The Role of the Periaqueductal Gray Matter in Lower Urinary Tract Function

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Abstract
The periaqueductal gray matter (PAG), as one of the mostly preserved evolutionary components of the brain, is an axial structure modulating various important functions of the organism, including autonomic, behavioral, pain, and micturition control. It has a critical role in urinary bladder physiology, with respect to storage and voiding of urine. The PAG has a columnar composition and has extensive connections with its cranially and caudally located components of the central nervous system (CNS). The PAG serves as the control tower of the detrusor and sphincter contractions. It serves as a bridge between the evolutionary higher decision-making brain centers and the lower centers responsible for reflexive micturition. Glutamatergic cells are the main operational neurons in the vlPAG, responsible for the reception and relay of the signals emerging from the bladder, to related brain centers. Functional imaging studies made it possible to clarify the activity of the PAG in voiding and filling phases of micturition, and its connections with various brain centers in living humans. The PAG may be affected in a wide spectrum of disorders, including multiple sclerosis (MS), migraine, stroke, Wernicke’s encephalopathy, and idiopathic normal pressure hydrocephalus, all of which may have voiding dysfunction or incontinence, in certain stages of the disease. This emphasizes the importance of this structure for the basic understanding of voiding and storage disorders and makes it a potential candidate for diagnostic and therapeutic interventions.

Keywords Periaqueductal gray matter · Bladder · Incontinence · Micturition · Brain

Introduction
The PAG is the central gray matter of the midbrain, in continuity with the circumventricular organs, and is to a large extent analogous to the gray matter of the spinal cord. Due to its axial location, the PAG is involved in various important functions, including autonomic [1, 2], behavioral [3], pain [4], and micturition control. The role of the PAG in the control of the bladder function encompasses both downstream connections, as well as connections with the higher brain centers involved in decision-making. Barrington was the first one to discover the function of the pontine micturition center (PMC) (Barrington’s nucleus), and the role of the midbrain in the control of micturition, though he did not mention the PAG [5].

The PAG has been found to bear functionally separate columns [6], which are also different histopathologically in the human brain [7]. There are three pairs of columns, namely ventrolateral (vlPAG), lateral (lPAG), and dorsolateral (dlPAG), as well as a single dorsomedial column (dmPAG), in the PAG (Fig. 1). These columns can be functionally divided into two groups, having opposite autonomic functions: the ventrolateral column, which has parasympathetic functions, and the lateral and dorsolateral columns which have sympathetic functions [8, 9]. Even considering the amount of expression of specific markers, or distant connections, there exist two distinct components: the dorsolateral pair of columns, and the remaining columns, including the ventrolateral, lateral, and dorsomedial pairs [10]. dlPAG is functionally more closely...
related to the midbrain tectum, and the remaining columns may be considered more closely related to the periventricular structures [10]. All of the PAG columns have ipsilateral and contralateral reciprocal connections [11]. Internal connections also exist within each column [11]. Below, we will discuss the role of the PAG in the control of micturition.

Connections of the PAG

The PAG has extensive connections with the cortex (prefrontal, cingulate, and insular gyri), diencephalon (thalamus and medial preoptic area of hypothalamus (MPO)), brainstem (PMC), and the spinal cord (sacral segments) (Fig. 2) [15, 20–25]. Caudal connections of the PAG have been investigated by tracing techniques, which are briefly mentioned in this section. The cranial connections with cortical regions have been discovered mainly by connectivity analysis in functional imaging studies, which will be described in the following sections.

PMC, as another important micturition control center, has bilateral connections with vPAG [23, 24, 26], as well as receiving afferents from dPAG and the MPO [23, 27, 28] (Fig. 2). In parallel, MPO has direct connections with the PAG [29–31]. The MPO, rich in androgen and estrogen receptors, is integral to the limbic, or the so-called emotional motor system, regulating sexual behavior. Since micturition is an important behavioral signal in animal territorial demarcation, and the scent of urine may additionally serve as a sexual message, the PAG may integrate the micturition and mating functions.

The dPAG along with vPAG receive afferents from spinal parasympathetic and dorsal commissural nuclei of the lumbo-sacral cord [12, 32, 33], which have ipsilateral dominance [32]. This pathway contributes to the awareness of the PAG to the state of the bladder fullness. Efferent PAG connections project indirectly via PMC to the spinal segments and are distributed via somatic or autonomic nerves and ganglia to the detrusor muscle or sphincters [34].

The vPAG as the Major PAG Column with Connections to the Bladder

The vPAG has direct connections with the spinal segments [35]. It is also the main column receiving afferents from the lumbo-sacral cord, which have ipsilateral dominance [32] and originate from laminae V, VII, and VIII [35]. Spinal neurons throughout the cord projected more to the IPAG and the vPAG rather than to the dPAG or the dmPAG, and specially segments from the S1-S3 projected to the central portion of the IPAG and the vPAG [13]. Indeed, only few neurons, mainly located in the lumbo-sacral segments, project to the dorsomedial and dorsolateral PAG columns [36]. The quantity of projections weighs toward the ventrolateral column, more than to the other columns, and there exists evidence showing the higher significance of the role of the vPAG, regarding the control of bladder function.

Further evidence regarding the importance of vPAG in micturition control is as follows. Most c-Fos reactivity is induced in the vPAG after chemical irritation [37] or electrical stimulation (unpublished data) of the bladder. c-Fos is a transcription factor expressed after neuronal activation. Electrical stimulation of the pelvic nerves in the cat evoked maximum field potentials in vPAG [38]. Studies in cats confirm that it is the vPAG, of which the electrical or chemical (DL-homocysteine) stimulation results in the contraction of the bladder [24]. Chemical stimulation of the vPAG by D,L-homocysteic acid (DLH) in rats increases the frequency of micturition [39]. On the other hand, stereotaxic injection of the inhibitory mediator cobalt chloride into the caudal vPAG reversibly attenuates bladder contractions and external urethral sphincter (EUS) electromyographic activity in rats [40]. Injection of other inhibitory or stimulatory agents, CoCl2 and L-glutamate, into the vPAG leads to suppression or stimulation of voiding in rats, respectively [41]. Taking these studies into account, it is most likely that the vPAG would be the primary station of the ascending PAG afferents, and it may secondarily relay these signals to the other PAG columns [11].
PAG and the Function of the Bladder

Functional studies regarding the control of the PAG over the bladder encompass electrical or chemical stimulation of the PAG, single-unit recordings in the PAG, or indirect evaluation of the function by measuring the neuronal markers c-Fos or nerve growth factor (NGF). We can differentiate two major columns, the ventrolateral and the dorsolateral columns, for playing the main role in the micturition-related PAG functions.

Studies Featuring the Function of the Ventrolateral Column of PAG

Some studies have a top-down design, in such a way that central electro-stimulation at PAG is accompanied by peripheral evaluation of function at bladder level. Electrical stimulation of the vlPAG elicits either contraction or inhibition of the bladder [42]. The optimum sites for evoking bladder contractions were located in and close to the laterodorsal tegmental nucleus (LDT) (which is the same structure functionally called PMC), and in the PAG, just dorsal or dorsolateral to the LDT, in rats [42]. Electrical stimulation of the ventral PAG elicits neuronal firing at the postganglionic nerves of the bladder [43]. Studies in cats confirm that electrical or chemical (DL-homocysteine) stimulation of vlPAG results in contraction of the bladder [24].

