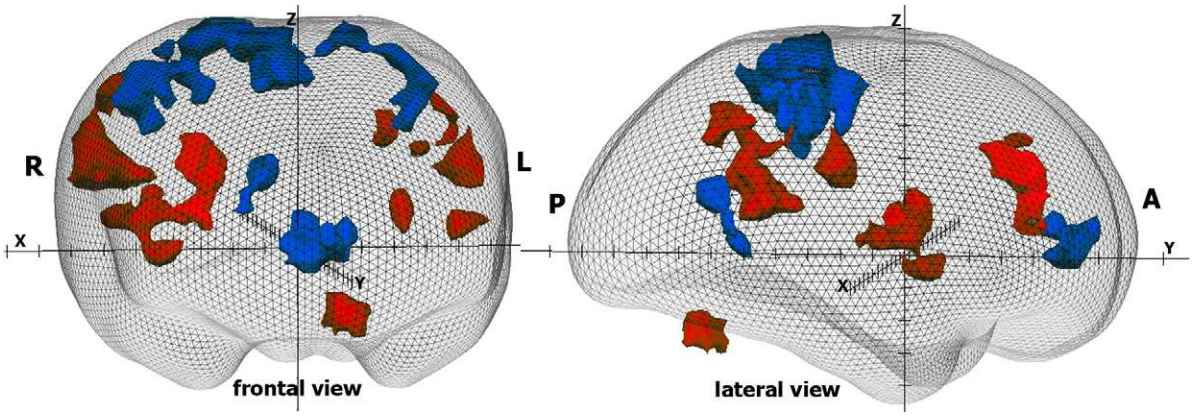

Fig. 5. Frontal and lateral view on a glass brain, showing activations in brain regions during the COMBINED stimulation condition at q(FDR)=0.001 (p=0.000034) and a cluster size of ≥200 anatomical voxels. For detailed description and coordinates of activated regions consider Table 1. Talairach axes (X, Y, Z) are displayed for easier orientation. Red cluster indicates stronger activation during COMBINED compared to REST, and blue cluster indicates lesser activation respectively. R = right hemisphere, L = left hemisphere, P = posterior, and A = anterior.

Central effects of the simultaneous DCN stimulation (COMBINED>REST)

The group analysis of all subjects at q(FDR)<0.001 (p=0.000034) revealed activations in the middle frontal gyrus bilaterally (Brodmann area 9, 10), the left insula, the bilateral parietal operculum, the bilateral postcentral gyrus and the left cerebellum (Fig. 5, Table 1). Deactivations were observed bilaterally in the middle frontal gyrus (Brodmann area 10), the post- and paracentral gyri (Brodmann areas 2–5) and the right posterior cingulate gyrus (Brodmann area 23) (Fig. 5, Table 1).

Contrasting the bladder filling condition vs. the simultaneous stimulation of the dorsal clitoral nerve during bladder filling (FILLING>COMBINED) at q(FDR)<0.05 (p=0.0002) resulted in a positive contrast (=stronger activation during FILLING) bilaterally in the inferior frontal gyrus (Brodmann area 45, 46) and unilateral in the right anterior cingulate gyrus (Brodmann area 24) and putamen. A negative contrast (=stronger activation during COMBINED) was observed unilaterally for the right insula region.

No activations for the pons or the periaqueductal grey region could be found.

Discussion

Stimulation of the dorsal genital nerves during bladder filling is able to reduce the desire to void (Oliver et al., 2003). Our hypothesis was that we can demonstrate the influence of dorsal clitoral nerve stimulation on the desire to void using fMRI, showing a decrease in activation of supraspinal regions involved in the perception and processing of bladder sensations.

