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Organization of the neural switching circuitry underlying reflex micturition

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Abstract

The functions of the lower urinary tract to store and periodically eliminate urine are regulated by a complex neural control system in the brain and spinal cord that coordinates the activity of the bladder and urethral outlet. Experimental studies in animals indicate that urine storage is modulated by reflex mechanisms in the spinal cord, whereas voiding is mediated by a spinobulbospinal pathway passing through a coordination centre in the rostral brain stem. Many of the neural circuits controlling micturition exhibit switch-like patterns of activity that turn on and off in an all-or-none manner. This study summarizes the anatomy and physiology of the spinal and supraspinal micturition switching circuitry and describes a computer model of these circuits that mimics the switching functions of the bladder and urethra at the onset of micturition.

Keywords

brain stem; spinal cord; switching circuit

The functions of the lower urinary tract to store and periodically release urine are dependent upon neural circuitry in the brain and spinal cord (Barrington 1925, Kuru 1965, de Groat *et al.* 1993, Fowler *et al.* 2008, Birder *et al.* 2009). This dependence on central nervous control distinguishes the lower urinary tract from many other visceral organs that maintain a certain level of activity even after elimination of extrinsic neural input. The lower urinary tract is also unusual in regard to its pattern of activity and the complexity of its neural regulation. For example, the urinary bladder has two principal modes of operation: storage and elimination. Thus, many of the neural circuits controlling the bladder exhibit switch-like patterns of activity (de Groat 1975) in contrast to tonic patterns occurring in autonomic pathways to the cardiovascular organs. Micturition also depends on the integration of autonomic and somatic efferent mechanisms within the lumbosacral spinal cord which is necessary to coordinate the activity of visceral organs (the bladder and urethra) with that of urethral striated muscles. In addition, micturition is under voluntary control and depends upon learned behaviour that develops during maturation of the nervous system; whereas many other visceral functions are regulated involuntarily. This and the companion paper (Griffiths & Fowler 2012) will review the central neural mechanisms controlling the lower urinary tract with a major focus on the brain stem and forebrain circuits that mediate reflex and voluntary control of micturition

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Conflict of interest
The authors have no conflict of interest and nothing to disclose.

Innervation of the lower urinary tract

Storage and elimination of urine are dependent upon the coordinated activity of two functional units in the lower urinary tract: (1) a reservoir (the urinary bladder) and (2) an outlet, consisting of bladder neck, urethra and striated muscles of the external urethral sphincter (rhabdosphincter) (Fry *et al.* 2009). These structures are, in turn, controlled by three sets of peripheral nerves: sacral parasympathetic (pelvic nerves), thoracolumbar sympathetic (hypogastric nerves and sympathetic chain) and sacral somatic nerves (pudendal nerves) (Fig. 1).

Sacral parasympathetic pathways

The sacral parasympathetic outflow provides the major excitatory input to the urinary bladder. Cholinergic preganglionic neurons located in the intermediolateral region of the sacral spinal cord (de Groat & Ryall 1968a, Nadelhaft *et al.* 1980) send axons via the pelvic nerves to ganglion cells in the pelvic plexus and in the wall of the bladder. The ganglion cells in turn excite bladder smooth muscle via the release of cholinergic (acetylcholine) and non-adrenergic, noncholinergic transmitters and also inhibit urethral smooth muscle via the release of nitric oxide (Fry *et al.* 2009).

Thoracolumbar sympathetic and sacral somatic efferent pathways

Sympathetic pathways to the lower urinary tract that originate in the lumbosacral sympathetic chain ganglia as well as in the prevertebral inferior mesenteric ganglia pass to the bladder via the hypogastric (Morgan *et al.* 1986) and pelvic nerves (Kuo *et al.* 1984). Sympathetic efferent pathways elicit various effects including: (1) inhibition of detrusor muscle, (2) excitation of the bladder base and urethra (Fry *et al.* 2009) and (3) inhibition and facilitation in bladder parasympathetic ganglia (de Groat & Saum 1972, Saum & de Groat 1972, de Groat & Theobald 1976, Keast *et al.* 1990).

The efferent innervation of the urethral striated muscles originates from cells in a circumscribed region of the lateral ventral horn that is termed ‘Onuf’s nucleus’. Sphincter motoneurons send their axons through the pudendal nerves and excite sphincter muscles via the release of acetylcholine (Thor & de Groat 2010).

Afferent pathways

Afferent axons innervating the urinary tract are present in the three sets of nerves (de Groat & Yoshimura 2009). The most important afferents for initiating micturition are those passing in the pelvic nerve to the sacral spinal cord. These afferents are small myelinated (Aδ) and unmyelinated (C) fibres, which convey information from receptors in the bladder wall (Habler *et al.* 1993, Sengupta & Gebhart 1994, Shea *et al.* 2000, Gillespie *et al.* 2009, Kanai *et al.* 2011) to second-order neurons in the spinal cord (Fig. 1). Aδ bladder afferents in the cat respond in a graded manner to passive distension as well as active contraction of the bladder and exhibit pressure thresholds in the range of 5–15 mmHg, which are similar to those pressures at which humans report the first sensation of bladder filling. These fibres also code for noxious stimuli in the bladder. On the other hand, C-fibre bladder afferents in the cat are insensitive to mechanical stimuli and commonly do not respond to even high levels of intravesical pressure (Habler *et al.* 1990). However, activity in these afferents is unmasked by chemical irritation of the bladder mucosa. These findings indicate that C-fibre afferents in the cat have specialized functions, such as the signalling of inflammatory or noxious events in the lower urinary tract. In the rat, A-fibre and C-fibre bladder afferents cannot be distinguished on the basis of stimulus modality; thus both types of afferents consist of mechanosensitive and chemosensitive populations (Birder *et al.* 2009).

Anatomy of the spinal pathways controlling the lower urinary tract

The reflex circuitry controlling micturition consists of four basic components: primary afferent neurons, spinal efferent neurons, spinal interneurons and neurons in the brain that activate or modulate spinal reflex pathways (Fig. 1).

Afferent projections in the spinal cord

Afferent pathways from the bladder project into Lissauer's tract at the apex of the dorsal horn and then send collaterals laterally and medially around the dorsal horn into laminae V–VII and X at the base of the dorsal horn (Morgan *et al.* 1981, Steers *et al.* 1991). The lateral pathway terminates in the region of the sacral parasympathetic nucleus.

Efferent neurons in the spinal cord

Parasympathetic preganglionic neurons (PGN) are located in the intermediolateral gray matter (laminae V–VII) in the sacral segments of the spinal cord (Nadelhaft *et al.* 1980), whereas sympathetic preganglionic neurons are located in medial (lamina X) and lateral sites (laminae V–VII) in the rostral lumbar spinal cord. External urethral sphincter (EUS) motoneurons are located in lamina IX in Onuf's nucleus in the cat and in the dorsolateral motor nucleus in the rat (Thor & de Groat 2010).

Parasympathetic PGN exhibit dendrites projecting to four major areas: (1) the lateral and dorsolateral funiculus, (2) lamina I on the lateral edge of the dorsal horn, (3) the dorsal gray commissure and (4) the gray matter and lateral funiculus ventral to the autonomic nucleus (Morgan *et al.* 1993). It has been speculated that these dendritic projections reflect the major synaptic inputs to the PGN, that is, lamina I dendrites receiving primary afferent projections, lateral funiculus dendrites receiving bulbospinal projections and medial dendrites receiving interneuronal projections from the dorsal commissure and inputs from the contralateral side of the spinal cord. EUS motoneurons have a similar dendritic pattern (Thor & de Groat 2010).

The most striking feature of the sacral PGNs in the cat is an extensive axon collateral system that projects bilaterally to various regions of the dorsal and ventral horns including the area around the central canal, the intermediolateral gray matter, the dorsal commissure and the lateral dorsal horn (Morgan *et al.* 1991). These axon collaterals are likely to be involved in a bilateral recurrent inhibitory pathway that regulates the parasympathetic outflow to the bladder (de Groat & Ryall 1968b, de Groat 1976) (Fig. 1).

