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ORIGINAL BASIC SCIENCE ARTICLE

Parcellation of human periaqueductal gray at 7-T fMRI in full and empty bladder state: The foundation to study dynamic connectivity changes related to lower urinary tract functioning

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Abstract

Aims: The periaqueductal gray (PAG) is a brain stem area involved in processing signals related to urine storage and voiding. The PAG is proposed to be responsible for projecting afferent information from the bladder to cortical and subcortical brain areas and acts as a relay station projecting efferent information from cortical and subcortical areas to the pons and spinal cord. Here, we use 7-Tesla functional magnetic resonance imaging to parcellate the PAG into functionally distinct clusters during a bladder filling protocol.

Methods: We assess the similarity between parcellation results in empty and full bladder states and show how these parcellations can be used to create dynamic response profiles of connectivity changes between clusters as a function of bladder sensations.

Results: For each of our six healthy female participants, we found that the agreement between at least one of the clusters in both states resulting from the parcellation procedure was higher than could be expected based on chance ($p \leq .05$), and observed that these clusters are significantly organized in a symmetrical lateralized fashion ($p \leq .05$). Correlations between clusters change significantly as a function of experienced sensations during bladder filling ($p \leq .05$).

Conclusions: This opens new possibilities to investigate the effects of treatments of lower urinary tract symptoms on signal processing in the PAG, as well as the investigation of disease-specific bladder filling related dynamic signal processing in this small brain structure.

KEYWORDS
bladder sensation, fMRI, functional connectivity, PAG, parcellation

1 | INTRODUCTION

The control of micturition is hierarchically organized within the central nervous system (CNS) and the peripheral nervous system.[1] In healthy adults, storage and voiding of urine is controlled by dedicated areas on the level of the spinal cord (SC; e.g., Onuf's nucleus [ON]), the brain stem (e.g., pons and periaqueductal gray [PAG]), and higher cortical and subcortical areas (e.g., prefrontal cortex, anterior cingulate cortex, insula, thalamus). The main purpose of this system is to facilitate storage of urine and to initiate a micturition episode once a time and place is found where it is safe and socially acceptable to void.

The PAG is centrally located in the hierarchical system of micturition control, and is assumed to serve as a relay station projecting afferent information from the bladder to cortical and subcortical brain areas and as a gatekeeper projecting efferent information from these higher areas to the pontine micturition center and ON.[2-4] The PAG is organized in a symmetrical columnar fashion, and the ventrolateral PAG and dorsolateral PAG are indicated to be involved in the control of voiding and storage of urine respectively.[5-8] Patients with selective lesions of the PAG are known to present with micturitional disturbances.[9-11] This indicates the relevance of gaining a better understanding of the role of the PAG, both as a whole and at the level of individual columns, on the control of micturition.

Neuroimaging studies have consistently observed activity in the PAG related to bladder fullness and bladder sensations,[12-15] and have underpinned the importance of the role of the PAG in the switching mechanism during the initiation and interruption of voiding.[16] Altered CNS processing has been indicated to be related to lower urinary tract symptoms (LUTS) like urge incontinence.[17-19]

The utilization of ultra-high-field functional magnetic resonance imaging (fMRI) at 7 T will enable us to investigate PAG activity in human subjects at a submillimeter resolution. We aim to parcellate the PAG into clusters based on their fMRI blood-oxygen-level-dependent (BOLD) signal using connectivity-based parcellation methods. This approach allows the identification of anatomical regions that differ from one another based on correlations in BOLD fluctuations during resting-state.[20] Resting-state-based parcellations reflect an underlying anatomical construct,[21] and this approach enables us to parcellate the PAG into anatomically distinct clusters. Since the underlying anatomy remains constant we hypothesize that PAG parcellations based on resting-state fMRI data during an empty and a full bladder state show a high overlap, and show a symmetrical organization that corresponds to what has been observed in prior histochemical work.

To demonstrate the clinical potential of a robust functional parcellation of the PAG, we propose an exploratory application that can be used to study connectivity changes between different subregions of the PAG related to changes in bladder fullness and bladder sensations. To this end, we introduce a novel methodological approach to identify dynamic response profiles of PAG activity that, when established in healthy controls, could serve as a benchmark to which LUTS patients' response profiles can be compared.