Inversely, some other studies have a down-top design, i.e., peripheral electro-stimulation is accompanied by central evaluation. Electrical stimulation of the pelvic nerves in the cat evokes maximum field potentials in vlPAG [38]. Since the regions activated by the pelvic nerve stimulation differ from those activated by stimulation of the sensory pudendal or superficial perineal nerves, it is possible that specific pathways
exist for different nerve inputs to the PAG [38]. However, in another study, electrical stimulation of afferents in the pelvic nerve of the rat evoked field potentials in the dorsal part of the PAG [43].

Single-unit recordings in PAG columns either with or without stimulation of the bladder derived some information regarding the PAG neuronal activity. The vlPAG shows three different patterns of neuronal firing rate during the micturition: increased firing rate, decreased firing rate, or no correlation with the micturition [44]. These neurons may correspond to specific functions they have during the micturition, with some of them contracting the detrusor, some relaxing the sphincter, some receiving afferent sensory signals, and others unrelated to the micturition. A similar study accompanied by intravesical pressure recordings was conducted in cats [45]. However, only 16 out of 84 neurons that were recorded were located in the PAG. The rest of the neurons were in adjacent midbrain areas. Nevertheless, this study emphasizes the existence of neurons in ventrolateral and lateral columns of the cat PAG, with firing patterns which change, corresponding to specific phases of micturition [45].

Chemical stimulation or suppression of the PAG exerts similar effects over the bladder. As detailed above, chemical stimulation of vlPAG increases the frequency of micturition in rats [39, 41] and contracts the detrusor muscle in cats [24]. On the other hand, chemical inhibition of vlPAG attenuates the detrusor and EUS contractions in rats [40, 41]. Similarly, bilateral electrolytic lesion of vlPAG and IPAG in cat attenuates detrusor contractions [45].

Neuronal activation may also be evaluated by specific markers such as c-Fos or NGF expression. Either anatomical manipulation or chemical irritation of the bladder may induce increased neuronal activity in the PAG. Increased c-Fos [46] or NGF [47] reactivity in vlPAG was observed after the induction of stress urinary incontinence by transabdominal urethrolysis in rats. Chemical bladder irritation, which is a painful stimulus, induced c-Fos expression in the PAG, though the exact PAG column was not stated [48].

Studies Featuring the Function of the Dorsolateral Column of PAG

Some studies proposed the possibility of the existence of a specific micturition-suppressing region in dlPAG, acting via GABAergic inhibition of PMC [49]. Electrical stimulation of dlPAG elicits various types of reactions from the bladder. Electrical stimulation of the dorsal part of the PAG, including the dorsomedial and the dorsolateral columns, in rats, resulted in a higher frequency of voiding, as well as some behavioral responses such as tense immobility, accompanied by exophthalmos and running and jumping responses [50]. A similar result, as bladder contraction, was encountered after electrical stimulation of points concentrated at the superior collicular and intercollicular levels, in an area involving the deep layers of the superior colliculus, the dlPAG, and the tegmental reticular formation, neighboring the most lateral border of the PAG, in cat [51]. Nevertheless, this may be an erroneous conclusion due to inadvertent diffusion of electrical current to other PAG columns. In fact, electrical stimulation or glutamate microinjection within either ventrolateral, lateral, or dorsolateral columns evoked the rise of the intravesical pressure, as well as an increase in blood pressure [52]. Another reason behind this discrepancy between different studies may be the difference in cranio-caudal location of the stimulation sites. For example, a similar study in cat mentioned rostral part of dorsal PAG and caudal part of ventral PAG to be the main sites, at which high-frequency electro-stimulation causes inhibition of micturition [45].

Other Functional Studies

Some other studies show that stimulation of either the vl- or dlPAG would suppress the contraction of the bladder. Deep brain stimulation (DBS) in vlPAG attenuates or completely suppresses the voiding in rats and humans [53]. Similar physiological activities such as rhythmic straining reflexes or defecation, alongside with micturition, are inhibited by electrical stimulation of the ventral or dorsal PAG, dorsal raphe nucleus, and central tegmental field, with similar threshold intensities, in dogs [54]. These studies show that the electrical stimulation of various midbrain structures, and not only specific PAG columns, may suppress some pelvic functions. Such equivocal results may be due to a possible jamming effect over the normal electrical circuitry, contributing to the negative impact of DBS on micturition [39]. One possible explanation is the disruption of the normal coordinated voiding activity in the detrusor and sphincter muscles, after DBS.

Since the micturition reflex is under unconscious control during sleep, and nocturnal enuresis is a common associated disorder, it would be interesting to investigate the function of PAG during sleep, and the associated changes in detrusor contractions. Simultaneous recordings of the detrusor pressure, EUS electromyogram (EMG), cortical electroencephalogram (EEG), and single-unit activity in the PAG in rats reveal that during slow-wave EEG activity (SWA), voiding becomes more irregular and detrusor voiding pressure threshold and voiding volume threshold, and the duration of the bursting activity in the EUS EMG is raised, all in line with maintaining continence during sleep [55]. SWA is associated with slower neuronal firing rate in the PAG as well. Different sleep-like brain states are associated with changes in urodynamic properties, suggesting changing excitability of the micturition circuitry in the PAG. This may uncover some underlying factors in the pathophysiology of nocturnal enuresis [55].

In summary, the PAG receives ascending sensory signals from the bladder and can modulate the bladder function by its...
descending efferent connections. Such bilateral connections may be important in conducting reflexive micturition in rodents, or the immature human. This comprises a feedback loop, with PAG continuously monitoring the state of bladder fullness, and induction of voiding in a suprathreshold-filled bladder (Fig. 2).

The Role of Distinct Cell Groups in PAG Function

To better understand the organization of neural circuits, different cell populations contributing to various neural pathways are investigated (Table 1). The PAG, like most other brain regions, has a variety of distinct cell groups and has immense connections with its cranially and caudally located CNS structures, related to the control of the micturition. Among these cell groups are dopaminergic, serotoninergic, glutamatergic, and GABAergic neurons, and cells expressing neuronal nitric oxide synthase (nNOS). Dopamine, serotonin, and glutamate are stimulatory neurotransmitters, and GABA and nNOS are inhibitory neurotransmitters. PAG neurons express receptors for all of these neurotransmitters (Table 1).

The vlPAG has distinct groups of glutamatergic cells which can stimulate other centers [62]. Chemogenetic or optogenetic stimulation of glutamatergic neurons in the vlPAG leads to detrusor contraction and voiding [64]. By contrast, chemogenetic or optogenetic activation of vlPAG GABAergic neurons delayed detrusor contraction and inhibited voiding [64]. The vlPAG GABAergic cells stimulated in this experiment were most probably interneurons [75]. On the other hand, the main inhibitory GABAergic input to the vlPAG, relevant to the micturition, projects from dlPAG [49]. Glutamatergic cells of the vlPAG also control other important functions, including freezing [75] and nociception [76], which are controlled by two separate cell groups in the vlPAG [75]. Whether these cell groups are different from those vlPAG glutamatergic cells controlling voiding, or have some overlap, remains to be elucidated.