Spinal interneurons

Spinal interneurons involved in lower urinary tract function have been identified by retrograde transneuronal labelling after injection of pseudorabies virus (PRV) into the urinary bladder, urethra or external urethral sphincter of the rat. PRV which is transported from peripheral efferent terminals to efferent neurons in the spinal cord crosses multiple synapses to infect interneuronal circuitry throughout the central nervous system. PRV labelled spinal neurons are located in the same general regions of the spinal cord that receive afferent input from the bladder including the dorsal commissure, laminae I and V and lamina VII just dorsal and medial to the PGN (Nadelhaft *et al.* 1992, Nadelhaft & Vera 1995, 1996, 2001, Vizzard *et al.* 1995, Marson 1997, Sugaya *et al.* 1997). Spinal interneurons in these locations receiving afferent input from the lower urinary tract have also been identified by firing in response to stimulation of bladder efferents (de Groat *et al.* 1981, McMahon & Morrison 1982a,b) or by the expression of the immediate early gene, c-*fos* after chemical or mechanical stimulation of the bladder and urethra (Birder & de Groat 1993, Birder *et al.* 1999). Some of these interneurons make excitatory and inhibitory synaptic connections with

PGN (Araki & de Groat 1996, 1997, de Groat *et al.* 1998, Miura *et al.* 2003) and participate in segmental spinal reflexes (de Groat *et al.* 1998), whereas others send long projections to supraspinal centres, such as the periaqueductal gray (PAG) (Fig. 1), pontine micturition centre (PMC, Barrington's nucleus), the hypothalamus and thalamus that are involved in the supraspinal control of micturition (McMahon & Morrison 1982a, Blok *et al.* 1995, Ding *et al.* 1997, Birder *et al.* 1999, Duong *et al.* 1999, Blok & Holstege 2000, Holstege & Mouton 2003).

Axonal projections from the brain to the spinal cord

Transneuronal PRV tracing methods have also identified many populations of neurons in the rat brain that are involved in the control of bladder (Nadelhaft *et al.* 1992, Nadelhaft & Vera 1995, 2001, Sugaya *et al.* 1997), urethra (Vizzard *et al.* 1995) and the external urethral sphincter (Nadelhaft & Vera 1996, 2001, Marson 1997), including the PMC, PAG, medullary raphe nuclei, which contain serotonergic neurons; the locus coeruleus, which contains noradrenergic neurons, and the A5 noradrenergic cell group. More rostral regions in the hypothalamus (lateral medial preoptic and paraventricular nucleus), dorsal thalamus, the primary and secondary motor cortices and entorhinal and piriform cortices also exhibit virus-infected cells (see companion paper by Griffiths & Fowler 2012 for further description of the forebrain circuitry). In the cat PRV tracing from the urinary bladder or the EUS identified efferent neurons and interneurons in the spinal cord as well as a cluster of neurons extending from the PMC ventrolaterally into the pontine reticular formation (de Groat *et al.* 1998).

Other anatomical studies in which anterograde tracers were injected into areas of the cat brain revealed labelled axon terminals in regions of the brain and spinal cord consistent with the virus tracing data. Tracer injected into the paraventricular nucleus of the hypothalamus labelled terminals in the sacral parasympathetic nucleus as well as the sphincter motor nucleus (Holstege & Mouton 2003, Beckel & Holstege 2011). Injections of tracers into the anterior hypothalamus or PAG (Blok & Holstege 1994) labelled terminals in the PMC, whereas tracers in the PMC labelled axonal projections to the sacral parasympathetic nucleus, the lateral edge of the dorsal horn and the dorsal commissure (Blok & Holstege 1997, Blok *et al.* 1997), areas containing dendrites of preganglionic neurons, sphincter motoneurons and afferent inputs from the bladder. Conversely, projections from neurons in the ventrolateral pons in the cat, an area identified as the pontine urine storage centre (Kuru 1965), terminate rather selectively in the sphincter motor nucleus (Holstege *et al.* 1986). Thus, the sites of termination of descending projections from the pons are optimally located to regulate reflex mechanisms at the spinal level.

Activity of the lower urinary tract during storage and voiding

The neural pathways controlling lower urinary tract function are organized as simple on–off switching circuits that maintain a reciprocal relationship between the urinary bladder and urethral outlet (de Groat 1997, 1975, Fowler *et al.* 2008, Birder *et al.* 2010, Drake *et al.* 2010, Thor & de Groat 2010, Sadananda *et al.* 2011). Intravesical pressure measurements during bladder filling in both humans and animals reveal low and relatively constant bladder pressures when bladder volume is below the threshold for inducing voiding. The accommodation of the bladder to increasing volumes of urine is primarily a passive phenomenon dependent upon the intrinsic properties of the vesical smooth muscle and quiescence of the parasympathetic efferent pathway (Fowler *et al.* 2008). In addition, in some species, urine storage is facilitated by sympathetic reflexes that mediate an inhibition of bladder activity, closure of the bladder neck and contraction of the proximal urethra (de Groat & Lalley 1972, de Groat & Theobald 1976). During bladder filling, the activity of the sphincter electromyogram (EMG) also increases, reflecting an increase in efferent firing in

the pudendal nerve and an increase in outlet resistance that contributes to the maintenance of urinary continence (Thor & de Groat 2010).

The storage phase of the urinary bladder can be switched to the voiding phase either involuntarily or voluntarily. The former is readily demonstrated in the human infant when the volume of urine exceeds the micturition threshold. At this point, increased afferent firing from tension receptors in the bladder produces firing in the sacral parasympathetic pathways and inhibition of sympathetic and somatic pathways. The expulsion phase consists of an initial relaxation of the urethral sphincter followed by a contraction of the bladder, an increase in bladder pressure and flow of urine. Relaxation of the urethral outlet is mediated by activation of a parasympathetic reflex pathway to the urethra that triggers the release of an inhibitory transmitter, nitric oxide, as well as by removal of adrenergic and somatic excitatory inputs to the urethra (Birder *et al.* 2009, Thor & de Groat 2010).

Urine storage mechanisms

Sympathetic storage reflex

Although the sympathetic input to the lower urinary tract is not essential for the performance of micturition, it does contribute to the storage function of the bladder. Surgical interruption or pharmacological blockade of the sympathetic innervation can reduce urethral outflow resistance, reduce bladder capacity and increase the frequency and amplitude of bladder contractions recorded under constant volume conditions (de Groat *et al.* 1993).

Sympathetic reflex activity is elicited by a sacrolumbar intersegmental spinal reflex pathway that is triggered by vesical afferent activity in the pelvic nerves (de Groat & Lalley 1972) (Fig. 1). The reflex pathway is inhibited when bladder pressure is raised to the threshold for producing micturition. This inhibitory response is abolished by transection of the spinal cord at the lower thoracic level, indicating that it originates at a supraspinal site, possibly the PMC (Fig. 1). Thus, the vesico-sympathetic reflex represents a negative feedback mechanism that allows the bladder to accommodate larger volumes during bladder filling but is turned off during voiding to allow the bladder to empty completely.

Urethral sphincter storage reflexes

Motoneurons innervating the striated muscles of the external urethral sphincter (EUS) exhibit a tonic discharge that increases during bladder filling (Thor & de Groat 2010). This activity is mediated in part by a spinal reflex pathway activated by low-level afferent input from the bladder (Fig. 1). Studies in cats have also suggested that neurons in the ventrolateral region of the pontine reticular formation provide a tonic excitatory input to the EUS motoneurons (Holstege *et al.* 1986, Holstege & Mouton 2003). Electrical stimulation in this region (termed the pontine urine storage center, PUSC) (Kuru 1965, Kuru & Iwanaga 1966) excites the EUS motoneurons and induces contractions of the EUS (Kuru 1965, Koyama *et al.* 1966, Holstege *et al.* 1986). Contraction of the EUS induces firing in afferent axons in the pudendal nerve which in turn activate inhibitory interneurons in the spinal cord that suppress reflex bladder activity (McGuire *et al.* 1983, de Groat *et al.* 2001) by inhibiting PGN and interneurons on the micturition reflex pathway (Fig. 1) (de Groat 1978, de Groat *et al.* 1982). Thus, the bladder-to-EUS-to-bladder reflex pathway represents a second negative feedback mechanism in the spinal cord that promotes urinary continence. During micturition, the firing of sphincter motoneurons and the negative feedback is inhibited. This inhibition which is mimicked by electrical stimulation of the PMC and activation of bulbospinal pathways (Fig. 1) (Kruse *et al.* 1990, 1991) is less prominent in chronic spinal animals (Thor & de Groat 2010); and is therefore dependent in part on supraspinal mechanisms.

Brain stem storage mechanisms

Electrical stimulation of the rostral pontine reticular formation (RPRF) ventral to the PMC in an area also known as the nucleus reticularis pontis oralis inhibits reflex bladder contractions in cats and rats (Sugaya *et al.* 1987, 2005, Kimura *et al.* 1995, Nishijima *et al.* 2005). Neurons in this region project to the spinal cord and also to nucleus reticularis gigantocellularis located in the rostrodorsal medulla. The RPRF projects to lumbosacral glycinergic inhibitory neurons that may mediate the inhibitory effects of RPRF stimulation (Sugaya *et al.* 2005).

