Committee 3

Neural Control

Chair

L. Birder (USA)

Co-Chairman

M. Drake (UK)

Members

W. de Groat (USA),

C. Fowler (U.K),

E. Mayer (USA),

J. Morrison (UAE),

J. Paton (U.K)

Consultants

D. Griffiths (Canada),

I. Mills (U.K),

K. Thor (USA)

CONTENTS

Neural Control

L. Birder, M. Drake,

W. de Groat, C. Fowler, E. Mayer, J. Morrison, J. Paton

Consultants

D. Griffiths, I. Mills, K. Thor

OVERVIEW

This chapter deals with individual components regulating the neural control of the urinary bladder. This chapter has been completely remodeled and updated with the focus on factors and processes involved in the two models of operation of the bladder: storage and elimination. There has been significant new information since the last consultation in a number of fields:

- The urothelium and its roles in sensor and transducer functions including interactions with other cell types within the bladder wall ('sensory web')
- The location and properties of bladder afferents including factors involved in regulating afferent sensitization
- The neural control of the pelvic floor muscle and pharmacology of urethral and anal sphincters (focusing on monoamine pathways)
- Efferent pathways to the urinary bladder
- Abnormalities in bladder function including mechanisms underlying co-morbid disorders associated with bladder pain syndrome and incontinence

The next sections deal with the current understanding of brainstem neuronal networks, which regulate lower urinary tract function (1-4). The importance is reflected in the current advances in functional brain imaging (both positron emission topography or PET and functional magnetic resonance imaging or fMRI). These advances have had a major impact on understanding CNS control of the human bladder. This section has been completely revised to also include the recent advances in understanding of bidirectional brain-gut interactions in addition to discussion of homeostatic function and neural mechanisms underlying fecal and anal continence.

LEVELS OF EVIDENCE

This book attempts to use Levels of Evidence throughout. The Oxford Centre for Evidence Based Medicine has laid down guidelines that apply to Levels of Therapeutic Interventions and Grades of Recommendations to Patients; the existence of dispute regarding each major conclusion should be documented. However this advice does not really apply to the basic sciences, where randomized controlled trials are not a common format of investigation, and acute studies with internal controls are more common.

Within this chapter we intend to be selective and report scientific evidence that has appropriate controls and achieves statistical significance. Other categories of evidence, e.g. uncontrolled studies, anecdotal information, hypothesis or speculation will be referred to as such.

Of some importance in this field are species differences, and efforts have been made to make it very clear when each new topic is introduced in which species the observation was made with special emphasis as to the extent comparable data exists for humans.

In this report, we intend to indicate whether the conclusions are based on (A) peer-reviewed papers in reputable journals (B) evidence in book chapters or reviews, and (C) Abstracts: abstracts will only be mentioned if they refer to a systematic study with good statistical methodology.

I. THE UROTHELIUM

There is evidence that a number of functional pain syndromes are associated with changes in the epithelial layer. Alterations of bladder urothelium at the molecular and structural levels have been reported in both patients and animals modeled for various bladder disorders. It is likely that many therapies currently used in the treatment of bladder disease may target urothelial receptors and/or their release mechanisms.

1. INTRODUCTION TO THE ANATOMY AND BARRIER FUNCTION OF THE UROTHELIUM

The urothelium is the epithelial lining of the lower urinary tract between the renal pelvis and the urinary bladder. Urothelium is composed of at least three layers: a basal cell layer attached to a basement membrane, an intermediate layer, and a superficial or apical layer composed of large hexagonal cells (diameters of 25-250 μm) known as 'umbrella cells' [5, 6] (**Figure 1**) The umbrella cells are interconnected by tight junctions (which are composed of multiple proteins such as the claudins) and are covered on their apical surface (nearly 70-80%) by crystalline proteins called uroplakins that assemble into hexagonal plaques [7-10]. Uroplakins and other urothelial cellular differentiation markers, such as cytokeratin 20, are not expressed in the stratified epithelium of the urethra. In some species, the umbrella cells and perhaps also the intermediate cells have projections to the basement membrane [5].

