The organization of the central control of micturition in cats and humans
Blok, Bertil Feddo Maarten

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1998

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Chapter 10

Brain Activation during Micturition in Women

Bertil F.M. Blok, Leontien M. Sturms, and Gert Holstege

INTRODUCTION

Micturition or urination is a coordinated action between the urinary bladder and its external urethral sphincter. When the bladder contracts, the sphincter relaxes. Although the motoneuronal cell groups of both bladder and sphincter are located in the sacral spinal cord, their coordination takes place in the pons. This brainstem organization is best shown in patients with spinal cord injuries above the sacral level. They have great difficulty emptying the bladder, because when their bladder contracts, their urethral sphincter contracts also, a disorder called detrusor-sphincter dyssynergia. Such disorders never occur in patients with neurologic lesions rostral to the pons, which indicates that the coordinating neurons are located in the pontine tegmentum (Blaivas, 1982).

As early as 1925 Barrington in the cat showed that the neurons involved in micturition control are probably located in the dorsolateral part of the pontine tegmentum, because bilateral lesions in this area produced an inability to empty the bladder leading to urinary retention. Tracing studies in cat (Holstege et al., 1979) and rat (Loewy et al., 1979) revealed that a distinct cell group in the dorsal pontine tegmentum, called Barrington’s area or pontine micturition center (PMC) or M-region, projects to the sacral cord intermediolateral cell col-
Fig. 1. Left: Significant differences in rCBF in the right dorsal pontine tegmentum (indicated by pmc = pontine micturition center) after the comparison between the conditions “Successful micturition” (scan 2) and “Empty bladder” (scan 3). Right: Significant differences in rCBF in the right ventral pontine tegmentum (indicated by L-region) after the comparison between the conditions “Unsuccessful micturition” (scan 2) and the condition “Empty bladder” (scan 3). Threshold used for display uncorrected P<0.005. The number -28 refers to the distance in millimeters relative to the horizontal plane through the anterior and posterior commissures (z-direction). The numbers on the color scale refer to the corresponding Z-scores. L = left side of the brain; R = right side.

Fig. 2. Significant differences in rCBF in cortical areas after the comparison between the conditions “Successful micturition” (scan 2) and “Urine withholding” (scan 1). Note the activations in the right anterior cingulate gyrus (acg) in z planes +8 to +16, and the right inferior frontal gyrus (gfi) in the z-planes 0 to +12. For other details see Fig. 1.
PET study on micturition control in women

umn (IML). Blok and Holstege (1997) have shown that this projection is excitatory in nature and contacts dendrites and somata of parasympathetic preganglionic bladder motoneurons. Electrical stimulation in the PMC produces bladder contractions (Holstege et al., 1986; Mallory et al., 1990) and bilateral destruction of the PMC leads to chronic urinary retention (Griffiths et al., 1990).

Another area, important during the filling phase, is located more ventrally and laterally in the dorsolateral pontine tegmentum. This area, called L-region, maintains direct projections to the nucleus of Onuf in the sacral cord (Holstege et al., 1979; 1986). In the cat (Sato et al., 1978; Kuzuhara et al. 1980), monkey (Roppolo et al., 1985) and humans (Onufrowicz, 1899; Schröder, 1981) Onuf’s nucleus contains motoneurons innervating the pelvic floor, including the anal and urethral sphincters. Stimulation of the L-region in the cat results in a contraction of the pelvic floor, including the external urethral sphincter (Holstege et al., 1986). Bilateral lesions in the L-region cause an extreme form of “urge” incontinence (Griffiths et al., 1990).

Experiments in the cat have led to a concept about the basic micturition control systems in the central nervous system (Fig. 3). Information about the degree of bladder filling is conveyed by the pelvic nerve to neurons in the lumbosacral cord (Morgan et al., 1981), which in turn project to the periaqueductal gray (PAG; Noto et al., 1991; Blok et al., 1995; VanderHorst et al., 1996). When the bladder is filled to such a degree that voiding is appropriate, the PAG activates neurons in the PMC (Blok and Holstege, 1994), which, in turn, excite the sacral preganglionic parasympathetic bladder motoneurons, and inhibit the bladder sphincter motoneurons. The bladder excitation is achieved by way of the direct projection to the parasympathetic motoneurons (Blok and Holstege, 1997), the sphincter inhibition by way of PMC projections to GABA-ergic interneurons in the sacral cord dorsal gray commissure (Blok et al., 1997; Blok and Holstege, 1998). These GABA cells, in turn, project to the motoneurons in Onuf’s nucleus (Konishi et al., 1985; Nadelhaft et al., 1996).