Electrical stimulation of the PUSC ventrolateral to the PMC not only excites the EUS but also inhibits reflex bladder activity, increases bladder capacity and the inhibits the bladder excitatory effect of PMC stimulation (Sugaya *et al.* 2005). Neurons in the region of the PUSC project to the nucleus raphe magnus (NRM) in the medulla which contains neurons that project to the lumbosacral spinal cord. Electrical or chemical (Chen *et al.* 1993) stimulation in the NRM induces serotoninergic inhibition of reflex bladder activity. Thus, neurons in the PUSC may activate descending inhibitory pathways to the sacral parasympathetic nucleus (Sugaya *et al.* 2005).

Voiding mechanisms

Spinobulbospinal micturition reflex pathway: role of PMC

Voiding, which can be initiated voluntarily or reflexly, is mediated by the activation of the sacral parasympathetic efferent pathway to the bladder and urethra as well as reciprocal inhibition of the somatic pathway to the urethral sphincter. In contrast to storage mechanisms that are dependent on spinal reflex pathways, voiding is dependent on neural circuitry in the brain and spinal cord (Barrington 1925, Langworthy *et al.* 1940, Ruch & Tang 1956, Kuru 1965, de Groat & Ryall 1969, de Groat 1975).

Studies in cats using brain-lesioning and electrophysiological techniques revealed that reflex micturition is mediated by a spinobulbospinal pathway consisting of an ascending sensory limb that passes from the sacral spinal cord to circuitry in the rostral brain stem leading to activation of neurons in the PMC that send excitatory signals back to the sacral spinal cord to complete the reflex circuit (Fig. 1). In animals, reflex micturition is preserved after removal of the forebrain by supracollicular decerebration but is abolished after bilateral destruction of the PMC or transection of the neuraxis at any level caudal to the PMC (Kuru 1965).

Recordings of electrical activity in bladder efferent nerves support the concept that the micturition reflex is mediated by a pathway passing through a switching centre in the rostral pons. Stimulation of bladder afferent nerves evokes long latency discharges (120–150 ms) on bladder postganglionic nerves (Fig. 2) that persist after supracollicular decerebration but not after transection of the spinal cord at the thoracic level (de Groat & Ryall 1969, de Groat 1975). The evoked reflexes are unmasked by partial filling of the bladder to elicit a basal level of afferent firing. They also exhibit an unusual temporal facilitation in which the first stimulus during a train (0.5–1 Hz frequency) does not evoke a response and the next few stimuli evoke gradually increasing responses (wind-up), eventually producing a self-sustaining micturition reflex (Fig. 2). These observations indicate that even under optimal conditions with tonic afferent input from bladder mechanoreceptors, electrical stimulation of bladder afferents only activates the micturition switching circuit after a delay of several seconds.

Bladder afferent nerve stimulation evokes neuronal firing in the PMC at latencies ranging from 30 to 40 ms, and electrical stimulation in the PMC evokes bladder contractions and

postganglionic nerve firing at latencies of 60–75 ms (de Groat 1975, Noto *et al.* 1991b). The sum of the latencies of the putative ascending (afferent-pontine) and descending limbs (pontine-efferent) of the reflex approximates the latency of the entire reflex pathway (120 ms). The reflex firing elicited in cats and rats is not altered following supracollicular decerebration but is eliminated by acute transection of neuraxis at any level caudal to the PMC (de Groat & Ryall 1969, de Groat 1975, de Groat *et al.* 1981).

Role of the PAG

Early studies in cats (Langworthy & Kolb 1935, Kabat *et al.* 1936, Skultety 1959, Koyama *et al.* 1962, Gjone 1966) revealed that stimulation at sites in the PAG could either excite or inhibit bladder activity. The effects of stimulation were dependent on the state of the bladder. For example, when stimulation was applied with the bladder partially full and relatively inactive, excitatory effects were commonly elicited; however, when the bladder was full and exhibiting large amplitude, reflex contractions stimulation at the same site produced inhibition. Reflex bladder activity was also enhanced by elimination of parts of the PAG by focal lesions or serial transections through the mesencephalon (Langworthy & Kolb 1933, Tang 1955, Ruch & Tang 1956). This finding raised the possibility that a mesencephalic bladder inhibitory centre tonically controlled micturition. The inhibitory region seems to be located in the dorsolateral margin of the rostral PAG (Numata *et al.* 2008) because chemical or electrical stimulation at this site inhibits reflex bladder contractions and the contractions induced by electrical stimulation of the PMC. Injection of bicuculline a $GABA_A$ receptor antagonist into the PMC blocks the PAG-induced inhibition of PMC stimulation indicating that GABA is the transmitter in the inhibitory pathway (Numata *et al.* 2008).

Other sites in the PAG seem to have a facilitatory role in micturition. Electrical stimulation in the ventrolateral region of the PAG evokes bladder contractions (Noto *et al.* 1989, Matsuura *et al.* 2000, Taniguchi *et al.* 2002) and firing on bladder postganglionic nerves (Noto *et al.* 1991b), whereas injections of cobalt chloride, a synaptic inhibitory agent (Matsuura *et al.* 1998) or an opioid receptor agonist (Matsumoto *et al.* 2004), into this region suppresses reflex micturition. These data raised the possibility that the ventrolateral PAG is an essential component of the micturition reflex.

Electrical recordings in the PAG indicate that it may serve as a relay and coordinating centre on the ascending limb of the micturition reflex pathway. In the rat electrical stimulation of bladder afferents in the pelvic nerve elicits negative field potentials in the dorsal PAG at a mean latency of 13 ms which is considerably shorter than the mean latency of field potentials in the region of the PMC (42 ms) (Noto *et al.* 1989). In the cat, a similar difference between latencies of pelvic afferent evoked field potentials in the PAG (11 ms) (Duong *et al.* 1999) and PMC in the (30–40 ms) (de Groat 1975) has been noted.

Subsequent studies in the cat and rat provided further support for the idea that bladder afferent information is relayed through the PAG. Axonal tracing studies in the cat revealed that spinal tract neurons located in lamina I on the lateral edge of the sacral dorsal horn, a region receiving primary afferent input from the bladder (Morgan *et al.* 1981), send a prominent direct axonal input through the lateral funiculus to the PAG (Blok *et al.* 1995, Holstege & Mouton 2003) (Fig. 1). Injections of retrograde tracers into the lateral funiculus at the lumbar level labels the same group of sacral spinal tract neurons (de Groat *et al.* 1981). The PMC on the other hand receives a weaker input directly from the spinal cord, and this input does not terminate on the PMC output neurons that send information back to the sacral parasympathetic nucleus. Axonal tracing methods also identified projections from the PAG to the PMC (Blok & Holstege 1994, Kuipers *et al.* 2006), raising the possibility that ascending afferent information from the bladder is relayed through synapses in the PAG

to the PMC. Thus, it has been proposed that the PAG has an essential role in the spinobulbospinal micturition reflex pathway (Noto *et al.* 1989, Holstege & Mouton 2003).

However, experiments in cats by Takasaki *et al.* (2011) have raised questions about the importance of the PAG in reflex micturition. When the mesencephalon was serially transected at various levels that interrupted the connections between the PAG and the PMC reflex bladder contractions persisted after transections at rostral levels that eliminated connections with the dorsal half of the PAG. Reflex micturition also persisted after more caudal transections that eliminated connections with both the dorsal and ventral half of the PAG or eliminated the most rostral part of the PMC. On the other hand, transections caudal to the PMC abolished reflex micturition. The authors concluded that the PAG does not have an essential role in reflex micturition but rather is involved in transmitting bladder filling information to higher brain centres. Subsequently, the techniques used in transection experiments were questioned by other investigators (Stone *et al.* 2011) who noted that the PAG lesions in the experiments of Takasaki *et al.* (2011) were often incomplete and a part of the caudal ventrolateral PAG was preserved in some experiments.

In the rat, the role of the PAG is even less clear because prominent ascending projections from the lumbosacral spinal cord have been detected in the PMC as well as the PAG (Ding *et al.* 1997, Blok & Holstege 2000). Thus, the organization of the ascending limb of the micturition reflex is uncertain and may vary in different species.

Brain imaging studies (Tai *et al.* 2009) in the rat revealed that neuronal activity in the PAG increases during slow bladder filing indicating that afferent activity from the bladder is received and processed in the PAG prior to micturition; however, a similar signal was not detected in the PMC during filling. On the other hand, during micturition, signals were detected in the PAG and the PMC. Similar results have been reported during brain imaging in humans (see companion paper Griffiths & Fowler 2012). These results suggest that the PAG serves as a relay station for transmitting afferent information from the bladder to the PMC but that the switch from urine storage to voiding occurs in the PMC.