The ability of the bladder to maintain the barrier function, despite large alterations in urine volume and increases in pressure during bladder filling and emptying, is dependent on several features of the umbrella cell layer. These features include tight-junction complexes that reduce the movement of ions and solutes between cells and specialized lipid molecules and uroplakin proteins in the apical membrane, which reduce the permeability of the cells to small molecules (water, urea, protons) [5,11]. The apical surface of the urothelium is also covered with a sulfated polysaccharide glycosaminoglycan (GAG) or mucin layer that is thought to act as a nonspecific anti-adherence factor and as a defense mechanism against infection [12-14]. In addition, during bladder filling the umbrella cells become flat and squamous and this shape change is accompanied by vesicular traffic (i.e. exocytosis/endocytosis), adding membrane to the apical surface thereby increasing overall urinary bladder surface area [7,15,16]. There is evidence that this stretch-induced exocytosis is dependent on activation of epidermal growth factor receptor (EGFR) [17,18]. These processes allow the bladder to accommodate increasing volumes of urine during filling without compromising the barrier function. Exocytosis/endocytosis (vesicular recycling) may also play an important role in modulating the release of a number of neurotransmitters/mediators as well as regulation of the function of many receptors and ion channels in urothelial cells [19,20].

2. RESPONSE OF THE UROTHELIUM TO INJURY

Basal cells, which are thought to be precursors for other cell types, normally exhibit a low (3-6 month) turnover rate, in fact the slowest turnover of any mammalian epithelial cells [7,21]. It has been shown that neither urine-derived factors nor cyclic mechanical changes contribute to urothelial proliferation and differentiation; however accelerated proliferation can occur in pathology. For example, using a model (protamine sulfate) that selectively damages the umbrella cell layer, it has been shown that the urothelium rapidly undergoes both functional and structural changes in order to restore the barrier in response to injury [22]. The initiation of urothelial proliferation is thought to involve up-regulation of growth factors such as fibroblast growth factor and nerve growth factor (NGF) [23,24].

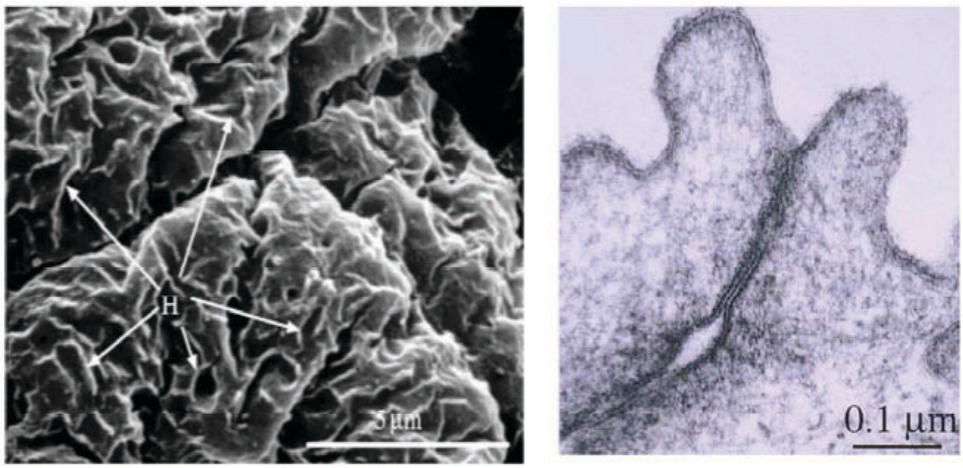


Figure 1 : Ultrastructural features of umbrella cell apical membrane. Left panel: Scanning electron micrograph (high magnification) of apical surface of rabbit umbrella cell layer (hinges "H" marked with arrows). Right panel, high power view of tight junctions. (from Apodaca, 2004; Truschel et al., 1999).

Though the urothelium maintains a tight barrier to ion and solute flux, a number of local factors such as tissue pH, mechanical or chemical trauma, or bacterial infection can modulate the barrier function of the urothelium [7,25]. Other conditions such as bladder pain syndrome/interstitial cystitis (BPS/IC) or spinal cord injury are also associated with changes in urothelial barrier [26,27]. When the barrier is compromised, water, urea and toxic substances can pass into the underlying tissue (neural/muscle layers) resulting in urgency, frequency and pain during bladder filling and voiding. In some pathological conditions the disruption of the urothelial barrier is associated with ultrastructural changes and alterations in the levels of chemical mediators such as nitric oxide (NO) and adenosine triphosphate (ATP) that may alter epithelial function and/or integrity. Disruption of urothelial barrier integrity has also been linked to the expression of substances such as antiproliferative factor (APF), which also slows urothelial cell growth [28-30]. APF, a frizzled 8 protein detected in the urine of patients with BPS/IC, is secreted by bladder epithelial cells obtained from these patients. Treatment of urothelial cells from normal patients with purified APF decreases the expression of adhesion and tight junction proteins.