Excitatory Signaling

In this section, we mention glutamatergic, dopaminergic, and serotoninergic neuronal signaling, arising from PAG and influencing micturition.

Glutamate serves as an excitatory neurotransmitter [77]. Its extracellular levels have been shown to be increased in PAG, after bladder distension or voiding, by microdialysis studies [58, 61]. Glutamatergic cells may project back to brainstem structures related to the control of the bladder contraction, to

| Table 1 | Synthesis of neurotransmitters and the expression of their receptors in the PAG, and their functional significance |
|-----------------|-----------------|-----------------|
| Synthesis | Receptor | Role in micturition (all experiments were performed on rats) |
| Dopamine | ✓ [56] | ✓ [57] | Inhibitory:  
  ✷ Application of a D1 receptor antagonist into the PAG facilitated the micturition reflex [58]. |
| Serotonin | ✓ [59] | ✓ [60] | No role detected so far:  
  ✷ It seems to be responsible for nociception in the PAG [61]. |
| Glutamate | ✓ [62] | ✓ [63] | Excitatory:  
  ✷ Chemogenetic or optogenetic stimulation of vlPAG glutamatergic neurons leads to voiding and detrusor contraction [64].  
  ✷ Glutamatergic vlPAG cells were activated after bladder electro-stimulation [65].  
  ✷ Glutamate microinjection within the PAG evoked a rise of intravesical pressure [41, 52].  
  ✷ Saline infusion into the bladder with consequent induction of the micturition reflex resulted in increased extracellular glutamate levels in the PAG [61]. |
| GABA | ✓ [66, 67] | ✓ [68, 69] | Inhibitory:  
  ✷ Chemogenetic or optogenetic activation of vlPAG GABAergic neurons delays detrusor contraction and inhibits voiding [64].  
  ✷ Microinjection of a GABA agonist into the vlPAG of the rat depressed reflex voiding frequency, whereas microinjection of a GABA antagonist into the same region increased reflex voiding frequency [70]. |
| Opioid | ✓ [71] | ✓ [72] | Inhibitory:  
  ✷ Injection of a µ receptor agonist into the caudal vlPAG abolished volume-evoked micturition [73].  
  ✷ Intracerebroventricular injection of morphine or a µ agonist showed consistent inhibition of spontaneous urinary bladder contractions [74]. |

The check (✓) mark positively denotes that the chemical mediator identified is either synthesized in the PAG, or the PAG bears receptors for them, in conjunction with their respective references

1 Capability to synthesize and the bearing of specific receptors for a particular neurotransmitter imply the existence of efferent or afferent pathways incorporating that particular neurotransmitter in the PAG.
fulfill a micturition reflex, or to higher cortical regions to undergo further analysis and decision-making. The vlPAG receives afferents from the lumbosacral cord [32] and has direct connections with the PMC [32], which then controls the micturition by its efferents to sacral parasympathetic segments. This circuit is especially very active in rodents and probably in the immature human infant, while higher decision-making brain centers are still underdeveloped. vlPAG glutamatergic cells probably stimulate the PMC, after receiving suprathreshold sensory signals from a full bladder. As a clinical correlate, multiple system atrophy (MSA), which is an extrapyramidal disease, has micturition symptoms including frequency, urgency, incontinence, or incomplete bladder emptying, as part of its manifestation. The number of glutamatergic cells in the ventrolateral, lateral, dorsomedial, and to a lesser extent dorsolateral PAG columns was shown to be decreased in MSA [78].

The role of dopaminergic neurotransmission over micturition is controversial. Microinjection of SCH-23390, a dopamine antagonist, into the PAG, had contrary results over micturition in two different studies [58, 79]. The PAG dopaminergic neurons mostly project rostrally to higher brain regions [80, 81]. There is a loss of putative wake-active PAG dopaminergic neurons in patients suffering from either MSA or dementia with Lewy bodies, which may contribute to excessive daytime sleepiness in these disorders [82]. Both of these conditions have micturition problems. As micturition is in coordination with the sleep-wake cycle, their possible role on micturition must be indirect, via influence over other autonomic systems.

Serotonergic cells are abundant in the brain. The evidence regarding the role of serotonin in the regulation of micturition is very limited. Few serotonergic neurons are present in the PAG, which are particularly scattered in vlAPG, close to the dorsal raphe nuclei. This actually makes these few cell populations suspicious to be extra-raphe serotonergic cells, not being part of the main PAG columns. There is not much known about the micturition-related pathways within the PAG, involving serotonin for neurotransmission. The vlPAG serotonergic cells inhibit the ejaculation in rats and may contribute to SSRI (selective serotonin reuptake inhibitor)-induced inhibition of ejaculation [83]. Yet, regulation of the autonomic function of the genital organs is different from that of the bladder. Since glutamate modulates 5-HT release in the PAG [84], and serotonergic cells of the PAG also have projections toward other brainstem regions [85], there may be an indirect relation between the bladder stimulation and the serotonin system.

Inhibitory Signaling

Inhibitory signaling from PAG arises from GABAergic cells, or neurons expressing nNOS.

GABAergic transmission has an indirect role over vlPAG, by internal connections via the dorsolateral column of the PAG [11]. GABAergic cells of dlPAG project to vlPAG to suppress the micturition reflex [49]. PMC has reciprocal connections with the vlPAG [23, 24] and receives inhibitory GABAergic input from dlPAG [49] (Fig. 2). The existence of reciprocal connections between various columns of the PAG [11] emphasizes the existence of a micturition-suppressing region in dlPAG.

The bladder’s intramural ganglia, dorsal root ganglia, and spinal cord contain nitric oxide (NO), the expression of which shows plasticity, following pathological lesions, such as pelvic nerve injury, chronic bladder irritation, and urethral obstruction [86]. The rate of NO production in brain is dependent on dynamic regulation of its synthetic enzyme, nNOS [87]. There has been no report about the role of nNOS in the brain, related to the physiology of micturition. Most functions mediated by nNOS, including modulation of the cardiovascular, behavioral, or nociceptive functions, have been found to be mainly active at dorsal PAG [88–90]. The existence of any possible role over the micturition pathways in the PAG by NO would probably be mediated by its interactions with GABA [91]. Its inhibitory neuronal activity within the PAG [92] may also be explained accordingly, since GABA is an inhibitory neurotransmitter.

An overall comparison of various cell groups in a standardized setup shows that in contrast to serotonergic, dopaminergic, GABAergic, and nNOS-synthesizing cells, only vlPAG glutamatergic neurons are activated upon receiving afferent bladder sensory signals [65].

Table 1 summarizes some key cell types in the PAG, bearing particular neurotransmitters, and their corresponding receptors, and describes their functions in micturition.

Other Important Chemical Mediators of PAG Function

Since the PAG is also a center for pain control, endogenous opioids have significant presence in this region. Their potential engagement in the control of voiding has been investigated in various ways. Among the different types of opioid receptors and PAG columns, it is only μ (mu) receptors which have an inhibitory effect on the vlPAG, by abolishing volume-evoked micturition [73]. Intracerebroventricular injection of morphine or the μ agonist morphiceptin confirms the aforementioned findings, showing consistent inhibition of spontaneous urinary bladder contractions [74]. The rapid onset of action and its limited distribution, shown by the intraventricular dye injection studies, indicates that its actions are confined predominantly to the periventricular and periaqueductal or associated areas, and not to the spinal cord [74].