Chemical modulation of PMC and PAG pathways

Pharmacological studies indicate that circuitry in the PMC and PAG allows the spinobulbospinal micturition reflex pathway to function as a switch that is either in a completely ‘off’ mode (storage) or maximally ‘on’ mode (voiding). Injections of excitatory amino acids into the PMC (Mallory *et al.* 1991) or PAG (Taniguchi *et al.* 2002) evoke bladder contractions in cat and rat. On the other hand, microinjections of low doses of inhibitory agents such as $GABA_A$ receptor agonists (muscimol), opioid peptides at these sites increases the bladder volume threshold for inducing micturition without altering the magnitude of the micturition reflex measured as the amplitude of voiding contractions (Mallory *et al.* 1991, Noto *et al.* 1991a, Matsumoto *et al.* 2004, Stone *et al.* 2011). Conversely, injections of $GABA_A$ receptor (bicuculline) or opioid receptor antagonists (naloxone) reduce the bladder volume threshold indicating that tonic activation of inhibitory receptors in these centres can alter the set point of the micturition switch (Mallory *et al.* 1991, Noto *et al.* 1991a, Stone *et al.* 2011). Because pharmacologic modulation of the PAG circuitry clearly alters the bladder volume threshold, it seems reasonable to conclude that PAG input to the PMC switching circuit also regulates the set point for the micturition switch. Thus, the micturition switching function might not be dependent on the properties of a select population of neurons at one site but rather be dependent on a more complex circuitry consisting of a network of neurons located at several sites in the brain stem.

The pontine-mesencephalic network is in turn influenced by inputs from many other brain regions because lesions, electrical stimulation or application of drugs to the brain rostral to

the mesencephalon can markedly alter the set point of the micturition reflex (Langworthy *et al.* 1940, Kuru 1965, Gjone 1966, de Groat *et al.* 1993, Birder *et al.* 2009). Thus, the micturition switching circuitry is organized as a hierarchical system in which spinal pathways are modulated by pathways in the brainstem which are in turn modulated by pathways in the forebrain that control reflex voiding and initiate voluntary voiding (see companion paper by Griffiths & Fowler 2012 for more detailed description of forebrain mechanisms). Because the PMC receives relatively few inputs from other sites in the brain (Kuipers *et al.* 2006), while the PAG receives inputs from many sites (Beckel & Holstege 2011), it has been speculated that the forebrain control of the PMC and the micturition switch is mediated at the level of the PAG (Beckel & Holstege 2011).

Properties of neurons in the PMC

Single unit recording in the PMC of the cat (Bradley & Conway 1966, Koshino 1970, de Groat *et al.* 1998, Sasaki 2002, 2005a,b, Sugaya *et al.* 2003, 2005, Tanaka *et al.* 2003) and rat (Elam *et al.* 1986, Willette *et al.* 1988) with the bladder distended under isovolumetric conditions revealed several populations of neurons exhibiting firing correlated with reflex bladder contractions including: (1) neurons that are silent in the absence of bladder activity but fire prior to and during reflex bladder contractions (direct neurons, 21%), (2) neurons that are active during the period between bladder contractions and are inhibited during contractions (inverse neurons, 51%) and (3) neurons that fire transiently at the beginning of bladder contractions (on–off neurons, 4%) (Fig. 3). Tonic firing that was not correlated with bladder activity was also identified in a large percentage (25%) of PMC neurons (termed ‘independent neurons’) (Fig. 3). These neurons are localized primarily in the region of the locus coeruleus complex.

Subpopulations of direct and inverse neurons in the cat have also been identified based on slow changes in firing during and between bladder contractions (Sasaki 2004). Approximately 50% of direct neurons (type 2) exhibit tonic firing between bladder contractions, whereas the remainder (type 1) are quiescent until 0.5–1.2 s prior to a bladder contraction. The majority of inverse neurons (84%) stop firing during a bladder contraction after a delay of 4–11 s, whereas a small number exhibit only a reduction in firing. A large percentage of direct neurons project to the lumbosacral spinal cord (Sasaki 2002, 2005b, Sugaya *et al.* 2003), whereas only a small percentage of inverse neurons send projections to the cord. Thus, it has been speculated that inverse neurons function as local inhibitory neurons in the PMC. Both direct and inverse neurons exhibit excitatory synaptic responses to electrical stimulation of afferent axons in the pelvic nerve (de Groat *et al.* 1998). Direct neurons fire at a mean latency of 62 ms after a stimulus, whereas inverse neurons fire at a shorter latency of 25–30 ms followed by an inhibition at a latency of 80 ms and then a late excitation at 250–300 ms.

Properties of neurons in the pontine urine storage centre

A region of the pons located ventrolaterally to the PMC has been designated the pontine urine storage centre (PUSC) because electrical stimulation in this region inhibits micturition and activates the EUS (Kuru 1965, Koyama *et al.* 1966, Holstege *et al.* 1986, Nishizawa *et al.* 1987). Lesions in this area induce incontinence. Injections of anterograde tracers into the PUSC label axonal projections to the sphincter motor nucleus in the sacral spinal cord (Holstege *et al.* 1986). In decerebrate, unanesthetized cats when the bladder is distended to induce rhythmic reflex contractions under isovolumetric conditions, four major types of neurons can be detected in the PUSC including: (1) tonic storage neurons that are continuously active during storage/voiding cycles with increased firing during storage (38%), (2) phasic storage neurons that are only active during the storage phase (40%), (3)

tonic micturition neurons that are continuously active throughout the storage/micturition cycle but exhibit increased activity during micturition (9%) and (4) phasic micturition neurons that are only active during micturition (13%) (Sakakibara *et al.* 2002a). These neurons have been subclassified into augmenting, constant or decrementing according to the change in their discharge rate during either the storage or micturition phases. Some of the neurons increase their firing rate prior to the onset of the micturition or storage phases. The average interval between the onset of preceding neural activity and the change in bladder activity ranges from 2 to 10 s and is evident in the various types of neurons.

Properties of neurons in the PAG

Single unit recordings in the PAG and adjacent mesencephalic reticular formation in decerebrate unanesthetized cats during rhythmic reflex bladder contractions under isovolumetric conditions revealed firing patterns similar to those recorded in the PMC including (1) tonic storage neurons that are partially inhibited during bladder contractions (43%), (2) phasic storage neurons that are completely inhibited during bladder contractions (15%), similar to inverse neurons in the PMC, (3) phasic micturition neurons that are only active during micturition (13%) (Liu *et al.* 2004), similar to direct neurons in the PMC. A fourth type of neuron (29%) classified as tonic micturition neurons that are active throughout storage and micturition but increase their firing during bladder contractions may be similar to the transient neurons identified in the PMC. Among the 84 neurons recorded in this study, 16 were located in the PAG and the remainder were located just ventral to the PAG. In the PAG, storage neurons seemed to be located in the middle part of the PAG (H-C coordinates: P 0–1), whereas micturition neurons are distributed in a broader area. Simultaneous unit recordings in the PAG and PMC or in the PAG and the PUSC did not reveal significant time correlations in 100 ms windows between unitary activity in the these locations.

Properties of neurons in the substantia nigra pars compacta and ventral tegmental area

Single unit recordings in the substantia nigra pars compacta/ventral tegmental area (SN/ VTA) in ketamine anesthetized cats during rhythmic reflex bladder contractions under isovolumetric conditions revealed firing patterns similar to those recorded in the PUSC including (1) tonic (55%) and phasic (22%) storage neurons and (2) tonic (16%) and phasic (6%) micturition neurons. Augmenting, constant, decrementing and binary neurons that fired at the beginning and at the end of the storage phase were identified (Sakakibara *et al.* 2002b). The large percentage of micturition storage neurons is consistent with other observations indicating that the dopaminergic neurons in the SN/ VTA have a predominate inhibitory influence on micturition. For example, electrical stimulation in the SN terminates ongoing micturition (Yoshimura *et al.* 1992) and destruction of dopaminergic neurons using the toxin MPTP or 6-hydroxydopamine facilitates the micturition reflex (Yoshimura *et al.* 1998, 2003). Dopaminergic neurons in SN synapse with neostriatal GABAergic neurons that may be involved in micturition inhibitory mechanisms in the forebrain. In addition, D_1 dopaminergic receptors have been implicated in the control of GABAergic neurons in the PAG (Kitta *et al.* 2008), where GABAergic inhibition also plays an important role in the control of micturition (Stone *et al.* 2011).

Phasic micturition neurons in the SN/VTA might also have a role in the control of micturition because pathological conditions such as middle cerebral artery occlusion or decerebration have been shown to upregulate D_2 receptor facilitatory control of micturition in the rat (Yokoyama *et al.* 2002).