Urinary tract infections produced by uropathogenic *Escherichia coli* (UPEC) are initiated by bacterial adherence to uroplakin proteins on the apical surface of umbrella cells [25,31] (**Figure 2**). The UPEC express filamentous adhesive organelles (type 1 pili) that mediate both bacterial attachment and invasion of the urothelial cells. Internalization of UPEC in the umbrella cells and formation of intracellular colonies (biofilm-like pods) of UPEC in umbrella cells has been implicated in the mechanism of chronic urinary tract infections.

Disruption of urothelial function can also be induced by more remote pathological conditions that influence neural or hormonal mechanisms. For example, spinal cord transection in rats leads to a rapid alteration in the urothelial barrier including ultrastructural changes and increased permeability [26]. The changes are blocked by pretreatment with a ganglionic blocking agent, suggesting an involvement of efferent autonomic pathways in the acute effects of spinal cord injury on bladder urothelium. Other types of urothelial-neural interactions are also likely, based on the recent reports that various stimuli induce urothelial cells to release chemical mediators that can in turn modulate the activity of afferent nerves [5,20]. This has raised the possibility that the urothelium may have a role in sensory mechanisms in the urinary tract.

In summary, modification of the urothelium and/or loss of epithelial integrity in a number of pathological conditions can result in passage of toxic/irritating urinary constituents through the urothelium or release of neuroactive substances from the urothelium leading to changes in the properties of sensory nerves and in turn sensory symptoms such as urinary frequency and urgency. Thus chemical communication between the nervous system and the urothelial cells may play an important role in the generation of urinary bladder dysfunction.

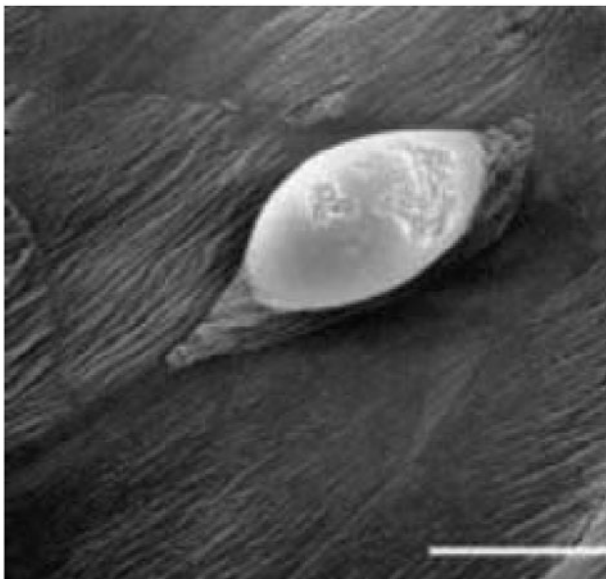


Figure 2 : Image depicts an intracellular bacterial "pod" on the surface of a C3H/H3J mouse bladder infected with UT189. (from Anderson et al., 2003).

3. UROTHELIAL HETEROGENEITY

Studies (comparing a number of species) have shown that the major part of the urinary tract is lined with a fully differentiated urothelium [9]. Findings in cultured cells reveal a distinct difference in morphology of ureteral and bladder urothelial cells, supporting a difference in cell lineage. There seems to be no apparent difference between the urothelium of the trigone compared to the detrusor, in contrast to cells from the proximal urethra [9,32]. In this region, there is a transition from urothelium to a stratified or columnar epithelium accompanied by a lack of urothelial-specific differentiation markers. Taken together, present evidence suggests at least 3 urothelial lineages: 1) those of the ureter/renal pelvis, 2) detrusor/trigone and 3) bladder neck/proximal urethra [33]. The functional significance of these findings has yet to be determined.

4. ROLES FOR UROTHELIAL CELLS IN VISCERAL SENSATION

While urothelial cells are often viewed as bystanders in the process of visceral sensation, recent evidence has supported the view that these cells function as primary transducers of some physical and chemical stimuli and are able to communicate with underlying cells including bladder nerves, smooth muscle and inflammatory cells (**Figure 3**).