2 | MATERIALS AND METHODS

2.1 | Participants

This study was designed and conducted in line with the Declaration of Helsinki and was approved by our local ethics committee. Written informed consent was obtained from each of our participants. We enrolled nine female participants in our study (mean age: 47, *SD*: 20) without any clinically significant history neurological disease or dysfunction as judged by the medical investigator. We chose to include only female participants in this project to control for gender as a confounding factor. Future studies should assess sex-related differences in PAG activity related to bladder fullness and bladder sensations. We had to exclude data obtained from three participants due to technical difficulties. All participants were able to complete all steps of the bladder filling and scanning protocol. For the development of the exploratory application of PAG parcellations, we demonstrate our novel methodological approach on data obtained from a single subject (age: 24).

2.2 | Study design

After obtaining informed consent, participants were asked to fill out a 3-day micturition diary to gain baseline values of their voiding- and drinking patterns and to familiarize the participants with scoring their bladder fullness sensations on a visual analog scale (VAS) and their perception of urgency on the four-point Indevus Urgency Severity Scale (IUSS).[22]

Participants were then invited to visit our clinic for the urodynamic and fMRI studies. Upon arrival at our institute, participants were instructed to void until empty in private after which a nurse inserted a filling catheter (FR: 8) transurethrally. Participants were instructed to lie down in a supine position on the MRI bed while an

MRI-compatible syringe pump was connected to the filling catheter. Head motion was restricted using foam cushioning. Scanning was conducted on a 7-T MRI scanner (Siemens, MAGNETOM) using a 32-channel head coil (NOVA Medical).

For each participant; first, we ran an empty bladder resting-state fMRI scan during which we collected 420 T2*-weighted multiband echo planar imaging volumes (mb-EPI sequence, acceleration factor = 2, MB-factor = 2, TR = 1400 ms, TE = 22 ms, resolution = 1.1 × 1.1 × 1.1 mm). We scanned 40 slices covering the supramedullary portion of the brain stem. Next, we ran a T1-weighted whole-brain anatomical scan using an MP2RAGE sequence. After the anatomical scan, participants were instructed to report their experienced bladder sensations, using a joystick they could control from the scanner, on a VAS for their perceived bladder fullness and the IUSS for their perceived levels of urgency. Reported bladder sensations were visually presented to the researcher in the operating room in real-time and were logged every second using a custom-written MATLAB script. We manually prefilled the bladder with a syringe at a rate of approximately 30 ml/min to 50% of the volume of the micturition episode with the smallest volume reported on the micturition diary with a score of 1 on the four-point IUSS (range 0–3) to decrease individual differences in total filling time. Next, a filling resting-state fMRI scan was started simultaneously with an automatic syringe pump to fill the bladder at a rate of 30 ml/min (mb-EPI sequence, acceleration factor = 2, MB-factor = 2, TR = 1700 ms, TE = 22 ms, resolution = 0.9 × 0.9 × 0.9 mm). The bladder was filled until participants indicated urgency levels of 2 on the four-point IUSS. This is defined by Nixon et al.[22] as "enough urgency discomfort that it interferes with or shortens your usual activity or tasks" (p. 607). The length of this scan was variable and depended on each participant's individual bladder capacity. After the participants indicated a strong desire to void, the syringe pump and filling scan were stopped and a full bladder resting-state fMRI scan was started using the same scanning parameters as the empty bladder scan. The scanning protocol is visualized in Figure 1. After the full bladder scan finished, participants were assisted out of the scanner and instructed to void until empty in private while recording their voided volume.

2.3 | Data processing

Functional data were preprocessed using BrainVoyager 20.6 (Brain Innovation). Functional volumes were first corrected for slice scan-time differences and 3D head motion using three translation and three rotation parameters. Subsequently, linear trends and low-frequency temporal drifts were removed from the data using a high-pass filter, removing temporal frequencies below 0.1 Hz, which is suggested to be an appropriate threshold for preservation of functional connectivity information in the data.[23,24] Functional data were subsequently coregistered to the anatomical images and rotated to ACPC space.

2.4 | Periaqueductal gray parcellation

For each participant, we identified and segmented out the PAG in the anatomical data using BrainVoyager 20.6. For both the empty bladder and the full bladder