Does a micturition switching mechanism exist in the spinal cord?

Spinal cord injury rostral to the lumbosacral level eliminates voluntary and supraspinal control of voiding, leading initially to an areflexic bladder and complete urinary retention followed by a slow development of automatic micturition and bladder hyperactivity mediated by spinal reflex pathways (de Groat & Yoshimura 2006, 2012). However, voiding is commonly inefficient due to simultaneous contractions of the bladder and urethral sphincter (bladder–sphincter dyssynergia) as well as contractions of the proximal urethra and bladder neck mediated by sympathetic reflex pathways. Storage of urine is poor and a rapid switch from storage to voiding does not occur. On the contrary in many species after chronic spinal cord injury, slow bladder filling induces repetitive small amplitude, short duration reflex bladder contractions that increase in amplitude as bladder volume increases (Cheng *et al.* 1995, 1999). Thus, bladder reflex mechanisms clearly survive after the removal of brain circuitry and persist in the lumbosacral spinal cord. In some patients, reflexes activated by suprapubic tapping or tactile stimulation in the perineal area can be used to induce voiding (Haslam *et al.* 2010). However, the key features of the micturition switching circuit including (1) coordination between bladder and urethral smooth and striated muscle, (2) efficient urine storage and emptying, (3) the characteristic abrupt switch from bladder quiescence to maximal activity that is maintained until complete emptying are absent after elimination of the PAG-PMC circuitry.

Electrophysiologic studies in animals have shown that the spinal micturition reflex is clearly different than the normal reflex with the spinal cord intact. In cats, the spinal reflex occurs after a short central delay of approx. 5 ms and is triggered by a C-fibre afferent limb (de Groat *et al.* 1981, 1990, de Groat & Yoshimura 2012). This contrasts with the spinobulbospinal micturition reflex in normal animals that occurs after a much longer central delay (60–70 ms) and in response to myelinated Aδ afferent input from the bladder. Furthermore, in normal cats, capsaicin, a neurotoxin known to disrupt the function of C-fibre afferents, does not block reflex contractions of the bladder or the Aδ-fibre-evoked bladder reflex. However, in cats with chronic spinal injury capsaicin completely blocks C-fibre-evoked bladder reflexes (de Groat *et al.* 1990, Cheng *et al.* 1999). Evidence of the contribution of C-fibre bladder afferents to bladder hyperactivity and involuntary voiding in humans has been obtained in studies in which capsaicin or resiniferatoxin, another C-fibre afferent neurotoxin, were administered intravesically to patients with neurogenic detrusor overactivity due to multiple sclerosis or spinal cord injuries (Fowler 2006). In these patients, the toxins increased bladder capacity and reduced the frequency of incontinence.

The emergence of C-fibre bladder reflexes seems to be mediated by several mechanisms including changes in central synaptic connections and alterations in the properties of the peripheral afferent receptors that lead to sensitization of the ‘silent’ C fibres and the unmasking of responses to mechanical stimuli (de Groat 2006, Vizzard 2006, de Groat & Yoshimura 2012). In rats, it has been shown that bladder afferent neurons undergo both morphologic (neuronal hypertrophy) (Kruse *et al.* 1995, Yoshimura & de Groat 1997b) and physiologic changes (upregulation of TTX-sensitive Na^+ channels and downregulation of TTX-resistant Na^+ channels) following spinal cord injury (Yoshimura & de Groat 1997a, de Groat & Yoshimura 2009). It has been speculated that this neuroplasticity is mediated by the actions of neurotrophic factors such as nerve growth factor (NGF) released within the spinal cord or the urinary bladder (Vizzard 2006). The production of neurotrophic factors including NGF increases in the bladder after spinal cord injury, whereas chronic administration of NGF into the bladder or spinal cord of rats induces bladder hyperactivity and increases the firing frequency of dissociated bladder afferent neurons (de Groat & Yoshimura 2006, 2009, 2012, Yoshimura *et al.* 2006). On the other hand, intrathecal application of NGF antibodies

to neutralize NGF in the spinal cord suppresses detrusor hyperreflexia (Seki *et al.* 2002) and detrusor-sphincter dyssynergia (Seki *et al.* 2004) in spinal cord injured rats.

Spinal cord injury in cats and rats also causes the re-emergence of a neonatal exteroceptive micturition reflex that is activated by tactile stimulation of cutaneous afferent axons in the perineum (perineal-to-bladder reflex) (de Groat 2002). In neonatal animals, this reflex is activated by the mother licking the perineal region of the young animal. The stimulation is essential for survival of the neonate because isolation of the newborn from its mother leads to urinary retention. The adult form of reflex voiding that is triggered by bladder distension does not become functional until several weeks after birth. During this same period, the perineal-to-bladder reflex becomes progressively weaker and eventually disappears (de Groat *et al.* 1981, 1998). Thus, postnatal maturation of voiding function is associated with a prominent reorganization of synaptic connections in bladder reflex pathways leading to down-regulation of primitive spinal mechanisms and up-regulation of mature supraspinal mechanisms (Araki & de Groat 1997). It seems likely that this developmental switching mechanism is dependent upon competition between brain and spinal pathways because spinal cord injury in adult animals and humans which interrupts brain–spinal cord connections causes the re-emergence of the neonatal perineal-to-bladder reflex (de Groat 2002, de Groat & Yoshimura 2012). Spinal cord injury in humans also unmasks a bladder reflex elicited by instillation of cold water into the bladder (Geirsson *et al.* 1999). This cold-evoked bladder reflex, which is activated by C-fibre afferents (Fall *et al.* 1990, Geirsson *et al.* 1995), is not present in normal adults but is present in neonates (Geirsson *et al.* 1994) and then is suppressed during postnatal maturation of the nervous system. Not all spinal cord injured patients exhibit the cold-evoked bladder reflex (Geirsson *et al.* 1993, Chancellor *et al.* 1998) and the percentage of patients exhibiting the reflex is higher in those with complete cord injury than in those with an incomplete injury. However, repeated instillations of cold water increases the percentage of patients responding possibly by sensitizing the C-fibre-mediated reflex pathway (van Meel *et al.* 2007).

In summary, these observations support the traditional view that a spinal segmental micturition reflex pathway is not a major contributor to voiding in spinal cord intact animals and that the abrupt switch from storage to voiding during bladder filling is dependent on the properties of supraspinal circuitry.

Computer modelling of the switching circuitry in the PMC and PAG that underlies the spinobulbospinal micturition reflex in a decerebrate animal

On the basis of neuronal firing patterns recorded in the PMC and PAG during rhythmic bladder contractions as well as antidromic responses to stimulation of the spinal cord and synaptic responses to stimulation of bladder afferent nerves, a neural circuit has been designed in an attempt to model the switching properties of the spinobulbospinal micturition reflex in a decerebrate animal (Fig. 4). The circuit includes the peripheral afferent and efferent pathways between the bladder and the spinal cord plus connections between the spinal cord, PAG and PMC. The primary purpose of this computer simulation is to test the correctness of the neural circuitry theorized in this paper. If this theorized circuitry is accurate, a computer simulation based on it should replicate the storage-voiding cycle. If the computer simulation does not replicate the storage-voiding cycle, it suggests that the theorized circuitry contains invalid elements or that it is incomplete. Furthermore, once this simulation has been demonstrated to accurately model the micturition reflex, it can be used in the future to generate hypotheses that can then be tested experimentally.

The ascending sensory limb of the circuit consists of a mechanosensitive bladder primary afferent neuron that synapses with a second-order spinal tract neuron. The latter projects to

excitatory neurons in the PAG that in turn relay information to the PMC. The PMC contains several types of neurons. Direct neurons (indicated by D in Fig. 4) that send information back to the sacral parasympathetic nucleus represent the descending limb of the spinobulbospinal micturition reflex (Fig. 4). These neurons (type 1 direct neurons) are silent during bladder filling but activated prior to and during micturition. In the model, the type 1 direct neurons receive tonic inhibitory input from independent neurons and bladder volume-dependent inhibition from inverse neurons. Inverse neurons (I) that are activated by afferent input from the PAG and fire during bladder filling make inhibitory connections with direct neurons to provide feed-forward inhibition of the micturition reflex during bladder filling. The inverse neurons are inhibited during micturition which in turn removes inhibitory input to the direct neurons and facilitates the micturition reflex. Transient neurons (T) that are activated by bladder afferent stimulation via a relay (B) through the PAG and fire at the beginning of a bladder contraction are postulated to inhibit the inverse neurons and play an important role in the initiation of the micturition reflex. Type 2 direct neurons (indicated by R in Fig. 4) which exhibit continuous firing during bladder relaxation but are strongly activated at the onset of micturition are postulated to receive excitatory axon collaterals (pathway C) from type 1 direct neurons and mediate reciprocal inhibition of inverse neurons. This would further enhance the development of the micturition reflex by suppressing inhibitory input to the type 1 direct neurons.