There are at least 3 lines of evidence that suggest that

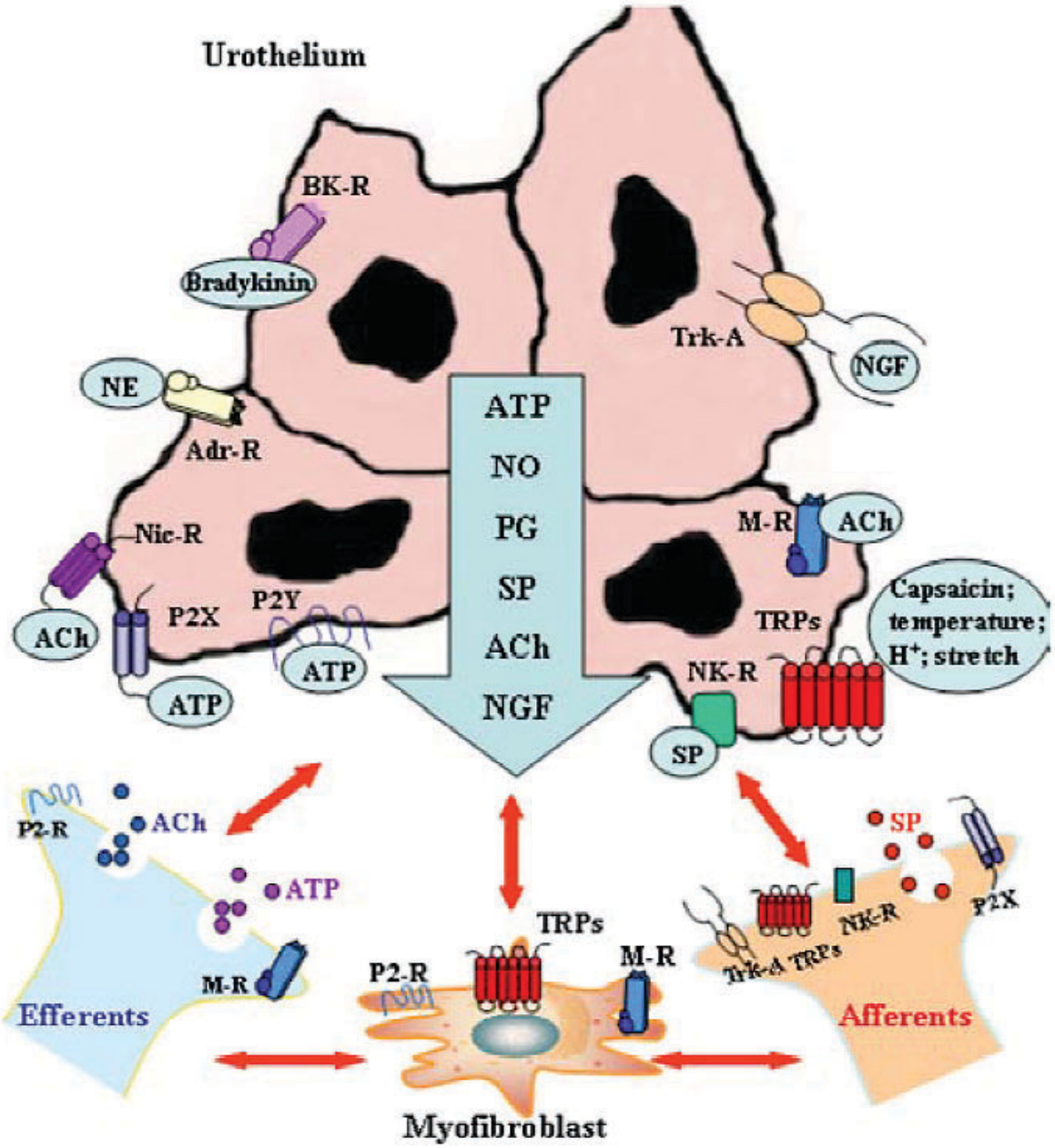


Figure 3 : Hypothetical model depicting possible interactions between bladder afferent and efferent nerves, urothelial cells, smooth muscle and myofibroblasts. Stimulation of urothelial receptors and channels can release mediators that target bladder nerves and other cell types; urothelial cells can also be targets for neurotransmitters released from nerves or other cell types. Urothelial cells can be activated by either autocrine (i.e. autoregulation) or paracrine (release from nearby nerves or other cells) mechanisms. Abbreviations: ACh, acetylcholine; AdR, adrenergic receptor; BR, bradykinin receptor; H+, proton, MR, muscarinic receptor; NE, norepinephrine; NGF, nerve growth factor; NR, neurokinin receptor; NicR, nicotinic receptor; NO, nitric oxide; P2R, purinergic 2 receptor unidentified subtype; P2X and P2Y; purinergic receptors; PG, prostaglandin; SP, substance P; Trk-A, receptor tyrosine kinase A, high affinity receptor for nerve growth factor; TRPs, transient potential channels.