The α1-adrenergic receptor antagonist tamsulosin, and the PDE-5 inhibitor sildenafil, significantly suppressed the increase in neuronal activities measured by the expression of
c-Fos and NGF, in the vPAG, in an overactive bladder rat model [93]. Further evidence for the role of phosphodiesterase system comes from studies using caffeine. Caffeine is a methylxanthine alkaloid chemically related to the adenine and guanine bases of DNA. Its mechanism of action is by phosphodiesterase inhibition and adenosine antagonism. Caffeine administration to rats for 14 days increased bladder smooth muscle contraction pressure and time, determined by cystometry [94]. Expression levels of c-Fos and NGF in the vPAG were also significantly increased following the administration of caffeine [94]. Hence, the phosphodiesterase system has possible regulatory role over the PAG and can influence the micturition.

**Functional Imaging of the PAG**

Functional imaging is a non-invasive tool for visualizing the activation of specific brain regions in response to various stimuli, respecting sensory afferent and motor efferent functioning. However, state-of-the-art functional imaging techniques usually lack adequate spatial resolution to make reliable statements about the function of a specific column of the PAG (Fig. 3a, b). Nevertheless, functional imaging studies have shed light on different connections of the PAG (Figs. 2 and 3b). They can also detect defects in some structural or functional pathologies involving the PAG. Functional MRI (fMRI), as the most common type of functional imaging, is the imaging modality widely used to determine the activated brain regions in different phases of micturition.

Since PAG is also a center for handling nociceptive signals, it would be interesting to differentiate pain from other afferent sensory information processing. Both distention of the bladder and painful stimuli may activate the PAG, with different patterns. The vPAG, among other regions, was activated in human PET scans after bladder distension, but not with intravesical ice bladder instillation, revealing different pathways in bladder distension and pain [98]. fMRI on healthy females shows that cold (pain) sensation is processed differently from bladder distension at the supraspinal level [99]. Parallel in vivo studies in mice revealed that selective optogenetic activation of bladder sensory fibers can differentially modulate nociceptive information and autonomic reflexes [100]. These findings show that bladder nociceptive and mechanosensory signals have separate pathways, both in the periphery and in the brain.

PAG is also activated in some similarly related physiological processes, like pelvic muscle contraction or rectal distension. In a non-voiding model of voluntary micturition control, ventral pons and the PAG showed more enhanced activation patterns by fMRI, in voluntary contraction than in the relaxation of the pelvic floor muscles [101]. Rectal distention in humans led to PAG activation detected by PET that was also associated with increased heart rate and with increased plasma adrenaline [102].

The PAG is active in both storage and voiding phases of the micturition cycle, but the extent of its activity differs between these two phases. During the storage phase, the PAG is activated, but the PMC is inactive, and during the voiding, the PMC maintains activation, and the activation of the PAG enhances [103].

Hereby, more evidence is provided supporting the activity of the PAG in storage and voiding phases of micturition.

**The PAG Activation During the Storage Phase:**

These experiments are usually designed in such a way that the brain would be scanned, while the bladder is being passively filled by intravesical infusion of saline. This way, mechanosensory signals arising from an expanding bladder...
would reach central micturition control centers. The activity in the right anterior insula and the PAG in human was enhanced at higher bladder volumes, in a non-voiding bladder, detected by fMRI [97] (Fig. 3b) and PET [104]. PAG activation after bladder filling is accompanied by the activation of the inferior parietal lobule, as well as the right insula and the dorsal anterior cingulate cortex (ACC) [105]. Passive filling and emptying of the bladder induce PAG activation as well [17, 106, 107]. Studies on Parkinson’s disease (PD) patients show that the activity of the PAG is enhanced in a full bladder, compared to an empty bladder [108]. Furthermore, DBS of subthalamic nucleus increases the PAG activity in PD patients [108]. This would indirectly influence the activity of other cortical regions related to micturition and ultimately restore aberrant bladder information processing [108]. These findings point to the fact that the PAG processes the sensory signals derived from a filling bladder. These signals may then flow to specific cortical regions and reach the awareness.

The PAG Activation During the Voiding Phase:

Initiation of Voiding

There is a special role for the PAG in the initial moments of the bladder emptying. The PAG is consistently active during “attempted micturition” [97] (Fig. 3b). During this maneuver, the individuals direct their attention to the sensations arising from the bladder and urethra and increase the desire to void as if initiating to allow the urine to pass [97]. Initiation of voiding induced significant activity in cortical regions, in addition to the PAG, as shown by fMRI, whereas actual micturition was associated with significantly less such activity [97]. Unsuccessful attempts at micturition result from inefficient activation of the PAG and PMC during the initiation phase, which itself may be secondary to inadequate antecedent activation of frontoparietal and cingulate cortices, involved in decision-making for the micturition behavior [109]. The same results can be found by PET scans [110]. fMRI studies found out that imitation or interruption of voiding by relaxation or contraction of the pelvic floor muscles in healthy women could induce activation patterns in the PAG, cortical regions, and some other micturition control centers [111]. The above findings emphasize the role of the PAG in the integration and relay of information coming from different areas (such as the spinal cord and the cortico-limbic system), which are essential in micturition physiology. This signifies the role of the PAG to be the trigger for implementing the decision for voiding.

Analysis of reflexive micturition in healthy rats by animal fMRI showed the activation of the PAG [112]. High-resolution animal fMRI in rabbit could specifically identify the vPAG for having an important role in bladder dysfunction biofeedback [113]. This is confirmed by a PET study in humans also showing the vPAG to be activated after distention of the bladder [98]. Columnar differentiation in human PAG could also be done by a PET scan, which identified the activation of the tegmental part of the PAG, denoting either ventrolateral or lateral columns, during micturition [114]. Nonspecific involvement of the PAG was mentioned in some other human PET scans [18, 115]. These discoveries are corroborated by a single-photon emission computed tomography (SPECT) in human, showing increased PAG activation during voiding [116].

With upcoming research by modern 7 Tesla and 9 Tesla MRI machines, we shall gain more precise information regarding the connections of individual PAG columns with specific parts of the brain.

Connectivity Analysis

It is often helpful to investigate the connections between one particular structure and other brain regions, and their temporal pattern of activation. The PAG has been found to be functionally connected with some other brain regions during voiding (Fig. 2). The physiophysiological interaction (PPI) is a sophisticated tool used to elucidate the effective connectivity between different brain regions and a particular task, i.e., the micturition. It is generally based on observing alterations in the slope of the regression line of two different brain regions, with respect to another region, or a particular task [117]. This method aids in revealing the cortical pathways, together with their associated functions (Fig. 2).

fMRI signals of the right insula/operculum change in parallel with PAG activity (indicating connectivity), when the subject perceives an enhanced urge to void during “attempted micturition” [97]. Indeed, positive functional connectivity exists between the vPAG and brain centers involved in micturition, such as the ACC or insula, shown by resting-state fMRI (rsfMRI) [118]. The PAG clearly shows some laterality in its effective connectivity, which is to right insula during the filling of the bladder [119, 120].