In the PAG, it is postulated that neurons tonically active during bladder filling or between micturition contractions represent relay neurons that transmit excitatory signals (pathway A) to inverse neurons in the PMC that in turn generate feed-forward inhibition of the micturition reflex. Conversely excitatory neurons in the PAG that relay bladder afferent information to PMC direct neurons are likely to be ‘phasic micturition neurons’ identified by Sakakibara *et al.* (Sakakibara *et al.* 2002a). It is known that a GABAergic inhibitory mechanism in the PAG tonically controls the bladder volume set point for initiating micturition (Stone *et al.* 2011). In the hypothetical circuit in Figure 4, this mechanism is represented by an independent inhibitory neuron synapsing with the PAG relay neuron. Similarly, GABAergic or enkephalinergic independent inhibitory neurons in the PMC are likely to control the set point for micturition by tonically inhibiting the type 1 direct neurons.

In summary, GABAergic or enkephalinergic inhibitory control of the micturition switching circuit may occur at several sites on the spinobulbospinal pathway including (1) the ascending limb in the spinal cord, (2) relay centres in the PAG, (3) synapses on type 1 direct neurons in the PMC. However, it is presumed that the switch from storage to voiding occurs at the level of the direct neurons because their firing is closely linked with PGN firing and reflex bladder contractions, indicating that transmission of descending signals through the sacral parasympathetic nucleus occurs with a high safety factor. Therefore, excitatory synapses on the descending limb of the micturition reflex pathway function as relays rather than switches and transmit signals from the PMC switch to the bladder with high fidelity.

Computer modelling of the spinal urine storage and micturition circuitry

As described in a previous section, spinal vesico-sympathetic and vesico-sphincter reflex mechanism can contribute to urine storage. For simplicity, these reflex mechanisms have been modelled (Fig. 1) as monosynaptic reflexes although it is probable that they are multisynaptic pathways. As indicated in the model, these two storage reflexes are inhibited by descending input from the type 1 direct neurons in the PMC, thereby promoting urethral outlet relaxation during micturition. The model includes a third storage mechanism in which sphincter afferents activate spinal inhibitory neurons that suppress bladder preganglionic neurons and interneurons on the ascending and descending micturition reflex pathway. The model also includes a recurrent inhibitory circuit in which preganglionic axon collaterals

activate interneurons that in turn inhibit excitatory interneurons on the ascending and descending limbs of the micturition reflex pathway (de Groat & Ryall 1968b, de Groat 1976). The spinal inhibitory mechanisms are suppressed by descending input from PMC direct neurons during micturition (Fig. 1). A urethral-bladder excitatory mechanism that facilitates micturition in response to flow of urine through the urethra (Barrington 1925, Kuru 1965) is shown as a urethral afferent making an excitatory synapse with a spinal tract neuron on the ascending limb of the spinobulbospinal pathway.

Computer simulation of the storage-voiding cycle using a model of the PMC and PAG switching circuitry

The model of the spinobulbospinal pathway consisting of the supraspinal components shown in Figure 4 and the spinal components shown in Figure 1 was used to simulate a reflex storage-voiding cycle and estimate various parameters including bladder pressure, bladder volume, bladder afferent firing and bladder efferent firing during filling of the bladder at a rate of 30 mL min^{-1} (Fig. 5). The model of the urinary tract used in this simulation has two interconnected components: a mechanical component that models the bladder and outlet, and an artificial neural network that models the neural reflex pathways controlling the lower urinary tract. This approach is similar to several other attempts to model the lower urinary tract (van Duin *et al.* 1998, Fry *et al.* 2011) but also includes putative supraspinal circuitry based on unit recordings in the PMC and PAG. The mechanical component was taken from the model produced by Bastiaanssen *et al.* (Bastiaanssen *et al.* 1996). The neural component was produced by abstracting the details of individual neurons into neuron groups, such as the PMC direct neurons, or Onuf's nucleus. This simplified approach was used because the goal of the simulation was to model clinical behaviour. Details of single nerve cells, when properly abstracted, are often not required for an understanding of the collective behaviour of a network of cells (Hertz *et al.* 1991).

The mathematical details of the neural component follow:

1. The output signal of each neuron group i is represented by a variable x_i, which can range between 0 (no firing) and 1 (maximal firing).
2. Each neuron group receives zero or more input signals. Input can come either directly from the mechanical component (e.g. firing from a bladder tension receptor) or from other neuron groups.
3. Each input signal j to a neuron group i is weighted by a constant factor w_{ji}. Positive weights model excitatory neurons, and negative weights model inhibitory neurons. A weight's absolute value represents the strength of the synapse.
4. Neuron output signal x_i is calculated by passing the weighted sum of its inputs into a sigmoid function $\sigma(n)$, which approaches 0 for sufficiently low values of n, and 1 for sufficiently large values of n; this models the manner in which a neuron's firing rate saturates when it reaches a certain level of excitatory input. Thus, for a neuron i that receives input from signals j through z, output is calculated according to the following formula:

$$x_i=\sigma(w_{ji}x_j+w_{ki}x_k+\ldots+w_{zi}x_z).$$

The topology of the neural network is based on evidence from physiological experiments. The numerical parameters (e.g. weights) in each neuron group were calibrated by simulating the effect of adding or removing various inputs to the neuron group and then comparing the result with behaviour observed in vivo when that same input is added or removed. In places

where this kind of data was unavailable, ad hoc sensitivity testing was performed to ensure that the overall behaviour of the system was robust to small changes in the numerical parameters.

As shown in Figure 5 and based on the Bastiaanssen *et al.* model (Bastiaanssen *et al.* 1996), we have assumed that during bladder filling bladder pressure remains low but bladder afferent firing slowly increases as bladder wall tension increases. Bladder efferent firing that represents activity in the spinobulbospinal micturition reflex pathway remains low during the filling because the PAG-PMC switching circuit is in the off mode. At a critical bladder volume threshold the PAG-PMC switch is turned on and efferent firing markedly increases inducing a prominent increase in bladder pressure followed by an increase in bladder afferent firing, a relaxation of the urethral outlet (not shown) and then voiding evident as a decrease in bladder volume. The model generates an all-or-none reflex response reflected as maximal efferent discharge throughout voiding even as bladder volume decreases. The model generates efficient voiding resulting in complete bladder emptying. Reducing the strength of the inhibitory input from the PMC tonically active independent neuron to the direct neuron reduces bladder capacity, while increasing the strength of the inhibition increases bladder capacity. The fact that the computer simulation replicates the storage-voiding cycle corroborates the validity of the neural circuitry theorized in this paper.

Conclusions

The neural control of micturition is organized as a hierarchical system in which spinal storage mechanisms are regulated by complex switching mechanisms in the rostral brain stem that initiate reflex micturition. The circuitry controlling reflex micturition is in turn modulated by input from the forebrain that underlies voluntary micturition.

Because reflex bladder activity can recover after complete transection of the thoracic spinal cord, it might be argued that all of the switching mechanisms required for control of the lower urinary tract are resident in the spinal cord. However, spinal circuits do not generate efficient storage or voiding and bladder reflexes appear to emerge after spinal cord injury due to the formation of new synaptic connections rather than the recovery of normal, pre-existing connections. Thus, it is reasonable to conclude the micturition switch is located in the brain stem.

The initiation of reflex micturition is driven by afferent signals arising in the bladder during bladder filling. These signals activate a spinobulbospinal reflex consisting of an ascending afferent limb that projects through the lateral funiculus of the spinal cord to sites in the mesencephalon (the PAG) and/or the rostral pons (the PMC). The descending limb of the reflex originates in the pons (PMC direct neurons) and carries signals back to the parasympathetic, sympathetic and sphincter motor nuclei in the lumbosacral spinal cord to coordinate the activity of the bladder and urethral outlet. It is assumed that output from the PMC is excitatory but can be converted to inhibitory inputs to sympathetic and sphincter pathways by activation of segmental inhibitory neurons in the spinal cord.

The function of the micturition switch is influenced not only by the properties of the pontine direct neurons but also by local modulatory circuits in the pons and by the level of afferent input from the bladder which can be regulated at various sites along the afferent pathway including the peripheral afferent terminals in the bladder, the spinal cord and the brain stem. Thus, it might be appropriate to view micturition switching as a property of a network of neurons at several sites in the brain stem rather than that of single population of neurons. Nevertheless, the ultimate decision to initiate reflex voiding must occur at the level of the PMC direct neuron that relays information to the spinal cord.