urothelial cells participate in the detection of both physical and chemical stimuli. First, bladder nerves (afferent and efferent) are localized in close proximity, and some within, the urothelium [20, 34-36]. A second line of evidence suggesting that urothelial cells play a role in sensory function is the expression of numerous receptors/ion channels similar to that found in both nociceptors and mechanoreceptors. And finally, these cells secrete a number of transmitters or mediators capable of modulating, activating or inhibiting sensory neurons.

a) Urothelial-Neuronal Signaling

Recent studies have shown that both afferent and autonomic efferent nerves are located in close proximity to the urothelium. Peptidergic, P2X- and TRPV1- immunoreactive nerve fibers presumed to arise from afferent neurons in the lumbosacral dorsal root ganglia are distributed throughout the urinary bladder musculature as well as in a plexus beneath and extending into the urothelium [20,34]. (**Figure 4**) In humans with neurogenic detrusor overactivity intravesical administration of resiniferatoxin, a C-fiber afferent neurotoxin, reduces the density of TRPV1 and P2X3 immunoreactive suburothelial nerves, indicating that these are sensory nerves [37,36]. In addition, immunohistochemical studies have also revealed both adrenergic (tyrosine hydroxylase) positive as well as cholinergic (choline acetyltransferase, ChAT) positive nerves in close proximity to the urothelium [35].

A network of cells with morphologic characteristics similar to those of myofibroblasts or interstitial cells is also detected in the suburothelial space of the bladder in both humans and animals [39-41]. These cells, which are extensively linked by gap junctions and have close contacts with nerves, can respond to neurotransmitters, such as ATP released from nerves or urothelial cells, suggesting that they could act as intermediaries in urothelial-nerve interactions [40-42]. Thus the anatomic substrates for bidirectional urothelial-neural communication exist within the urinary bladder.

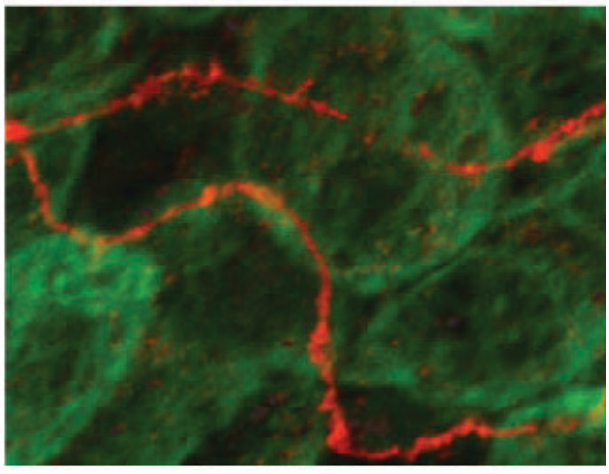


Figure 4 : Confocal image of the urothelium depicts afferent nerve fibers (red) located in close proximity to basal (green) urothelial cells.

b) Involvement of the Urothelium in "Sensing" Chemical and Mechanical Stimuli

The involvement of urothelial function in sensory signaling is suggested by the finding that urothelial cells express various receptors that are linked to mechano- or nociceptive sensations. Examples of neuronal "sensor molecules" (receptors / ion channels) that have been identified in urothelium include receptors for purines ($P2X_{1\text{-}7}$ and $P2Y_{1,2,4}$) adenosine (A_1, A_{2a}, A_{2b} and A_3), norepinephrine (α and β), acetylcholine (muscarinic and nicotinic), protease-activated receptors (PARs), amiloride- and mechanosensitive epithelial sodium channels (ENaC), bradykinin (B1 and B2), neurotrophins (p75, trkA, EGF family ErbB1-3), corticotrophin releasing factor (CRF1 and CRF2), estrogens ($Er\alpha$ and $ER\beta$), endothelins and various TRP channels (TRPV1, TRPV2, TRPV4, TRPM8 and TRPA1) [34, 43-51]. The expression of these various receptors enable the urothelium to respond to a number of "sensory inputs" from a variety of sources. These inputs include increased stretch during bladder filling, soluble factors (many found in the urine) such as epidermal growth factor (EGF), or chemical mediators/ peptides/ transmitters such as substance P, calcitonin gene-related peptide (CGRP), corticotrophin releasing factor (CRF), acetylcholine, adenosine or norepinephrine released from nerves, inflammatory cells and even blood vessels [5, 19, 20, 52, 53].