A recent PET study (Blok et al., 1997) in healthy human male volunteers revealed the same brainstem areas to be associated with micturition as in the cat. The first example is the dorsal pontine tegmentum, where in the cat the PMC is located. Other activated

![Fig. 3. Schematic overview of pathways between spinal and supraspinal structures involved in the control of micturition based on experiments in the cat and men. The locations of the micturition control areas (see text) in the brainstem and diencephalon were used in the null hypotheses for the present study in women. Ascending (sensory related) and descending (motor related) pathways are indicated on the respective left and right sides only.](image-url)
areas are the midbrain periaqueductal gray (PAG), the hypothalamus, the right inferior frontal gyrus, and the right anterior cingulate gyrus. Withholding of urine, despite vigorous attempts to micturate, was associated with increased regional cerebral blood flow (rCBF) in the ventral pontine tegmentum, an area corresponding with the L-region in the cat, and in the inferior frontal gyrus and anterior cingulate gyrus, all regions on the right side only.

Practical reasons had prevented to perform a PET scan study in women. This was unfortunate because the results of the PET scan studies are important for our understanding of the micturition control system and its abnormalities, and because in women the incidence of neurogenic related micturition disorders is much larger than in men (Resnick et al., 1989). Especially urge incontinence, which is the disorder in which a patient senses the urge to void, but is unable to delay micturition long enough to reach a toilet, is most frequently seen in the elderly, mostly female population (Jewett et al., 1981; Haschek, 1984; Resnick et al., 1989). Since, animal studies (for example Raisman and Field, 1971; Gorski et al., 1978; Breedlove, 1980) have provided ample evidence for important gender differences in the structural organization of neuronal cell groups in the central nervous system, the present study in women was designed to identify the brain regions involved in micturition and to compare the results with the PET scan findings in men, and the anatomical and physiological findings in the cat.

MATERIALS AND METHODS

Experimental design

The volunteers were adult women between 20 and 51 years of age (mean was 27). They all completed a general health questionnaire. Volunteers reporting a history of neurologic, psychiatric or gastroenterologic illness, had been excluded from the study (n=3). All remaining 18 subjects were right-handed, and gave their written informed consent, according to the declaration of Helsinki. The protocol of the study was approved by the research ethics committee of the University Hospital of Groningen. During each scan the lights were dimmed, the subjects had their eyes closed and did not move. Each scanning session consisted of four measurements and lasted 1.5 hours in total.

Experimental protocol and training

The volunteers were scanned during the following 4 conditions:

- **Condition 1.** filled bladder
- **Condition 2.** micturition
- **Condition 3.** empty bladder
- **Condition 4.** empty bladder

Eight seconds before the second scan and 15 seconds after the injection of the H$_2^{15}$O bolus, the right index finger of the volunteer was touched to let her know that she could start micturition. Prior to the other three scans no specific assignment was given. The urine was collected with a special urological device (Femicep; Wallace Ltd., Essex, England) attached to a plastic urine reservoir. The device was positioned close to the urethral orifice during all four scans. The floor of the Femicep was equipped with a self-made battery driven urine detector, which, during the second condition, indicated the onset of micturition with a small red light.