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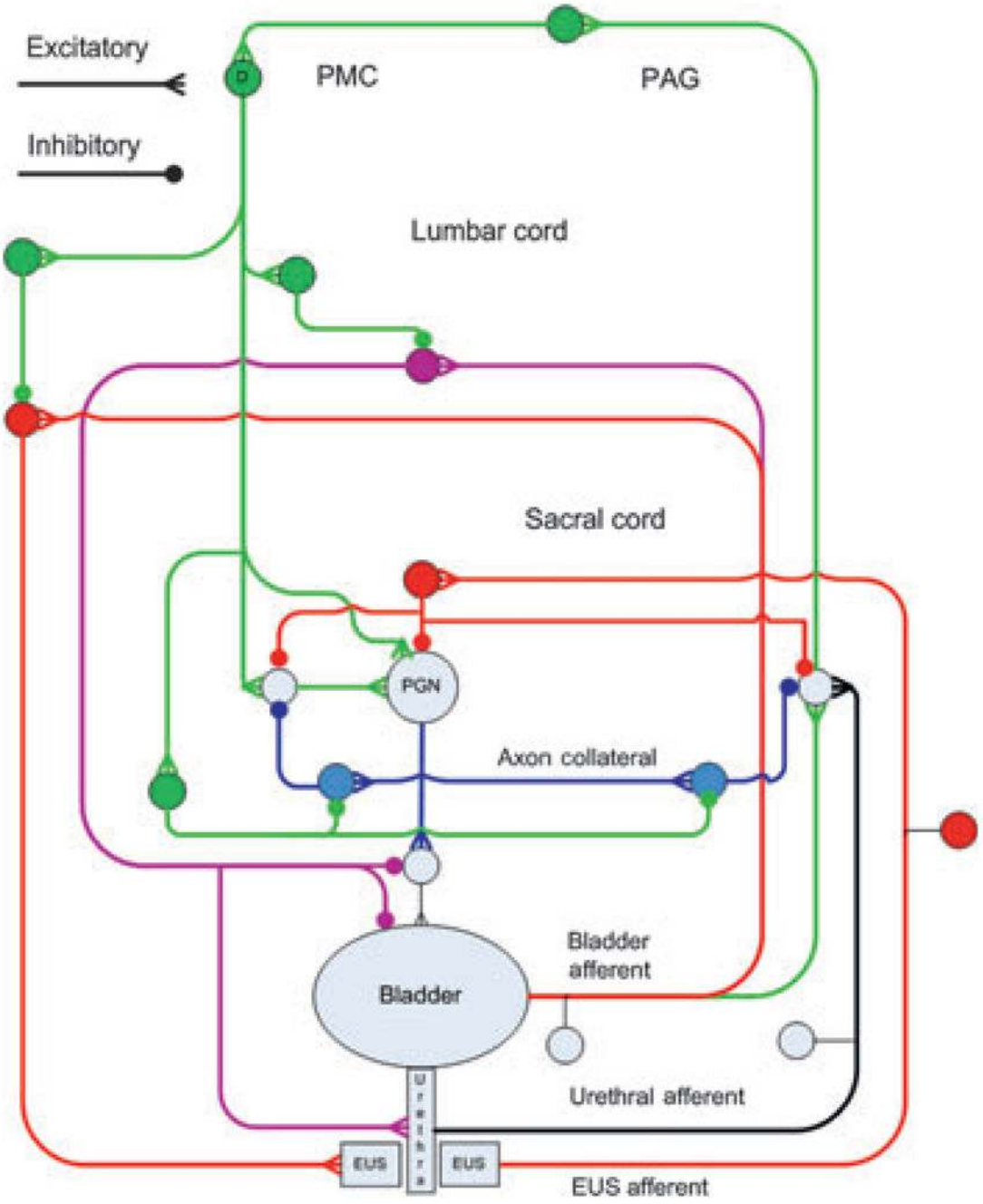

Figure 1.

Diagram summarizing the reflex pathways that regulate urine storage and voiding in a decerebrate cat. The major focus of the diagram is the organization of spinal circuitry. During the urine storage, distention of the bladder produces a low level of firing in vesical afferent axons in the pelvic nerve, which induces firing in lumbar sympathetic nerves innervating the bladder, bladder ganglia and urethra. This activity is mediated by an intersegmental sacral-lumbar reflex pathway (shown in purple) and produces inhibition of the bladder and contraction of the urethral smooth muscle. Bladder afferent firing also elicits reflex activation of motoneurons innervating the external urethral sphincter (EUS) (shown in red) which induces a EUS contraction (the guarding reflex). The EUS contraction in turn

induces firing in EUS afferents (shown in red) which activate inhibitory interneurons in the spinal cord leading to inhibition of bladder preganglionic neurons (PGN) and excitatory interneurons on the ascending and descending limbs of the spinobulbospinal micturition reflex pathway. The bladder-sympathetic and bladder-EUS reflex pathways are very likely multisynaptic but are shown as monosynaptic to simplify the diagram. A third spinal inhibitory mechanism (recurrent inhibition) is mediated by axon collaterals of the PGN which activate inhibitory interneurons (shown in blue) that in turn suppress the firing of excitatory interneurons on the ascending and descending limbs of the spinobulbospinal micturition reflex pathway. Reflex voiding in the decerebrate cat is triggered by bladder distension and afferent firing that activates spinal tract neurons (shown in light blue) in the sacral cord that send information to an excitatory circuit in the periaqueductal gray that in turn activates direct (D) neurons in the pontine micturition centre (PMC) that send excitatory signals back down the spinal cord to bladder PGN and/or excitatory interneurons that synapse with PGN (shown in green). PGN in turn excite peripheral ganglion cells that activate the bladder smooth muscle. Descending activity in the PMC direct neurons also suppresses sympathetic and EUS storage reflexes as well as recurrent inhibition by activating segmental inhibitory interneurons (shown in green). This suppression of spinal inhibitory mechanisms by the PMC enhances voiding efficiency.

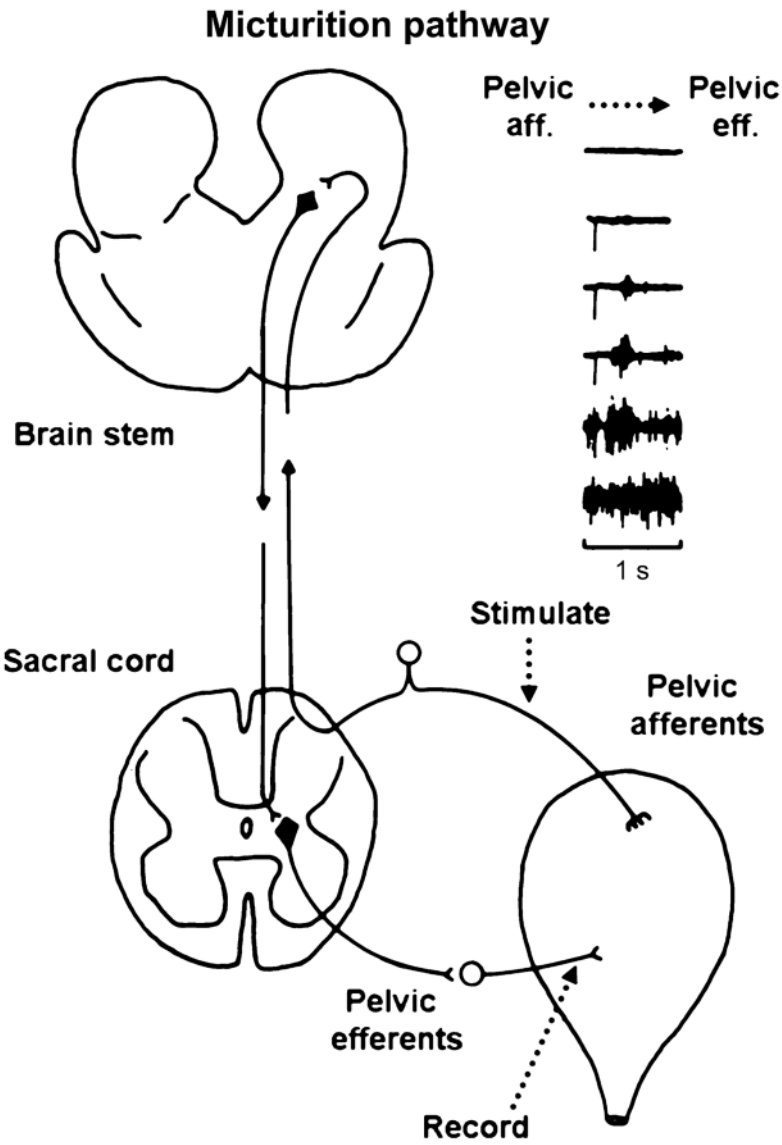

Figure 2.