Various stimuli can lead to secretion of numerous chemical substances such as neurotrophins, peptides, ATP, acetylcholine, prostaglandins, prostacyclin, nitric oxide (NO) and cytokines that are capable of modulating, activating or inhibiting sensory neurons. [19, 20]. For example, urothelial-derived NO can be released in response to mechanical as well as chemical stimulation and may either facilitate or inhibit the activity of bladder afferent nerves [20, 54]. Release of various factors from the urothelium can also modulate the spontaneous activity of the underlying smooth muscle [42, 55].

The mechanism underlying release of chemical mediators from the urothelium, including whether all sensory "inputs" stimulate membrane turnover (i.e. vesicular exocytosis) is not well understood. What little is known about the roles and dynamics of membrane-bound cytoplasmic vesicles in urothelial cell physiology is derived from measurements of membrane capacitance and microscopy of fixed tissues and cells. For example, there is evidence that once released, ATP can act as an important autocrine mediator, which can induce membrane turnover as well as enhance both stretch induced exocytosis and

endocytosis [56]. Alterations in membrane turnover can not only increase apical surface area (as described above) but also regulate the number and function of receptors and channels at the cell surface.

1. Purinergic Receptors

Since the first report of distension-evoked ATP release from the urothelium there is now abundant evidence supporting a role for urothelially-derived release of ATP in autocrine and paracrine signaling within the lower urinary tract. ATP is released from both the apical and basolateral urothelial surfaces in response to bladder stretch and can act on P2X2 and P2X3 urothelial receptors to stimulate stretch-induced exocytosis [56]. (**Figure 5**) The expression of both P2X and P2Y receptors in nerve fibers and myofibroblasts in close proximitiy to the bladder lumen and the sensitivity of these cells to ATP suggests that basolateral ATP release from the urothelium may also influence function of myofibroblasts and bladder nerves [57, 58]; The amiloride-sensitive apical sodium channel, ENaC, may be involved in mechano-transduction by controlling basolateral release of ATP [59]. In addition, intercellular communication mediated by gap junctions in myofibroblasts could provide a mechanism for long-distance spread of signals from the urothelium to the detrusor muscle [42]. Adenosine is also produced and released by the urothelium, and may play important roles in modulating sensory afferent function and smooth muscle contraction [60].

2. TRP Channels

The ability of capsaicin to evoke NO release from rat urothelium, reported in 1998, provided the first, albeit indirect, demonstration that TRPV1 channels are expressed in urothelial cells and that urothelial cells and afferent nerves, which also express these channels, share a number of common properties [61]. This ion-channel protein is activated by capsaicin, as well as to moderate heat, protons and lipid metabolites

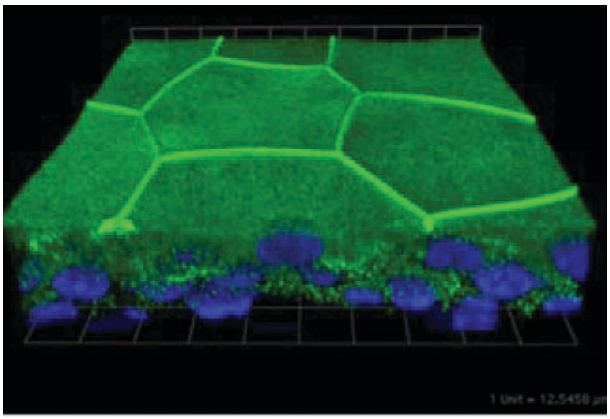


Figure 5 : Image is a 3-dimensional reconstruction (taken with a confocal microscope) depicting localization of P2X3 (green; nuclei blue) in the urothelium (from Wang et al., 2005).