A few days before the scanning session, the volunteers were asked to practice at home, urinating horizontally using the Femicep. When individual practicing was successful, the PET scan session during micturition was simulated at the subjects’ home under the guidance of one of the authors (LMS). Volunteers who were not able to micturate during this session at home were excluded from the study. They were asked to volunteer in a PET study on the voluntary control of the pelvic floor musculature (Bloks et al., 1998).
About 70 minutes prior to the first condition of the actual scanning session, the urine volume of the bladder was measured with the use of an ultrasonic device, called the Bladder Manager (Diagnostic Ultrasound Corporation, Seattle, Washington). If the Bladder Manager indicated that the bladder was hardly filled (<250 ml), and the volunteer affirmed that she did not have a sensation of a filled bladder, she was asked to drink an additional glass of water. In case of successful micturition during condition 2, the bladder volume was measured for a second time to determine the residual urine volume. When the bladder appeared to contain more than 200 ml, she was asked to micturate again. One volunteer was unable to do so, and she was catheterized by a trained nurse. Catheterization also took place in the eight volunteers in which micturition during condition 2 was not successful.

Data acquisition
The subjects were placed in a horizontal position in the PET camera (Siemens-CTI 951/31, Knoxville, TN, USA) parallel to and 5 cm below the orbitomeatal line (OM line) as determined by external examination. An individually constructed head mould was used to prevent substantial changes in head position. Because of the technical characteristics of the PET camera, the most caudal limit of the scanned area was the pons, and the most rostral the cingulate gyrus. It meant that images were obtained from 28 mm below and 48 mm above the intercommissural plane. This implied, for example, that the sensorimotor cortex could not be investigated.

In order to correct for absorption of gamma radiation by surrounding tissue, a 20 minutes transmission scan was made at the beginning of the scanning session. The relative attenuation factors, obtained from this transmission scan, were used for correcting the subsequent emission scans and image reconstruction.

After the transmission scan, the subjects were given 1.85 GBq of H215O diluted in saline for each of the four scans. The H215O bolus, followed by 40 ml saline, was injected in the right brachial vein using an automatic pump. Data acquisition continued for 90 seconds, and began 23 seconds after the beginning of the injection, at which time the peak in radioactivity was assumed to reach the cerebral circulation. To allow the radiation to return to background levels, there were 15 minutes intervals between the injections.

Data analysis
The data of each scan were summated and the resulting images were centered to prevent loss of information during sampling. Prior to the statistical procedure the data were sampled to a voxel size of 2.2 x 2.2 x 2.4 mm. The data were analyzed using the Statistical Parametric Mapping procedure (SPM 95 from the Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks Inc., Sherborn MA, USA) on a SPARC workstation (Sun Microsystems Inc., Surrey, UK). The SPM 95 software was used for the anatomical realignment, normalization, smoothing and statistical analysis.

Realignment corrected the images for the translational and rotational movements of the head, using the first scan as reference. Normalizing spatial transformation matches each scan to a reference or template, which conforms to the stereotactic standard space (Talairach and Tournoux, 1988). Finally, the images were smoothed with a Gaussian filter of 8x8x8 mm3 (full width, half maximum in the x-, y-, and z-axes, respectively). This relatively small filter was used because the main part of the study was aimed at relatively small brainstem and diencephalon sites, which were found to be involved in the central control of male micturition (Blok et al., 1997).
Statistical analysis
The differences in global activity within and between subjects were removed by analysis of covariance (ANCOVA) on a pixel-by-pixel basis with global counts as covariate. For each pixel in stereotactic space, the analysis of covariance generated a condition specific adjusted mean rCBF value (normalized to 50 ml/dl/min) and an associated adjusted error variance. A repeated measures ANCOVA was used for the comparison of all four conditions. The differences between conditions were assessed by weighting each condition with an appropriate contrast. The significance of each contrast was assessed with a statistic whose distribution has Student’s t distribution under the null hypothesis. For each contrast a t-statistic was computed for each and every voxel to form a statistical parametric map (SPM(t); Friston et al., 1991). Finally, the SPM(t) was transformed to the unit normal distribution (SPMz).

Since the location of the expected micturition related areas was predicted a priori on the basis of the PET study in males, an uncorrected threshold of P<0.001 was used for these areas. Trends in activation in the expected micturition control areas were reported when they reached a significance level of P<0.005 or a Z value of 2.5. This level of significance gives sufficient protection against false positives (Kosslyn et al., 1994; Warburton et al., 1996).