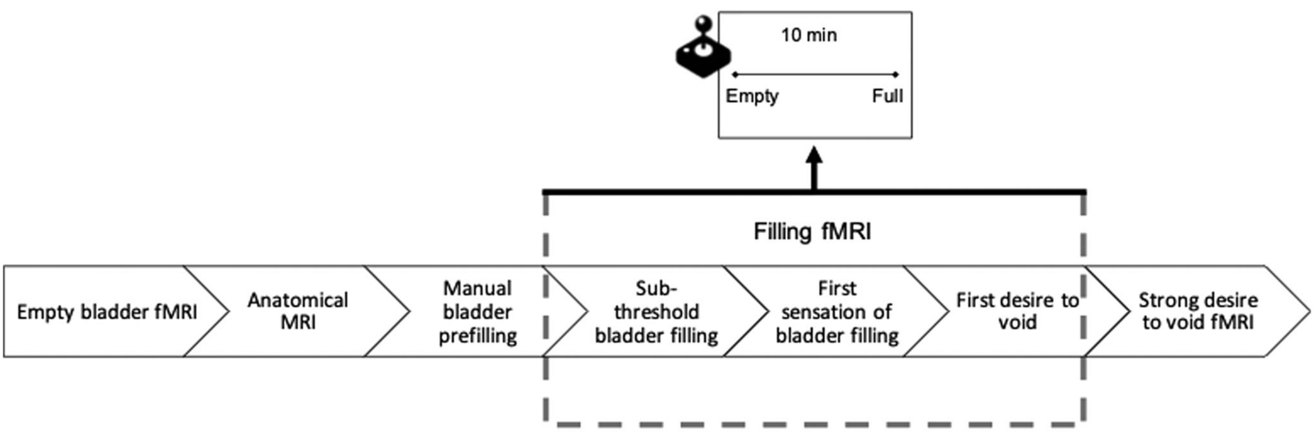

FIGURE 1 The scan paradigm consisted of an empty bladder resting-state fMRI scan which was followed by an anatomical scan. Next, we manually prefilled the bladder to a predetermined participant-specific level. A filling functional magnetic resonance imaging (fMRI) scan was then started, which could later be subdivided into different sensory states based on participant-reported data acquired using a joystick script. Lastly, a full bladder or strong desire to void fMRI scan was started

resting-state fMRI scans, the timecourse of each voxel within the PAG mask was correlated in a pair-wise fashion to obtain a voxel-by-voxel square connectivity matrix for both datasets. We removed the lowest 5% correlations that are assumed to represent spurious connections from our matrix.[25]

To generate parcellation maps of the PAG, we partitioned the connectivity matrices of both the empty bladder and full bladder data into clusters using a MATLAB implementation of the Louvain module detection algorithm.[26] This algorithm outputs clusters with stronger within-cluster connectivity than between-cluster connectivity.[27] The assignment of voxels to modules by the Louvain module detection algorithm has a stochastic nature, and to compensate for this, we ran the algorithm for 500 iterations and selected the parcellation with the largest modularity statistic (*Q*-value) for further analysis. The *Q*-value quantifies the extent to which a network has a modular underlying data structure.[28] Our parcellation algorithm then assigned a label to each voxel in the PAG assigning it to a module. These labels were then used to create a module map for each participant for, both, empty and full bladder datasets.

2.5 | Quantification of parcellations

We propose that if the clusters resulting from the parcellation procedure correspond to an underlying anatomical construct, these should not be influenced by subjective bladder sensations or objective bladder filling. To ascribe meaningful interpretations to our PAG parcellations and quantify our results we, therefore, aimed to assess the agreement between parcellations based on the empty and full bladder datasets.

Using the empty bladder parcellation, we computed random parcellations using a custom MATLAB script that randomly places N anchor points in the 3D space of the PAG mask, with N representing the number of clusters in the original dataset. From each of these anchor points, a cluster was grown until it reached a similar voxel count as the original cluster. We iterated this script to obtain 1000 different random parcellations. These random parcellations were subsequently used to assess the agreement between our parcellations based on fMRI data acquired during an empty and full bladder state compared to what could be expected based on chance. The Sørensen–Dice coefficient is a useful measure of spatial overlap which can be applied to studies of reproducibility and accuracy in image segmentation, it is based on the percentage of spatial overlap between two images.[29] For each participant, we computed the Sørensen–Dice coefficient between each cluster in the empty bladder parcellation and each cluster in the full bladder parcellation. Next, we computed the Sørensen–Dice coefficient between each cluster from the empty and full bladder parcellations and each cluster from the 1000 iterations of the random parcellation, obtaining a distribution of Dice coefficients under the null hypothesis. This allowed us to statistically test the extent to which the similarity between empty and full bladder parcellations was higher than could be expected based on chance on a single subject level using a nonparametric permutation test.