such as anandamide (an endogenous ligand of both cannabinoid and vanilloids receptors) [62, 63] TRPV1-positive nerves are in close contact with urothelial cells [64, 65]. Activation of urothelial cells with capsaicin or resiniferatoxin can increase intracellular calcium and evoke transmitter (nitric oxide, NO or ATP) release. Similar to that in sensory neurons, urothelial-response to vanilloids are enhanced by low pH, blocked by TRPV1 antagonists and eliminated in TRPV1 null mice [66]. In afferent neurons, TRPV1 is thought to integrate/amplify the response to various stimuli and to play an essential role in the development of inflammation-induced hyperalgesia. It seems likely that urothelial-TRPV1 might participate in a similar manner, in the detection of irritant stimuli following bladder inflammation or infection.

Though TRPV1-null mice are anatomically normal, they exhibit a number of alterations in bladder function, including a reduction in stretch-evoked and hypotonic-evoked ATP release and stretch-evoked increase in membrane capacitance [66]. In addition, TRPV1 knockout mice have a higher frequency of low-amplitude, non-voiding bladder contractions suggesting the possibility of a small but ongoing role for TRPV1 in normal urine storage function. These relatively benign changes may result from TRPV1 expression not only in afferent nerves that form close contacts with bladder epithelial (urothelial) cells but also in urothelial cells themselves. (**Figure 6**) These findings demonstrate that the functional significance of TRPV1 in the bladder extends beyond pain sensation to include participation in normal voiding function, and is essential for mechanically evoked purinergic signaling by the urothelium.

3. Additional TRP channels

Much less is known about the involvement of other TRPs in bladder function or disease. TRPV4 which is a nonselective cation channel activated by a number of stimuli including heat, shear stress, changes in osmolarity and lipid ligands is expressed mainly within the epithelium of the urinary bladder [67]. While a definitive role for TRPV4 in bladder function has not been established, there is evidence that null mice exhibit impaired voiding responses and, intravesical instillation of a TRPV4 agonist in the rat triggers a novel voiding reflex which could regulate the late phase of micturition [68, 69]. In addition, in the awake ewe, TRPV4 may also be involved in a urethra to bladder reflex, proposed to facilitate bladder emptying [70]. Another member of the TRP family, TRPA1 (characterized as a thermoreceptor activated by noxious cold), is expressed in C-fiber afferents as well as urothelium and agonists to this channel induce bladder hyperreflexia [71]. Of interest is the finding that hydrogen sulfide, which may be formed during infection/inflammation, is an activator of TRPA1 [72].

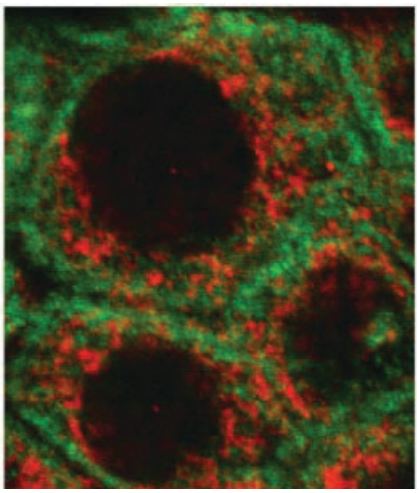


Figure 6 : TRPV1 expression detected within the rat urinary bladder urothelium. Image depicts basal cell TRPV1 (cy3, red) and cytokeratin (FITC, green) immunoreactivity.

4. Acetylcholine and the urothelium

There is evidence that the urothelium expresses the full complement of muscarinic receptors as well as enzymes necessary for the synthesis and release (except vesicular choline transporter) of acetylcholine. [53, 74]. Further, the urothelium is able to release acetylcholine following both chemical and mechanical stimulation [53]. Once released, urothelial-derived acetylcholine is likely to exert effects via a number of sites including smooth muscle, nerves as well as urothelial associated-muscarinic or nicotinic receptors, the latter that could contribute to feedback mechanisms modifying urothelial function. In addition, stimulation of urothelial-cholinergic receptors elicits release of mediators such as nitric oxide as well as ATP, which could alter bladder sensation by stimulating nearby sensory afferent nerves [73, 75, 76].

Thus, targeting muscarinic receptors and/or urothelial release mechanisms may play an important role in the treatment for a number of bladder disorders. In this regard, recent evidence suggests that botulinum toxins prevent the release of transmitters from the urothelium, which may suggest urothelial-released mediators contribute to sensory urgency [77].