White matter hyperintensities (WMH) are common in the older population and have been linked to demyelination, spongiosis, and glial proliferation, presumably after lacunar ischemic infarcts. Global WMH has been linked to impairments of mobility, cognition, affect, and continence [121]. These hyperintensities could be located in any of the central micturition control centers and are particularly related to the severity of the incontinence [122]. The PAG is one of the regions, of which activity shows positive correlation with the global WMH [123]. Hence PAG may be secondarily affected by WMH, contributing to the micturition symptoms.

The imaging modalities explained above may be used for experimental or diagnostic purposes. Putting various cortical connections of the PAG together, we find a circuit of continuous monitoring of the level of the bladder fullness, vigilance of the environmental circumstances, and deciding to void at the right
moment. This comprises a cortical feedback loop, completed by incorporating the ventrolateral and dorsolateral PAG columns, for thinking and implementation of the decisions (Fig. 2).

PAG in Neuropathological Conditions and Their Effects on Micturition

The PAG may be affected in several pathological states, and its role has been investigated in various disorders showing lower urinary tract problems as part of their spectrum of symptoms. Here, we explain some structural and functional disorders, affecting the PAG and compromising micturition.

Structural Pathologies

The so far reported structural pathologies include stroke, neoplastic lesions, hydrocephalus, and spinal cord injury. Spinal cord injury would secondarily alter PAG activity states, as discussed below.

Structural damage involving the PAG has been reported to be a cause of voiding problems. Cerebral stroke, which is a debilitating disorder in the elderly, may cause voiding dysfunction. Urinary retention was among the manifestations of a patient suffering from acute ischemic stroke of the brainstem [124]. Diffusion-weighted MRI showed hyperintensity over the left paramedian segment of the lower midbrain, which includes the PAG. The computed tomography also showed a hemorrhagic transformation and intraventricular hemorrhage in the fourth ventricle [124], which is directly beneath the aqueduct. Either indirect ischemic damage of the PAG or chemical irritation by a hemorrhagic cerebrospinal fluid (CSF) inside the aqueduct could explain the micturition-related symptomatology. In an intracerebral hemorrhage (ICH)-induced rat model of neurogenic voiding dysfunction, c-Fos and NGF expression levels in the neuronal voiding centers, including vlPAG, were significantly increased with induced ICH, as compared to the control rats [125].

Various other structural defects may affect the PAG and lead to micturition problems. We here mention two case reports of subjects with neoplastic lesions or hydrocephalus, which compromised normal micturition by affecting the PAG. A lesion in the midbrain and upper pons was detected by MRI scan of a 34-year-old man presenting with a history of delayed ejaculation, voiding difficulties, and diplopia, which was a developmental abnormality such as a dermoid or teratoma [126]. In evaluation of patients with possible idiopathic normal-pressure hydrocephalus (iNPH) with typical imaging features (ventricular enlargement) and normal CSF pressure, a small bladder capacity and detrusor overactivity were seen in 95% of patients [127]. Since the PAG completely encircles the aqueduct, there is a possibility that this structure would be affected by minor dimensional changes in iNPH [127].

Sacral neuromodulation is commonly used in various neurologically mediated continence problems. The bladder filling and rest contrast, in eight spinal cord-injured (SCI) participants, elicited clear activation, measured by fMRI, in the PAG and in a continuous area in the right midbrain [106]. Following 2 weeks of pudendal stimulation treatment, abnormal PAG overactivity was decreased in all six participants, as well as in the four clinically improved subjects. Thus, the PAG may be overactive in the SCI group, following the sudden loss of the spinal afferent inputs [106]. The vlPAG overactivity in the SCI subjects was also demonstrated in a rat model, by increased expression of c-Fos or NGF, relative to the sham-operated group [128]. Abnormal PAG function would be restored to normal by sacral neuromodulation, in individuals with urinary retention [129].

Functional Pathologies

These disorders encompass demyelinating diseases, PD, MSA, migraine, Wernicke’s encephalopathy, nocturnal enuresis, and urge incontinence, which will be elaborated below.

One of the most common demyelinating diseases is multiple sclerosis (MS), a common debilitating disorder with white matter plaques affecting any part of the brain. In 18.7% of MS patients, lesions were located in the PAG [130]. Thirty-six percent of these lesions were periventricular lesions of the third ventricle, extending toward the aqueduct. Bowel and bladder disability scores in MS patients are correlated with the volume of lesions in the medial frontal lobes, cerebellum, insula, dorsal midbrain including dorsal part of the PAG, and pons, areas known to be involved in the control of micturition [131]. As a clinical correlate, a 31-year-old man had suffered from sudden voiding difficulty and retention. A filling cystometrogram revealed anatomic bladder with diminished bladder sensation. Hyperintensities were shown in the PAG in T2W-MRI that were reduced after steroid therapy, with subsequent improvement of the voiding symptoms. He was suspected to have a demyelinating disease such as MS [132].

Patients with PD, which is an extrapyramidal disorder, may have lower urinary tract symptoms. Elimination of dopaminergic neurons by 6-OHDA microinjection into the PAG in a rat model of PD leads to altered micturition patterns [133]. Moreover, the reduction of the amplitude of the evoked potentials measured in the PAG, elicited by means of electrical stimulation of the pelvic nerve in the rat, is more pronounced in PD animals compared to sham animals, after the intravenous administration of an adenosine receptor antagonist [134]. Besides the direct effect dopaminergic lesions can have on the PAG, there is some evidence showing that the micturition problems in PD may be a consequence of a primary problem residing in the substantia nigra (SN), secondarily affecting the PAG by its projections toward the PAG. Increased c-Fos reactivity was observed in the PAG and ACC, in a PD rat model.
induced by 6-hydroxydopamine injection into SN [135]. Moreover, a 6-OHDA lesion in the SN produces a transient increase in voiding frequency within the first 2 weeks, with recovery of urinary function by 4 weeks post-lesion [136]. In PD patients, significant brain activation can be detected by PET in the PAG, during detrusor overactivity (measured by intravesical pressure monitoring) [137]. As another example of extrapyramidal disease, MSA, which includes micturition symptoms, shows neurochemical changes in the PAG (detailed above) [78].

Furthermore, a possible role of the PAG has been suggested in nocturnal enuresis. Single-unit activity in the lateral and ventrolateral columns of the PAG was linked to the occurrence of voids induced by continuous infusion of saline into the bladder of urethane-anesthetized rats, to mimic sleep-like brain states [55]. Almost a quarter of the recorded neurons were responsive during the micturition reflex. Their spontaneous firing rate in the absence of bladder stimuli decreased during slow-wave EEG states [55]. This suggests that the micturition reflex is reset centrally during the sleep. Failure of this mechanism could contribute to the development of nocturnal enuresis [138].

Various other functional disorders may have PAG malfunction together with micturition symptoms. Vegetative symptoms including increased micturition may occur in migraine [139]. More specifically, vlPAG was found in a PET study as a structure that may be implicated in migraine pathophysiology [140]. PAG dysfunction also has been shown in Wernicke’s encephalopathy [141]. A case report of Wernicke’s encephalopathy in a pregnant woman described decreased bladder volume and detrusor hyperreflexia in urodynamic studies. Brain MRI revealed abnormal intensities in medial thalamic-hypothalamic regions, and the PAG [141]. PAG [142] or midbrain [143] activation was shown by fMRI studies in urge incontinent patients as well. CBV analyzed by PET is decreased in the midbrain during sacral neuromodulation in chronically implanted urge incontinent patients [144].