Findings related to bladder filling

In our study we found activation in Brain areas during bladder filling without stimulation of the DCN that includes frontal and prefrontal cortices, the anterior cingulate gyrus, the insula, the precuneus, the pons and the cerebellum. These areas, especially the cingulate gyrus and the prefrontal cortex, have been described to be involved in the control of lower urinary tract function in several PET and fMRI studies (Athwal et al., 2001; Blok et al., 1997a,b, 1998; Griffiths et al., 2005; Kuhtz-Buschbeck et al., 2005; Matsuura et al., 2002; Nour et al., 2000; Seseke et al., 2006; Zhang et al., 2005). Griffiths reported that reduced perfusion of the frontal cortex is associated with decreased bladder sensation (Griffiths, 1998). In another fMRI study, Griffiths et al. used increasing bladder volumes to investigate brain control of the bladder in healthy subjects and in subjects with overactive bladder (OAB) (Griffiths et al., 2005). Individuals with OAB showed exaggerated fMRI responses at larger bladder volumes. These abnormalities were reported to be associated with inadequate activation response to bladder infusion in a specific bilateral region of the orbitofrontal cortex suggesting dysfunction of the orbitofrontal cortex as a common background for patients with overactive bladder symptoms (Griffiths et al., 2005).

The prefrontal areas, which include the orbitofrontal cortex, have strong projections towards the periaqueductal grey (PAG) region, which operates as a central relay station. Neurons of the PAG receive information related to bladder filling from neurons in the lateral dorsal horn of the sacral spinal cord, which in turn receives bladder sensory input from A-delta fibres originating in the bladder wall and travelling via the pelvic nerves. The PAG then processes incoming information and is able to initiate voiding via strong projections to the pontine micturition centre or M-region (Barrington, 1931, 1941; Blok, 2002; Holstege, 1998; Kuipers et al., 2006). The pontine micturition centre in turn projects to neurons in the intermediolateral column of the sacral spinal cord and is responsible for synergic micturition. But voiding is not initiated by PAG activation alone. In humans input of higher brain areas to the PAG is necessary to initiate voiding. Brain areas intensely connected to the PAG include the above mentioned limbic and prefrontal areas (Holstege, 1998; Holstege et al., 1986; Noto et al., 1991; Torrens and Morrison, 1987). The precuneus and the cingulate gyrus are strongly related to the limbic area and are responsible for functions like motivation, focusing attention on emotionally significant events, error correction and anticipation of events and tasks (Bush et al., 2002; Carter et al., 1998; Nieuwenhuis et al., 2001; Posner and DiGirolamo, 1998). The prefrontal area, which is also closely linked to the limbic system is involved in abilities to differentiate among conflicting thoughts, to work towards a specific aim, to think of future consequences and prediction of outcomes, to control social behaviour (e.g. the ability

to suppress urges that, if not suppressed, could lead to socially-unacceptable outcomes) and it plays a part in pleasure and addiction (Blok, 2002; Krawczyk, 2002; Miller and Cohen, 2001). Brain regions activated as a response to increasing bladder volume and the desire to void sensation, have been firstly reported in studies using positron emission tomography (PET) (Athwal et al., 2001). The periaqueductal grey matter, the central mid pons region, the cingulate cortex and the frontal lobes were implicated in the process of bladder perception and procession of sensations relayed through the PAG. A decreased activation in these brain regions would therefore suggest lesser input from the PAG. A decreased afferent activity towards the PAG from the bladder may result from inhibiting interneurons on sacral spinal cord level by activating pudendal afferents. However, this remains speculative.

Surprisingly, in the present study we did not find the expected activation in the PAG. Considering its central role in processing signals controlling bladder function, we can only speculate that this may be related to methodological reasons namely, inadequate spatial resolution to detect significant activation in the small brainstem structures and the increased susceptibility and movement related artefacts associated with functional imaging of this region.

Activation of the PMC or M-region could not be detected and was not expected, as this experiment did not include voiding. The activation in the ventral pons found in our study probably represents L-region activity, similar to findings in the study of Kuhtz-Buschbeck et al. (2005). This region has been suggested to serve as a continence centre since it has been proven to excite urethral sphincter motor neurons through direct projections. The L-region has direct long fibre projections to the external urethral sphincter, but no direct connection to M-region. These areas are therefore considered to be separate functional systems that act independently (Blok and Holstege, 1999).