Other brain areas than those predicted to be activated during micturition, were considered statistically significant only after a correction for multiple comparisons. This correction is necessary, because with so many voxel-by-voxel comparisons, many t-values will reach conventional levels of significance by chance. The problem was resolved by using a Bonferroni-like correction for the number of voxels studied and reporting only those voxels that achieved P<0.05 (corrected) level of significance after such a correction (Friston et al., 1991).

RESULTS
Ten of the 18 volunteers were able to micturate within 15 seconds after the beginning of scan 2. The mean collected urine volume of this group of volunteers was 423 ml +/- 97 ml (mean +/- standard deviation). One successful volunteer, who urinated 320 ml of urine during the second scan, had to be catheterized a urine volume of 380 ml prior to scan 3. The results obtained in this group will be referred to as “successful micturition”. The other eight volunteers tried to micturate during scanning, but did not succeed (the “unsuccessful micturition” group). Of the unsuccessful micturition group, all were catheterized (urine volume 780 ml +/- 162 ml). The data from the two groups are reported separately.

Successful micturition group
In the ten subjects, who were able to micturate during scanning, the sites showing significantly activation (uncorrected P<0.001) in brain areas previously implicated in micturition control, are presented in Table 1.

During micturition (scan 2), compared with the empty bladder condition (scan 3), the right dorsal pontine tegmentum (Fig. 1 left), and the right inferior frontal gyrus were significantly activated. Using the same comparison trends in activation (uncorrected P value between 0.001 and 0.005) were observed in the most caudal extension of the P AG (uncorrected P<0.002) and the rostral hypothalamus (uncorrected P<0.004). Other regions, previously not implicated in micturition control, were not found to be significantly activated (corrected P<0.05).

Comparing the micturition condition (scan 2) with the filled bladder condition (scan 1), increased rCBF was observed in the right inferior frontal gyrus (Fig. 2). A trend in activation was found in the dorsal pontine tegmentum (uncorrected P<0.005). With this comparison other regions, previously not implicated in micturition control, were not
found to be significantly activated (corrected $P<0.05$). The rCBF in the right anterior cingulate gyrus (Fig. 2) was significantly decreased during the filled bladder condition (scan 1) compared with the micturition condition (scan 2), but also compared with the empty bladder conditions (scans 3 and 4).

Similar significant decreases in rCBF (corrected $P<0.05$) were observed during micturition (scan 2) compared to the other three conditions in the left medial frontal gyrus and the right inferior frontal gyrus. Interestingly, the right anterior insula and/or the right frontal operculum, were strongly activated (corrected $P<0.003$) during the filled bladder condition (scan 1) compared with the empty bladder conditions, and, to a lesser degree, compared with the micturition condition (Z score =4.3).

**Unsuccessful micturition group**
Comparing the second condition (unsuccessful micturition; scan 2) with the condi-

---

**Table 1** Regional differences in cerebral blood flow during successful micturition

<table>
<thead>
<tr>
<th>Coordinates of peak activation (x, y, z in mm)</th>
<th>Z-score</th>
</tr>
</thead>
</table>

**Micturition, successful (scan 2) minus empty bladder (scan 3)**

**Cortical areas**

- Right inferior frontal gyrus (BA 45): +52, +24, +12, +3.1
- Right inferior frontal gyrus (BA 44): +46, +14, +24, -4.3
- Left medial frontal gyrus (BA 46): -42, +40, +20, -3.6

**Forebrain and brainstem areas**

- Dorsal pontine tegmentum: +12, -36, -28, +3.6
- Periaqueductal gray: +2, -38, -24, +2.8
- Hypothalamus: -8, +10, -8, +2.6

**Micturition, successful (scan 2) minus withholding urine (scan 1)**

**Cortical areas**

- Right inferior frontal gyrus (BA 45 and 47): +52, +22, +4, +4.4*
- Right anterior cingulate gyrus (BA 24 and 32): +14, +30, +16, +3.6
- Right inferior frontal gyrus (BA 44 and 45): +46, +14, +20, -4.7*
- Left medial frontal gyrus (BA 46): -42, +40, +20, -4.5*

**Forebrain and brainstem areas**

- Dorsal pontine tegmentum: +16, -40, -28, +2.5

**Withholding urine (scan 1) minus micturition, successful (scan 2)**

- Right frontal operculum and/or anterior insula: +38, +10, +8, +4.3

**Withholding urine (scan 1) minus empty bladder (scan 3)**

- Right frontal operculum and/or anterior insula: +38, +10, +12, +5.1*

Peak activations indicated by x, y, and z coordinates, according to the stereotactic atlas of Talairach and Tournoux (1988). BA = estimated Brodmann’s area. * = significant after a multiple comparisons correction with a threshold of corrected $P<0.05$. 