By its involvement in a multitude of disorders leading to micturition problems, the PAG must be included in future diagnostic or therapeutic algorithms concerning neurological causes of the bladder dysfunction. Specifically, with the availability of future high-precision MRI machines, the PAG can be more specifically evaluated, for diagnostic purposes.

**Conclusion**

The PAG plays the role of a switchboard located in the brainstem, coordinating the evolutionary primitive and advanced brain centers. It has a broad spectrum of functions and has a paramount role in the control of micturition. The PAG functions as a sensory and motor relay station for the ascending afferents from the lower urinary tract, and descending afferents from the cortical areas. This is partly made possible by its special position at the intersection of the forebrain and the hindbrain. Two of the four columns of the PAG, namely the ventrolateral and dorsolateral columns, demonstrate more significant involvement in this respect. The vlPAG is more connected to the caudal structures, and the dlPAG is more connected to the cranial structures of the CNS [16]. The intercolumnar connections [11] traverse the information between vlPAG and dlPAG and thus complete a full circuit. Pathological conditions affecting the PAG may compromise the continence, and some of them may be detected by modern imaging techniques. Thus, the PAG will be a potential diagnostic and therapeutic target for specific incontinence problems and voiding dysfunctions. This may be done pharmacologically, by for example targeting its glutamatergic neurotransmission, or surgically, by DBS of particular PAG columns.

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**References**

1. Rathner JA, Morrison SF (2006) Rostral ventromedial periaqueductal gray: a source of inhibition of the sympathetic outflow to brown adipose tissue. Brain Res 1077(1):99–107. https://doi.org/10.1016/j.brainres.2006.01.035
2. Farkas E, Jansen AS, Loewy AD (1998) Periaqueductal gray matter input to cardiac-related sympathetic premotor neurons. Brain Res 792(2):179–192
3. Mouton LJ, Holstege G (1994) The periaqueductal gray in the cat projects to lamina-Viii and the medial part of lamina-vii through-out the length of the spinal-cord. Exp Brain Res 101(2):253–264
4. Budai D, Harasawa I, Fields HL (1998) Midbrain periaqueductal gray (PAG) inhibits nociceptive inputs to sacral dorsal horn nociceptive neurons through alpha(2)-adrenergic receptors. J Neurophysiol 80(5):2244–2254
5. Barrington FF (1925) The effect of lesions of the hind- and mid-brain on micturition in the cat. Q J Exp Physiol 15(1):81–102. https://doi.org/10.1113/expphysiol.1925.sp000345
6. Bender R, Shipley MT (1994) Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? Trends Neurosci 17(9):379–389
7. Parviz I, Van Hoesen GW, Damasio A (2000) Selective pathologi-cal changes of the periaqueductal gray matter in Alzheimer’s disease. Ann Neurol 48(3):344–353. https://doi.org/10.1002/1531-8249(20000948)48:3<344::Aid-Ana9>3.0.Co;2-S
8. Benarroch EE (2012) Periaqueductal gray: an interface for behav-ioral control. Neurology 78(3):210–217. https://doi.org/10.1212/WNL.0b013e31823edee
44. Matsumoto Kengo ST, Gen Y (2006) Characterization of micturition-related single neuron activities and their distribution in periaqueductal gray matter (PAG) in anesthetized rats. J Pharmacol Sci 100:251
45. Liu Z, Sakakibara R, Nakazawa K, Uchiyama T, Yamamoto T, Ito T, Hattori T (2004) Micturition-related neuronal firing in the periaqueductal gray area in cats. Neuroscience 126(4):1075–1082. https://doi.org/10.1016/j.neuroscience.2004.04.033
46. Ko IG, Kim SE, Kim CJ, Jung JH, Lee SJ, Kim DH, Lee KY, Kim KH (2010) Effect of treadmill exercise on leak-point pressure and neuronal activation in brain of rats with stress urinary incontinence. Int Neurolour J 14(3):141–148. https://doi.org/10.5213/inj.2010.14.3.141
47. Ko IG, Kim SE, Kim BK, Shin MS, Kim CI, Yim SJ, Bang YJ, Choi IH et al (2011) Swimming: effects on stress urinary incontinence and the expression of nerve growth factor in rats following transabdominal urethrostomy. Int Neurolour J 15(2):74–81. https://doi.org/10.5213/inj.2011.15.2.74
48. Zhi LYL, Libing M, Dan W, Limin W, Jun M, Tomoyuki U, Ryui S The different relationship with nerve center from normal micturition—comparing between normal and cystitis model rat by using c-fos method Paper presented at the International Continence Society, Beijing, 2012
49. Numata A, Iwata T, Iuchi H, Taniguchi N, Kita M, Wada N, Kato Y, Kakizaki H (2008) Micturition-suppressing region in the periaqueductal gray of the mesencephalon of the cat. Am J Physiol Reg I 294(6):R1996–R2000. https://doi.org/10.1152/ajpregu.00393.2006
50. Vargas LC, Marques TD, Schenberg LC (2000) Micturition and defensive behaviors are controlled by distinct neural networks within the dorsal periaqueductal gray and deep gray layer of the superior colliculus of the rat. Neurosci Lett 280(1):45–48. https://doi.org/10.1016/S0304-3904(99)00985-4
51. Koyama Y, Iwata T, Iuchi H, Taniguchi N, Kita M, Wada N, Kato Y, Kakizaki H (2008) Micturition-suppressing region in the periaqueductal gray of the mesencephalon of the cat. Am J Physiol Reg I 294(6):R1996–R2000. https://doi.org/10.1152/ajpregu.00393.2006
52. Rocha I, Burnstock G, Spyer KM (2001) Effect on urinary bladder receptors in the rat periaqueductal gray participates in the micturition to morbid micturition The different relationship with nerve center from normal micturition. Ann Neurol 72(1):144–150. https://doi.org/10.1002/ana.23571
53. Green AL, Stone E, Sitsapesan H, Turney BW, Coote JH, Aziz TZ, Hyam JA, Lovick TA (2012) Switching off micturition using electrodes. Brain Res 1902(1):61–69. https://doi.org/10.1016/j.neuroscience.2015.11.027
54. Liu Z, Sakakibara R, Nakazawa K, Uchiyama T, Yamamoto T, Ito T, Hattori T (2004) Micturition-related neuronal firing in the periaqueductal gray area in cats. Neuroscience 126(4):1075–1082. https://doi.org/10.1016/j.neuroscience.2004.04.033
55. Clements JR, Beitz AJ, Fletcher TF, Mullett MA (1985) Immunocytochemical localization of serotonin in the rat periaqueductal gray: a quantitative light and electron microscopic study. J Comp Neurol 236(1):60–70. https://doi.org/10.1002.cne.902360106
56. Pazos A, Palacios JM (1985) Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. Brain Res 346(2):205–230
57. Gutstein HB, Mansour A, Watson SJ, Akil H, Fields HL (1998) Contribution of mu opioid receptors in the periaqueductal gray matter mediate sensory inputs after bladder stimulation in freely moving rats. Int J Urol. https://doi.org/10.1111/j.1440-179X.1998.tb03213.x
58. Stone E, Coote JH, Allard J, Lovick TA (2011) GABAergic control of micturition within the periaqueductal grey matter of the male rat. J Comp Neurol 505(5):478–492. https://doi.org/10.1002/jne.21509
59. Neumann A, Iwata T, Iuchi H, Taniguchi N, Kita M, Wada N, Kato Y, Kakizaki H (2008) Micturition-suppressing region in the periaqueductal gray of the mesencephalon of the cat. Am J Physiol Reg I 294(6):R1996–R2000. https://doi.org/10.1152/ajpregu.00393.2006
60. Gumbert AL (1991) Regional subdivisions in the midbrain periaqueductal gray of the cat revealed by in vivo receptor autoradiography. In: Depaulis A, Bandler R (eds) The midbrain periaqueductal gray matter: functional, anatomical, and neurochemical organization. Springer US, Boston, MA, pp. 449–464. https://doi.org/10.1007/978-1-4615-3302-3_23
61. Verstegen H, Mathai J, Zeidel M, Geertling J (2017) Brain networks controlling bladder filling and voiding. J Urolgy 197(4):E1150–E1150
62. Zare A, Jahanshahi A, Meriaux C, Steinbusch HW, van Kooeveringe GA (2018) Glutamatergic cells in the periaqueductal gray matter mediate sensory inputs after bladder stimulation in freely moving rats. Int J Urol. https://doi.org/10.1111/jiu.13562
63. Williams FG, Beitz AJ (1990) Ultrastructural morphometric analysis of GABA-immunoreactive terminals in the ventrocaudal periaqueductal grey: analysis of the relationship of GABA terminals and the GABAA receptor to periaqueductal grey-raphe magnus projection neurons. J Neurocytol 19(5):686–696
64. Rechling DB, Basbaum AI (1990) Contribution of brainstem GABAergic circuitry to descending anti-nociceptive controls: I. GABA-immunoreactive projection neurons in the periaqueductal gray and nucleus raphe magnus. J Comp Neurol 302(2):370–377. https://doi.org/10.1002/jne.903020213
65. McCabe RT, Wamsley JK (1986) Autoradiographic localization of subcomponents of the macromolecular GABA receptor complex. Life Sci 39(21):1937–1945
66. Barbareis P (2007) Cellular and subcellular localization of the GABA(B) receptor 1α/b subunit in the rat periaqueductal gray matter. J Comp Neurol 505(5):478–492. https://doi.org/10.1002/cne.21509
67. Stone E, Coote JH, Allard J, Lovick TA (2011) GABAergic control of micturition within the periaqueductal grey matter of the male rat. J Physiol-London 589(8):2065–2078. https://doi.org/10.1113/jphysiol.2010.202614
68. Kwock CHF, Devoshime IM, Bennett AJ, Hathway GJ (2014) Postnatal maturation of endogenous opioid systems within the periaqueductal grey and spinal dorsal horn of the rat. Pain 155(1):168–178. https://doi.org/10.1093/pain/pain.tsw022
69. Gutstein HB, Mansour A, Watson SJ, Akil H, Fields HL (1998) Mu and kappa opioid receptors in periaqueductal gray and rostral ventromedial medulla. Neuroreport 9(8):1777–1781. https://doi.org/10.1097/00001092-199807070-00019
70. Matsumoto S, Levendusky MC, Longhurst PA, Levin RM, Millington WR (2004) Activation of mu opioid receptors in the ventrolateral periaqueductal gray inhibits reflex micturition in anesthetized rats. Neurosci Lett 377(1):116–119. https://doi.org/10.1016/j.neulet.2004.03.055
71. Dray A, Metsch R (1984) Opioid receptor subtypes involved in the central inhibition of urinary bladder motility. Eur J Pharmacol 104(1–2):47–53
75. Tovote P, Esposito MS, Botta P, Chaudun F, Fadok JP, Markovic M, Wolff SB, Ramakrishnan C et al (2016) Midbrain circuits for defensive behaviour. Nature 534(7606):206–212. https://doi.org/10.1038/nature19796