Most activated areas showed a right dominance, which is in agreement with the findings and explanations of Blok et al. (1997b).

Findings related to bladder filling and simultaneous stimulation of the DCN

In our study, simultaneous clitoral stimulation during bladder filling was used to modulate central bladder control resulting in a reduced activity of a well defined network of areas that are normally activated during bladder filling (prefrontal areas, right anterior cingulated cortex and putamen) (Fig. 6). This finding is in agreement with the only other study to date investigating the effect of neuromodulation on central bladder control (Herzog et al., 2006). In this study, subthalamic deep brain stimulation in Parkinson patients also resulted in comparably less activation in the prefrontal and the anterior cingulate cortex during bladder filling.

In a previous fMRI study we studied the effect of voluntary pelvic floor contraction on brain activation induced by bladder filling and found activations in the SMA, bilateral putamen, right parietal cortex, right limbic system, and right cerebellum. This was the first study to suggest that afferent input from the pelvic floor (carried via the pudendal nerve) might modulate central perception and processing of bladder filling sensations (Zhang et al., 2005).

The influence of somatic afferent fibres on bladder function can be explained by the gate-control-theory in pain, described by Melzack and Wall (1965).

The underlying theory is, that bladder afferents travelling through the lateral dorsal horn of the sacral spinal cord can be inhibited by interneurons, which are activated by afferent sensory fibres of the pudendal nerve (Craggs and McFarlane, 1999). Although there is still a lack of a concrete evidence of such pathways, spinal interneurons have been described in close proximity to bladder and urethral afferents in the lateral dorsal horn and dorsal commissure of the spinal cord (de Groat, 2006). Some of these interneurons send long projections to the brain (Birder et al., 1999), whereas others make local connections in the spinal cord and participate in segmental spinal reflexes (Araki and De Groat, 1996). Electrophysiological studies in cats showed that activation of pudendal afferent input from various sites including penis, vagina, anal canal/rectum and sphincters inhibited micturition by suppressing interneuronal pathways in the spinal cord as well as by directly inhibiting parasympathetic preganglionic neurons (de Groat, 1978, 1981). In accordance with these findings, several clinical studies in humans showed reduction of detrusor overactivity using transcutaneous electrical stimulation of the pudendal nerve via electrodes on the anal or vaginal mucosa, perineal skin and penis (Fall et al., 1978; Madersbacher et al., 1995; Primus and Kramer, 1996; Vodusek et al., 1986). Lindstrom et al. were able to show in animal experiments that