101
tion during an empty bladder (scan 3) significantly increased rCBF (uncorrected P<0.001) was found in the right ventral pontine tegmentum (peak activation x = 8 mm, y = -22 mm, z = -28 mm; Z score = 3.8; Fig. 1 right) and a trend in significant activation in the right inferior frontal gyrus (BA 45; peak activation x = +36 mm, y = +20 mm, z = +16 mm; Z score = 2.8; P<0.002).

Comparing the first condition (withholding of urine) with the unsuccessful micturition condition (scan 2) a significant decrease in rCBF (uncorrected P<0.001) was found in the right anterior cingulate gyrus (BA 32; peak activation x = +16 mm, y = +36 mm, z = 16 mm; Z score = 3.2), but also compared with the empty bladder conditions (scans 3 and 4). A trend in significant activation was found in the right inferior frontal gyrus (BA 47; peak activation x = +44 mm, y = +44 mm, z = +12; Z score = 2.6; P<0.004), but not in ventral pontine tegmentum. The ventral pontine tegmentum (peak activation x = -8 mm, y = -24 mm, z = -28; Z score = 4.5; corrected P<0.05) was also strongly activated during the filled bladder condition (scan 1) compared with the empty bladder conditions (scans 3 and 4). The right anterior insula and/or the right frontal operculum were also strongly activated (peak activation x = +36 mm, y = +22 mm, z = +12; Z score = 5.1; corrected P<0.005) during the filled bladder condition (scan 1) compared with the empty bladder conditions (scan 3 and 4), but not in comparison to the unsuccessful micturition condition (scan 2).

**DISCUSSION**

The present study was designed to determine the brain regions in women activated during micturition and to compare the results with those obtained in the previous study in men. It appeared that the micturition related brain regions in the pons and cerebral cortex in men and women are the same, but that the PAG and the hypothalamus, found to be significantly activated during micturition in men, only showed trends in increased activation in women.

**Areas related to micturition**

**Dorsal pontine tegmentum**

A distinct area in the dorsal pontine tegmentum was activated during micturition (scan 2) when compared with the empty bladder condition (scan 3). The location of this pontine area is identical with the pontine region found to be activated in men. Since this dorsal pontine region in the cat represents the pontine micturition center (PMC), it seems most likely that a similar group of neurons in the dorsal pons exists in humans (men and women).

**Right inferior frontal gyrus**

The activation in men and women of the right inferior frontal gyrus during micturition (scan 2) in women, in comparison with a full (scan 1) or empty bladder (scan 3), is the same, although in women much less extensive. The inferior frontal gyrus is involved in attention mechanisms (Pardo et al., 1991) and response selection (Jenkins et al., 1994). In respect to micturition, the area might play a role in deciding whether or not micturition can take place. The observation that this region is activated during micturition is in agreement with a specific involvement of the right prefrontal cortex in micturition control (Kuroiwa et al., 1987; Griffiths, 1998).

**Right anterior cingulate gyrus**

The right anterior cingulate gyrus showed a significantly decreased rCBF during the withholding of urine condition (scan 1) compared with successful micturition (scan 2) and empty bladder conditions (scan 3 and 4). This observation is similar to that in the PET study on micturition in men. Possibly, the decrease of rCBF in the right cingulate gyrus during urine withholding results in a decrease in the urge to void. Another argu-
ment for the anterior cingulate gyrus to play a role in micturition control is that lesions in the forebrain have been reported to cause urge incontinence (Andrew and Nathan, 1964; Maurice-Williams, 1974). Moreover, studies using single photon emission computer tomography (SPECT) scanning indicate that urge incontinence is associated with a hypoperfusion of the right forebrain (Griffiths, 1998).