76. Samineni VK, Grajales-Reyes JG, Copits BA, O’Brien DE, Trigg SL, Gomez AM, Bruchas MR, Gereau RW (2017) Divergent modulation of nociception by glutamatergic and GABAergic neuronal subpopulations in the periaqueductal gray. eNeuro 4(2). https://doi.org/10.1523/ENEURO.0129-16.2017

77. Clements JR, Madl JE, Johnson RL, Larson AA, Beitz AJ (1987) Localization of glutamate, glutaminase, aspartate and aspartate aminotransferase in the rat midbrain periaqueductal gray. Exp Brain Res 67(3):594–602

78. Benarroch EE, Schmeichel AM, Low PA, Parisi JE (2010) Differential involvement of the periaqueductal gray in multiple system atrophy. Auton Neurosci : Basic Clin 158(1–2):111–117. https://doi.org/10.1016/j.autneu.2010.07.009

79. Stone E (2012) Midbrain control of micturition in the rat chapter 5 phd thesis. University of Birmingham

80. Lu J, Jhou TC, Saper CB (2006) Identification of wake-active dopaminergic neurons in the ventral periaqueductal gray matter. J Neurosci 26(1):193–202. https://doi.org/10.1523/JNEUROSCI.2244-05.2006

81. Li S, Shi Y, Kirouac GJ (2014) The hypothalamus and periaqueductal gray are the sources of dopamine fibers in the paraventricular nucleus of the thalamus in the rat. Front Neuronaat 8:136. https://doi.org/10.3389/fnana.2014.00136

82. Benarroch EE, Schmeichel AM, Daguer BN, Sandroni P, Parisi JE, Low PA (2009) Dopamine cell loss in the periaqueductal gray in multiple system atrophy and Lewy body dementia. Neurology 72(2):106–112. https://doi.org/10.1212/WNL.0b013e3181ad3e7

83. Normandin JI, Murphy AZ (2011) Serotoninergic lesions of the periaqueductal gray, a primary source of serotonin to the nucleus paragigantocellularis, facilitate sexual behavior in male rats. Pharmacol Biochem Be 98(3):369–375. https://doi.org/10.1016/j.pbb.2011.01.024

84. Montez S, Pinto E, de Novellis V, Stella L, Leyva J, Rossi F (1998) Metabotropic glutamate receptor modulates serotonin release in the rat periaqueductal gray matter. N-S Arch Pharmacol 358(4):411–417. https://doi.org/10.1007/Pt00055272

85. Beitz AJ, Shepard RD, Wells WE (1983) The periaqueductal gray-raphe magnus projection contains somatostatin, neurotensin and serotonin but not cholecystokinin. Brain Res 261(1):132–136. https://doi.org/10.1016/0006-8993(83)91292-1

86. Zhou L, Zhu DY (2009) Neuronal nitric oxide synthase: structure, localization of NOS and function in the brain. Brain Res 1248:369–377. https://doi.org/10.1016/j.neuropharm.2007.12.008

87. Lovick TA, Key BJ (1996) Inhibitory effect of nitric oxide on neuronal activity in the periaqueductal grey matter of the rat. Exp Brain Res 108(3):382–388

88. Ishide T, Amer A, Hepburn S, Eston M, Wolff SB, Ramakrishnan C et al (2016) Midbrain circuits for bladder pain and voiding function detected by an fMRI study. J Neurophysiol 102(5):2719–2730. https://doi.org/10.1152/jn.00700.2009