To evaluate the anatomical characteristic of a lateralized organization of the human PAG, we assessed the lateralization of each of the identified clusters during the full bladder scan. We identified the midline of the PAG mask for each participant and divided all clusters in the full bladder parcellation into a left and right homolog. We then computed the center of mass (CoM) of the whole PAG and used this as a reference point to plot the CoM of the left and right homolog of each cluster (Figure 2). This enabled the assessment of the difference in distance of the CoM from each left cluster to the CoM of the PAG versus the distance of the CoM from each right cluster to the CoM of the PAG. We expected the difference in distance of the CoM of the matching clusters in each hemisphere to the CoM of the PAG to be significantly lower than for non-matching clusters. This effect was statistically tested using a Wilcoxon signed-rank test.

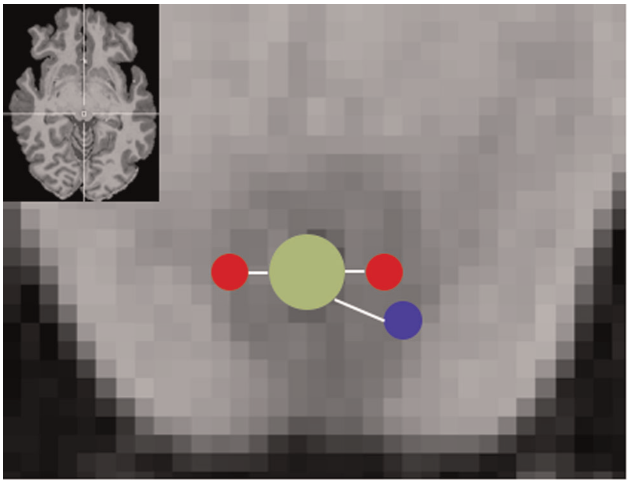

FIGURE 2 The relative location of the periaqueductal gray (PAG) in transversal view is indicated by the crosshairs in the top left corner of the figure. The center of mass (CoM) of the whole PAG is indicated in green. The red dots represent the left and right CoM of homologs of the same cluster. The blue dot represents the CoM of a nonmatching cluster. We compared the distance of the CoM of the cluster in left/right PAG to the CoM of the whole PAG for matching and nonmatching clusters

2.6 | Dynamic response profiles

From the filling resting-state fMRI scan, we could delineate three different states of bladder sensation using the data regarding participants' perceived levels of bladder fullness and urgency. The period before participants indicated any experienced bladder fullness was classified as "sub-threshold bladder fullness," once participants moved the cursor along the VAS the sensory state was classified as "first sensation of bladder filling," and when participants switched the IUSS indicator from 0 to 1 this was classified as "first desire to void." Together with the empty and full bladder resting-state scans, we had five different states of interest: (1) empty bladder; (2) partially filled bladder without sensation of bladder filling; (3) first sensation of bladder filling; (4) first desire to void; (5) strong desire to void. To obtain a dynamic response profile of PAG connectivity changes related to bladder sensations, we computed the correlations between the different clusters identified in the parcellation procedure of the full bladder dataset for each of the five different sensory states. To investigate how connectivity between PAG clusters changes during bladder filling, we fitted a second-degree polynomial to the data points and statistically tested the amplitude of the polynomial using a nonparametric permutation test.

3 | RESULTS

After completion of the scanning protocol, all participants voided a volume that was higher than the average volume of voids with an urgency level of 2 reported on the micturition diary, indicating that the interoceptive nature of the study did not significantly influence the tolerated infused volume.

3.1 | Parcellation analysis

We found that for each of our participants, the agreement between at least one of the clusters resulting from the empty and full bladder resting-state fMRI parcellation procedures was higher than could be expected based on chance ($p \leq .05$, corrected for multiple comparisons using a false discovery rate [$q = .05$]; Table 1). The mean similarity for modules identified in empty and full bladder parcellations, when compared to random iterations, was 69.4%, and for two of our participants this percentage was as high as 100% (Figure 3). This confirms our hypothesis that resting-state fMRI parcellations of the PAG are stable despite changes in subjective bladder sensations and bladder fullness.

The distance of the center of mass of clusters in each side of the PAG to the center of mass of the whole PAG mask was significantly lower for matching clusters than for non-matching clusters over our participants ($p = .016$). These results indicate that the clusters resulting from our parcellation procedures are organized in a lateralized fashion.