**Areas related to continence**

**Ventral pontine tegmentum**

Eight of the eighteen volunteers were unable to micturate when requested, although they tried vigorously (scan 2). In this group the ventral pontine tegmentum showed increased rCBF during scan 2 in comparison with scan 3 (empty bladder condition). During scan 2, the volunteers who were not successful in their attempt to micturate, probably for emotional reasons. Despite a full bladder they contracted their urethral sphincter and withheld their urine. The location of the activated region in the ventral pons is similar to the region which showed increased rCBF in the group of male volunteers, who were also unable to micturate during scanning. In the cat this same pontine region corresponds with the L-region, involved in continence control (see introduction). Interestingly, this same area in the ventral pontine tegmentum also showed strongly increased rCBF during the filled bladder condition (scan 1) compared with the empty bladder conditions (scans 3 and 4). Activation of the ventral pons, including the L-region, is probably responsible for the increased activity of the striated urethral sphincter during the continence phase in order to keep the bladder closed. This activity is at its highest just prior to micturition when the bladder is filled (De Groat, 1990).

**Right inferior frontal gyrus**

The right inferior frontal gyrus was activated during not successful micturition (scan 2) in women. This result is similar to that observed in the previous PET study in men. The importance of the right inferior frontal gyrus in attention mechanisms and response selection has been discussed in the previous paragraph.

**Right anterior cingulate gyrus**

Similar to the successful micturition group, the right anterior cingulate gyrus showed decreased rCBF during scan 1, compared with the second scan (micturition unsuccessful). This finding is identical to that in the PET study in men, who were not able to micturate during scanning.

**Insular and opercular activation during filled bladder condition**

The right frontal operculum and/or the right anterior insula were significantly activated (corrected for multiple comparisons) during the filled bladder condition (scan 1) compared with the other three conditions in the successful micturition group, and with the empty bladder conditions in the unsuccessful micturition group. The previous PET study on human male micturition did not comment on activation related to the filled bladder condition (Blok et al., 1997). In retrospect, unpublished results of this study revealed that, similar to the findings in the present study in women, the rCBF in the right anterior insula was also increased during the filled bladder phase (peak activation x = +32 mm, y = +24 mm, z = +12 mm; Z score = 4.1) compared with the empty bladder conditions. However, in men this activation was not significant for multiple comparisons (corrected P<0.05).

In a recent PET study (Aziz et al., 1997) the right human anterior insula has been associated with the processing of non painful and painful sensation from the esophagus. Possibly, the bladder filling information is another example of visceral sensation processed by the anterior insula.
Alternatively, activation of the right human insula results in an increased sympathetic tone (Oppenheimer et al., 1992). Activation of sympathetic fibers has been shown to inhibit bladder wall mechanoreceptor discharge (Vaughan and Satchell, 1992). The result is a bladder wall relaxation leading to an increased bladder capacity. This is exactly what happened during the first condition of the present study, when the volunteers had a filled bladder, but were not allowed to micturate.

**Activation differences during micturition between men and women**

In women only a slight increase in rCBF was observed during micturition in both the most caudal portion of the PAG and the rostral hypothalamus. In the cat the PAG and hypothalamus are involved in micturition control and these areas were also activated during micturition in male humans (Blok et al., 1997). It is possible that the present group of female volunteers was not large enough to reach significant levels of activation in these regions. Another possibility might be that the lack of activation in these two emotion related structures reflects a gender difference in micturition control.

**Predominance of the right brain in micturition control**

Similar to the previous study in men, the micturition related brain regions in women (frontal and cingulate cortices) were located predominantly on the right side of the brain. This finding corresponds with studies indicating that urge incontinence is specifically correlated with lesions in the right hemisphere (Maurice-Williams, 1974; Kuroiwa et al., 1987; Griffiths, 1998). Although this right sided predominance was also found in the pontine tegmentum, it should be kept in mind that, at least in cats, bilateral PMC lesions are necessary to induce urinary retention. Unilateral lesions are not sufficient to produce this effect (Griffiths et al., 1990; Mallory et al., 1991). It is, therefore, premature to conclude that only one side of the brain controls micturition.