89. Chumi Y, Ko IG, Hwan L, Shin MS, Kim CJ, Kim SH, Jin JJ et al (2014) Caffeine enhances micturition through neuronal activation in micturition centers. Mol Med Rep 10(6):2931–2936. https://doi.org/10.3892/mmr.2014.2646

90. Schmidbrink F, Scliar A, Mather H, Yakupov R, Speck O (2017) Data from: T1-weighted in vivo human whole brain MRI dataset with an ultrahigh isotropic resolution of 250 μm. Dried Data Repository. https://doi.org/10.5061/dryad.38s74

91. DeBerry JJ, Samineni VK, Sullivan CJ, Vogt SK, Albers KM, Davis BM, Gereau Iv Jv RW (2018) Differential regulation of bladder pain and voiding function by sensory afferent populations revealed by selective optogenetic activation. Front Integr Neurosci 12:5. https://doi.org/10.3389/finnt.2018.00005

92. Matsuura S, Kakizaki H, Mitsui T, Shiga T, Tamaki N, Koyanagi T (2002) Human brain region response to distention or cold stimulation of the bladder: a positron emission tomography study. J Urology 168(5):2035–2039. https://doi.org/10.1016/S0022-5347(02)02022-6

93. Mehnert U, Michel L, Zempleni MZ, Schuch B, Kollias S (2011) The supraspinal neural correlate of bladder cold sensation—an fMRI study. Hum Brain Mapp 32(6):835–845. https://doi.org/10.1002/hbm.21070

94. Cho YS, Ko IG, Kim SE, Hwan L, Shin MS, Kim CJ, Kim SH, Jin JJ et al (2014) Caffeine enhances micturition through neuronal activation in micturition centers. Mol Med Rep 10(6):2931–2936. https://doi.org/10.3892/mmr.2014.2646

95. Xing J, Li DP, Li J (2008) Role of GABA receptors in nitric oxide inhibition of dorsolateral periaqueductal gray neurons. Neuropharmacology 54(4):734–744. https://doi.org/10.1016/j. neuropharm.2007.12.008

96. Suzuki H, Watanabe S, Hamaguchi T, Mine H, Terui T, Kanazawa A (2017) Cortical substrate of bladder control in SCI and the effect on the afferent pathways of micturition between tamsulosin and sildenafil. J Biomed Sci 20. DOI: https://doi.org/10.1186/s12284-012-05.2006
107. Tadic SD, Tannenbaum C, Resnick NM, Griffiths D (2013) Brain responses to bladder filling in older women without urgency incontinence. Neurourology 32(5):435–440. https://doi.org/10.1002/nau.22320

108. Herzog J, Weiss PH, Assmus A, Wefer B, Seif C, Braun PM, Pinsker MO, Herzog H et al (2008) Improved sensory gating of urinary bladder afferents in Parkinson’s disease following subthalamic stimulation. Brain 131(Pt 1):132–145. https://doi.org/10.1093/brain/avm254

109. Michels L, Blok BF, Gregorini F, Kurz M, Schurch B, Kessler T, Dechent P (2006) Voluntary pelvic floor muscle control—an fMRI study. NeuroImage 31(4):1399–1407. https://doi.org/10.1016/j.neuroimage.2006.02.012

110. Kong J, Tu PC, Zyloney C, Su TP (2010) Intrinsic functional brainstem changes in response to bladder function: results from an fMRI study. Cereb Cortex 20(1):216–226. https://doi.org/10.1093/cercor/bhu140

111. Nour S, Svarer C, Kristensen JKI, Paulson OB, Law I (2000) Changes in excitability of midbrain micturition center in brain activity following sacral neuromodulation for urinary dysfunction: analysis of lower urinary tract function. Mol Med Rep 19(3):532–536. https://doi.org/10.1023/A:1012869811584

112. Fukuyama H, Matsuzaki S, Ouchi Y, Yamauchi H, Nagahama Y, Wong K, Boone TB, Wong STC, Munoz A (2015) Functional brainstem changes in response to bladder function alteration elicited by surgical reduction in bladder capacity: a functional magnetic resonance imaging study. J Urology 195(3):532–544. https://doi.org/10.1016/j.juro.2015.03.042

113. Papadopoulou A, Naegelin Y, Weiher K, Amann M, Hirsch J, von Felten S, Yaldizli O, Sprenger T et al (2014) MRI characteristics of periaqueductal lesions in multiple sclerosis. Mult Scler Relat Disord 3(4):542–551. https://doi.org/10.1016/j.msard.2014.01.001

114. Crook JJ, Lovick TA Changes in excitability of midbrain micturition control circuitry during alterations in sleep-like brain states in rats &#x2013; implications for nocturnal enuresis. Auton Neurobiol (2019) 56:920–934

115. Kitta T, Chancellor MB, de Groat WC, Shinohara N, Yoshimura N (2016) Role of the anterior cingulate cortex in the control of micturition in brain activity following sacral neuromodulation for urinary dysfunction. J NeuroImaging 6(3):218–229. https://doi.org/10.1006/nimr.1997.0291

116. Tadic SD, Griffiths D, Murrin A, Schaefer W, Aizenstein HJ, Resnick NM (2010) Brain activity during bladder filling is related to white matter structural changes in older women with urinary incontinence. NeuroImage 51(4):1294–1302. https://doi.org/10.1016/j.neuroimage.2010.03.016

117. Crook JJ, Lovick TA Changes in excitability of midbrain micturition control circuitry during alterations in sleep-like brain states in rats &#x2013; implications for nocturnal enuresis. Auton
Neurosci: Basic Clin 192:31–32. https://doi.org/10.1016/j.autneu.2015.07.348

139. Silberstein SD (1995) Migraine symptoms: results of a survey of self-reported migraineurs. Headache 35(7):387–396

140. Weiller C, May A, Limmroth V, Juptner M, Kautbe H, Schayck RV, Coenen HH, Diener HC (1995) Brain stem activation in spontaneous human migraine attacks. Nat Med 1(7):658–660

141. Sakakibara R, Hattori T, Yasuda K, Yamanishi T, Tojo M, Mori M (1997) Micturitional disturbance in Wernicke’s encephalopathy. Neurourology and urodynamics 16 (2):111-115. Doi:Doi https://doi.org/10.1002/(SICI)1520-6777(1997)16:2<111::Aid-Nau4>3.0.Co;2-H

142. Griffiths D, Tadic SD, Schaefer W, Resnick NM (2007) Cerebral control of the bladder in normal and urge-incontinent women. NeuroImage 37(1):1–7. https://doi.org/10.1016/j.neuroimage.2007.04.061

143. Tadic SD, Griffiths D, Schaefer W, Cheng CI, Resnick NM (2010) Brain activity measured by functional magnetic resonance imaging is related to patient reported urgency urinary incontinence severity. J Urology 183(1):221–228. https://doi.org/10.1016/j.juro.2009.08.155

144. Blok BF, Groen J, Bosch JL, Veltman DJ, Lammersma AA (2006) Different brain effects during chronic and acute sacral neuromodulation in urge incontinent patients with implanted neurostimulators. BJU Int 98(6):1238–1243. https://doi.org/10.1111/j.1464-410X.2006.06521.x