TABLE 1 Number of modules per participant for each parcellation and significant similarity ratio

Participant	Number of modules empty	Number of modules full	Significant similarity ratio
1	3	3	2/3
2	3	4	2/3
3	3	3	3/3
4	3	3	1/3
5	3	3	3/3
6	3	2	1/2

3.2 | Dynamic response profiles

The modularity Q-value resulting from our parcellation of the full bladder scan using the Louvain module detection algorithm was above 0.4, which is an indicator of a modular underlying data structure. The parcellation procedure in the representative subject used in the figures yields a subdivision of the PAG into three distinct clusters in the full bladder resting-state scan. The change in functional connectivity as a function of experienced bladder sensations between PAG Clusters 2 and 3 was significant ($p = .017$) after correcting for multiple comparisons using a false discovery rate ($q = 0.05$). The patterns of connectivity between Clusters 1 and 2, and 1 and 3 did not reach statistical significance (Figure 4). This suggests that connectivity between clusters within the PAG changes significantly as a function of experienced sensations during bladder filling, and gives an indication of the potential implications of these dynamic response profiles in studying PAG activity related to bladder fullness and participants' experienced bladder sensations.

4 | DISCUSSION

4.1 | Parcellation analysis

Our results indicate that PAG activity in an empty and full bladder state can reliably be subdivided in clusters that are largely independent of bladder state, and show a

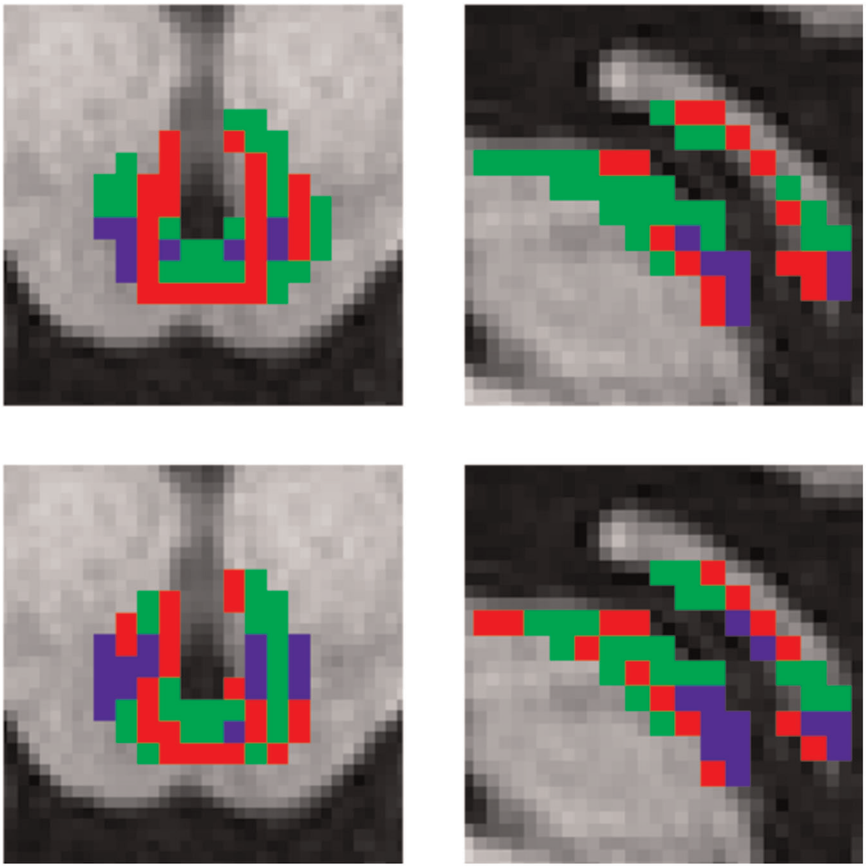

FIGURE 3 Visualization of the spatial correspondence between modules that show a significantly larger spatial overlap than could be expected on chance for a representative participant. Top row: transversal and sagittal view of parcellations based on data acquired during an empty bladder state. Bottom row: transversal and sagittal view of parcellations based on data acquired during a full bladder state. Colors indicate corresponding modules

higher similarity with each other than could be expected based on chance. Thereby, supporting the idea that voxels from clusters resulting from resting-state fMRI-based parcellation of the PAG have a high correlation in their BOLD timecourses related to an underlying anatomical construct.

As an additional measure of the extent to which the clusters from the parcellation procedures have an underlying anatomical construct, we observed that clusters are organized in a significantly more lateralized fashion than can be expected by chance. This implies that our parcellation correctly identified the symmetrical organization of the PAG.

These results imply that this method can be used to reliably parcellate the PAG using ultra-high-field resting-state fMRI data. This opens opportunities to use parcellation methods to study changes in dynamic connectivity between PAG clusters related to bladder fullness and participants' experienced bladder sensations.