Multiunit recordings of reflex activity on a bladder postganglionic nerve in a chloralose anesthetized cat during electrical stimulation (0.8 Hz, 3 v, 0.05 ms duration) of bladder afferent axons in the pelvic nerve. The bladder was distended with saline to a volume below the threshold for inducing micturition. First tracing in the upper right is a recording prior to the onset of stimulation showing that the efferent pathway is inactive. The next tracing shows lack of a response to the first stimulus in a train of stimuli. Further stimulation (lower tracings) induces a gradual increase in the magnitude of a long latency reflex and the eventual emergence of asynchronous firing (last tracing) which indicates the onset of reflex micturition. The diagram on the left shows the spinobulbospinal micturition reflex pathway and the sites of nerve stimulation and recording.

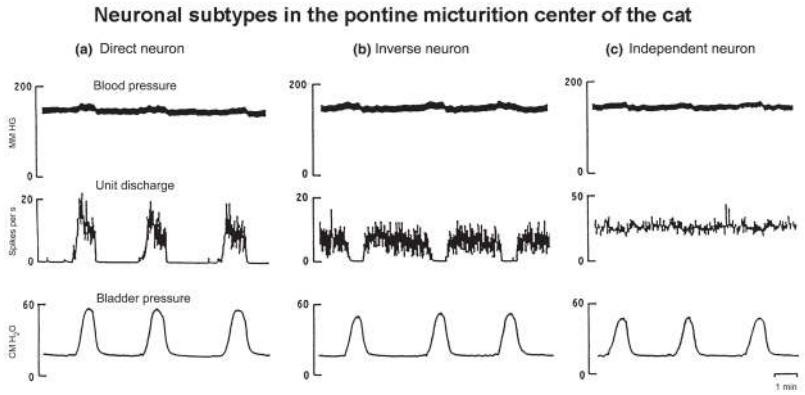

Figure 3.

Relationship between single unit activity in the PMC of a decerebrate, unanesthetized cat and reflex contractions of the urinary bladder. Top tracings are blood pressure, middle tracings are ratemeter recordings of unit activity in spikes per second and the bottom tracings are bladder pressure in cm H_2O. Three types of neuronal activity are illustrated: (a) a direct neuron that only fired during a bladder contraction, (b) an inverse neuron that fired between bladder contractions and was inhibited during contractions and (c) an independent neuron that exhibited continuous firing unrelated to bladder contractions. Small increases in blood pressure occurred during bladder contractions. The bladder was distended with saline and maintained under isovolumetric conditions. Horizontal calibration represents 1 min. The three neurons were studied at different times in the same animal.

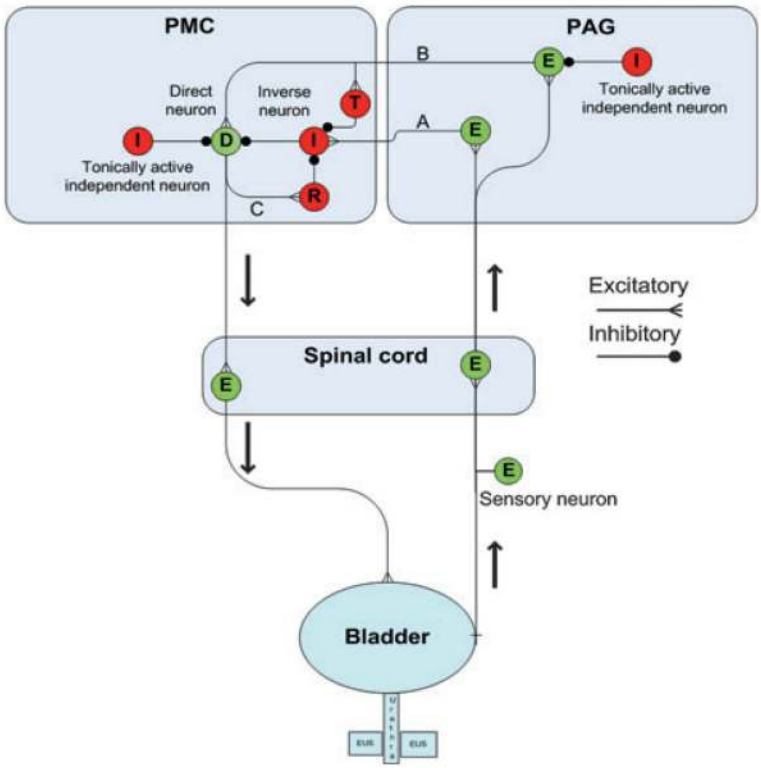

Figure 4.

Diagram illustrating the putative pathways in the periaqueductal gray (PAG) and pontine micturition centre (PMC) that contribute to urine storage and voiding. This circuitry shows the neuronal elements and connections used in our computer model. The right side illustrates the ascending afferent limb of the spinobulbospinal micturition reflex that projects to the PAG, and the left side shows the descending limb that connects the PMC direct neuron to the bladder efferent neuron in the sacral spinal cord. During urine storage as the bladder slowly fills low level of afferent activity activates an excitatory neuron (E) in the PAG which relays information (pathway A) to an inverse neuron (I) in the PMC that in turn provides inhibitory input to the type 1 direct neuron (D) to maintain continence. Bladder afferent input is also received by a second neuron in the PAG (E) that is on the excitatory pathway (pathway B) to the PMC type 1 direct neuron (D) and to a transiently active PMC neuron (T) that fires at the beginning of micturition. However, the PAG excitatory relay neuron (E) is not activated during the early stages of bladder filling because it is inhibited by a tonically active independent neuron (I). The PMC type 1 direct neuron is also inhibited by a tonically active independent neuron (I) located in the PMC. Bladder afferent firing gradually increases during bladder filling which increases feed-forward inhibition of the direct neuron via the PAG–PMC inverse neuron pathway. However, at a critical level of afferent firing, excitatory input to the PAG excitatory relay neuron surpasses the tonic inhibition of the independent neuron and sends signals to the PMC transient neuron which briefly inhibits the inverse neuron reducing inhibitory input to the direct neuron allowing it to overcome tonic inhibition and fire action potentials which activate by an axon collateral (pathway C) a reciprocal inhibitory neuron (R) that suppresses the inverse neuron (I) and further reduces inhibition of the direct neuron (D). The direct neuron then switches into maximal firing mode and sends excitatory input to the spinal efferent pathway to the bladder inducing a large bladder contraction and more afferent firing which further enhances synaptic transmission in the PAG-PMC micturition reflex pathways. The reflex circuitry

returns to storage mode as the bladder empties and afferent firing declines. Excitatory neurons are green and inhibitory neurons are red.

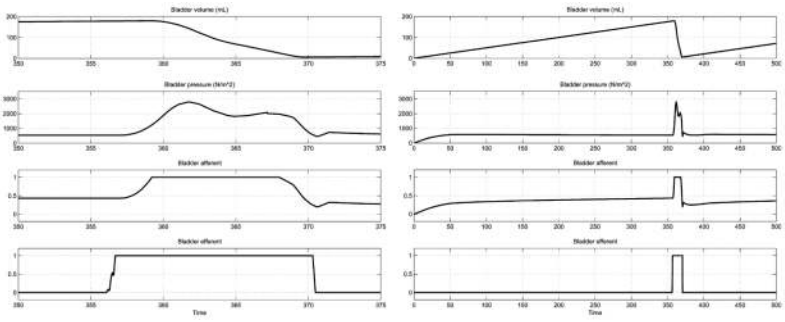

Figure 5.

Simulated bladder volume (top tracing) and pressure (2nd tracing), bladder afferent firing (3rd tracing) and bladder efferent firing (bottom tracing) during bladder filling (30 mL min^{-1}) and during reflex voiding using our computer model of spinal, PAG and PMC neural pathways and the myocybernetic model of Bastiaanssen *et al.* 1996 to predict the properties of the bladder, urethra and the afferent firing arising in these structures. Note that as bladder volume increases, bladder pressure remains low, bladder efferent firing is absent, but bladder afferent firing gradually increases eventually reaching a threshold for inducing a micturition reflex as evidenced by an abrupt increase in efferent firing, which induces an increase in bladder pressure and afferent firing and bladder emptying. Bladder efferent firing peaks early during micturition and is maintained until the bladder is empty. The voiding phase is shown on an expanded time scale in the tracings on the right side.