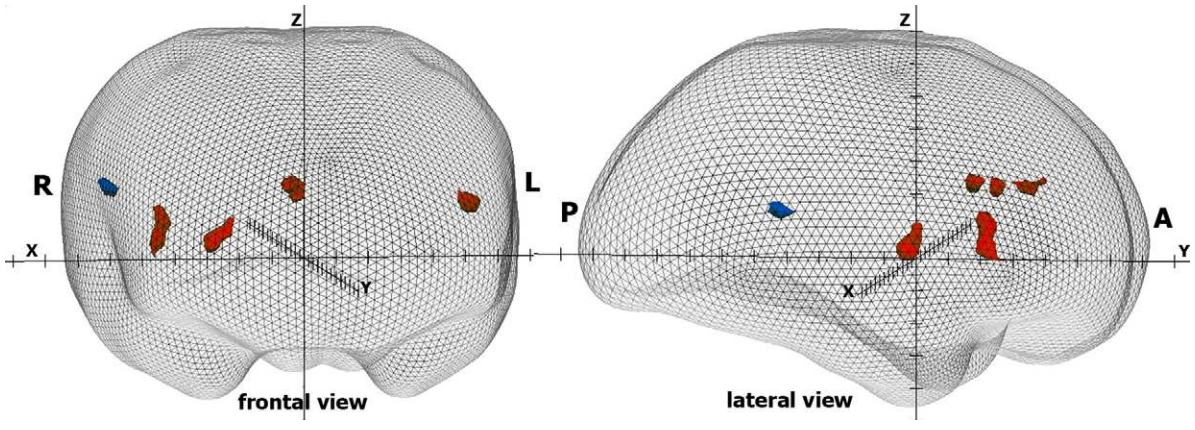

Fig. 6. Frontal and lateral view on a glass brain, showing activations in brain regions in the contrast calculation FILLING>COMBINED at q(FDR)=0.05 (p=0.0002) and a cluster size of ≥100 anatomical voxels. For detailed description and coordinates of activated regions consider Table 1. Talairach axes (X, Y, Z) are displayed for easier orientation. Red cluster indicates stronger activation during FILLING compared to COMBINED, and blue cluster indicates lesser activation respectively. R = right hemisphere, L = left hemisphere, P = posterior, and A = anterior.

the effect of intravaginal stimulation is mediated by pudendal afferents and has two components: a) suppression of pelvic nerve activity to the detrusor by inhibition of the sacral micturition reflex and b) activation of sympathetic neurons, which run in the hypogastric nerves and cause inhibition of the parasympathetic efferent motoneurons at the level of the pelvic ganglia (Lindstrom et al., 1983). Nevertheless, in healthy individuals the spinal micturition and storage reflexes are under regulatory control of supraspinal structures and sacral modulation is probably not the only mechanism involved in the effect of pudendal nerve stimulation. Lindstrom et al. found a lasting inhibitory effect of pudendal nerve stimulation, although stimulation was stopped (Lindstrom et al., 1983). Furthermore he found a prolonged neuromodulatory effect up to several hours following repeated bursts of stimulation (Lindstrom and Jiang, 2000).

Patients with detrusor overactivity, either idiopathic or due to incomplete spinal cord lesions, benefit much more from the pudendal neuromodulation as compared to patients with a complete spinal cord lesion (Schurch et al., 2003). Therefore, supraspinal regions seem to play a crucial role in facilitating the effect of pudendal neuromodulation although this issue remains quite controversial among experts.

Pudendal afferent input probably activates both spinal and supraspinal circuits. On the spinal level, the upcoming bladder activity is suppressed by inhibitory intersegmental reflexes whereas on the supraspinal level, the perception of desire to void is probably modulated for a limited period of time through a reduced input to the PAG and subsequently other cortical brain areas.

Whereas in the FILLING condition the parietal opercular activation (BA 40) might have resulted to some degree from the catheter, its strong bilateral activation during the COMBINED condition most probably resulted from the clitoral stimulation, as the parietal operculum is known to activate during somato-sensory stimulation in humans.

A limitation of the present study may be the fact that stimulation of the DCN reaches only a branch of the pudendal nerve, which cannot be considered as complete pudendal nerve stimulation. However, this might still be sufficient considering that most transcutaneous stimulation techniques reported in literature do not produce stimulation of the entire pudendal nerve. Moreover, studies of Oliver and Shah showed a significant effect on bladder function with electrodes just attached on the clitoris or penis (Kondo et al., 1982; Oliver et al., 2003; Shah, 1999a,b).

Conclusion

Our findings support the findings from previous PET and fMRI studies that a complex visceral sensory neuronal network including the right frontal and prefrontal cortices, the anterior cingulate gyrus, the insula, the precuneus, the pons and the cerebellum is responsible for supraspinal control of bladder filling sensations.

With the present work we could demonstrate that this network can be modulated by stimulation of the dorsal clitoral nerve. As expected from our hypothesis, the central effect of the stimulation was a reduced activity in the areas found to be involved in bladder afferent and efferent control, demonstrating the central effect of a simultaneous neuromodulation and suggesting a reduction in compensatory activity of supraspinal structures. Viscerosomatic afferent input via the pudendal nerve seems to be involved in the modulation of LUT function of healthy subjects, possibly on a spinal level, but more apparently on the supraspinal level.

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