The current study is the first to parcellate the human PAG using 7-T resting-state fMRI data utilizing a largely data-driven approach. We validated our methodological approach by running parcellation procedures on two datasets from the same subject in different physiological and sensory states, namely with an empty bladder and no desire to void versus a full bladder and a strong desire to

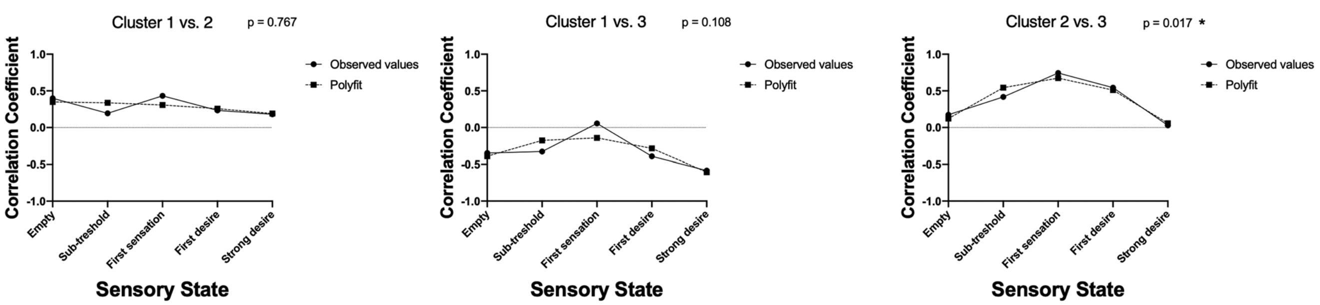

FIGURE 4 The observed correlations between periaqueductal gray clusters at different sensory states. The correlation between Clusters 2 and 3 was found to change significantly as a function of bladder sensations

void. Using ultra-high-field MRI methods, we are nearing spatial resolutions that allow for direct translations of invasive electrophysiological animal work to the human situation in noninvasive research designs. In view of our limited samples size, for the scope of this study, we have investigated the agreement between PAG parcellations at the within-subject level. To advance this line of research, future studies should assess whether an agreement can be found between PAG parcellations at the between-subjects level. Additionally, since the current study only included female participants, more research is needed to investigate the agreement between PAG parcellations across genders. This will allow for further validation of underlying (functional) anatomical organizations. Furthermore, the observation of such an agreement will enable the assignment of potential changes in PAG organization due to pathophysiological conditions to specific anatomical areas within this region.

4.2 | Dynamic connectivity profiles

Our results suggest that connectivity between PAG clusters identified in parcellations of full bladder resting-state fMRI data changes significantly between different sensory states during a bladder filling protocol. The observed changes in connectivity between PAG clusters agree with changes in PAG activity in animal studies,[8] and future studies should assess changes in connectivity between PAG clusters during the voiding phase. These distinct connectivity patterns can be seen as dynamic response profiles of PAG activity. These profiles, when established in healthy controls, can serve as a benchmark to which LUTS patients' response profiles can be compared. The separation of resting-state fMRI data by participants' reported bladder sensations is a completely new approach to study CNS activity related to bladder functioning. This approach will help to better understand CNS activity related to subjective bladder sensations and bladder filling. This technique will thus allow us to monitor bladder filling dependent activity in the reciprocal communication between the lower urinary tract and the CNS. Therefore, it can be utilized to develop a new and necessary imaging biomarker framework for translational research into new therapeutic targets, and new diagnostic strategies combined with treatment effectivity predictors and evaluators.

5 | CONCLUSIONS

In the present study, we aimed to assess whether the PAG can reliably be parcellated using a resting-state fMRI scanning protocol. Our results support that these parcellations are independent of participants' reported changes in bladder sensations or bladder filling. Additionally, we found that these parcellations show a symmetrical lateralized organization that could be expected based on previous postmortem neuroanatomical studies[30] and work in rodents.[31] Correlations between the identified clusters are indicated to change significantly as a function of experienced sensations during bladder filling. The current study lays the foundation to take an interdisciplinary and translational approach to find new and more optimally targeted treatment and diagnostic options for LUTS patients.

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CONFLICT OF INTERESTS

Gommert A. van Koeveringe received fees for consultancy from Astellas, Boston Scientific, Solace Therapeutics, Medtronic, and Laborie. He conducted trials for Astellas, Medtronic, Solace Therapeutics. None of these activities are related to this study. The other authors declare no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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