5. CLINICAL SIGNIFICANCE OF THE SENSORY WEB

Defects in urothelial sensor molecules and urothelial-cell signaling are likely to contribute to the pathophysiology of bladder diseases. For example, a number of bladder conditions (BPS/IC, spinal cord injury (SCI), chemically-induced cystitis) are associated with augmented release of urothelial-derived ATP, which is likely to result in altered sensations or changes in bladder reflexes induced by excitation of purinergic receptors on nearby sensory fibers [9, 10, 47, 73]. ATP can also act in an autocrine manner that would act to facilitate its own release from urothelial cells [10]. Once released, ATP can alter the threshold for activation of ion channels such as TRPV1. This novel mechanism, which likely reflects activation of intracellular protein kinases and phosphorylation of the TRPV1 channel, represents a means by which large amounts of ATP released from damaged or sensitized cells, in response to injury or inflammation, could trigger the sensation of pain. Changes in epithelial signaling/barrier function would not be unique to the urinary bladder. For example, airway epithelia in asthmatic patients as well as keratinocytes in certain types of skin diseases also exhibit a number of similar abnormalities and compromised repair processes [78-80]. This is particularly relevant given the high incidence of associated diseases that can include both visceral and somatic conditions, many of which exhibit a shared loss of epithelial barrier function. Taken together, epithelial cells can respond to a number of challenges (including environmental pollutants and mediators released from nerves or nearby inflammatory cells) resulting in altered expression and/or sensitivity of various receptor/channels as well as changes in release of mediators, all of which could impact function.

II. AFFERENT NEURONES

There have been a number of new developments in this area since the last consultation as a result of the introduction of *in vitro* preparations, knockout animals and an increasing influence of molecular techniques. Up until recently most experiments have taken place on cats and rats, but several studies have been reported in mice and guinea-pigs, mainly using *in vitro* preparations. These involve recordings from the pelvic or hypogastric (lumbar splanchnic) nerves at peripheral sites near the bladder; hence the conduction velocities of the single units, which provided information relevant to the degree of myelination, have not been measured. These preparations have however been able to increase the knowledge of the location of afferent endings within the bladder wall and some of the physiological/pharmacological properties in relation to synaptic transmission at these nerve endings. Drugs that are toxic in the whole animal have also been used in isolated preparations, and some authors have used drugs to minimise the smooth muscle movements and the generation of inflammatory mediators; the latter are useful in the analysis, but may give rise to differences in the properties studied either *in vivo* or *in vitro* in the absence of such agents.

1. PROPERTIES OF BLADDER AFFERENT NEURONS

Afferent axons in the pelvic, hypogastric (lumbar splanchnic) and pudendal nerves transmit information

from the lower urinary tract to the lumbosacral spinal cord [81-83] and studies in several species including cats, rats and mice have shown some similarities in properties.

The most sensitive afferents are excited by a physiological increase in volume and by detrusor contractions: it is believed that these low threshold afferents have small myelinated axons, are A-delta fibres (which are larger in diameter and conduct action potentials more rapidly than C-fibres) and that their endings are located in the detrusor smooth muscle. They have been called 'in series tension receptors' (84) because they are excited by bladder wall tension caused either by distension or by contraction, and neurons with this range of conduction velocities are less likely to contain peptides.

There have been a number of systematic studies recently in mice and guinea pigs that have provided detailed information about the classes of receptors in the pelvic and hypogastric nerves (**Figure 7**). In the mouse, it seems there may be at least four classes of mechanosensitive afferents, which include myelinated and unmyelinated fibres, and distributed in the serosa, muscle and urothelial layers of the organ; one class has mechanosensitive endings in both the muscle and urothelial layers. The lumbar splanchnic nerves contain principally serosal and muscular afferents whereas all four classes of afferents are present in the pelvic nerve (63% of which are muscular afferents) [85]. Another study found endings in the urothelium and in the muscle layers, as well as stretch-insensitive afferents and chemosensitive afferents [86]. The small myelinated afferents are involved in two processes: (a) sensing bladder volume, and (b) reinforcing reflex function by monitoring the contractile state of the detrusor. In particular these afferents, which form the most sensitive distension receptors, are most probably responsible for the sensation of fullness, and mediate the normal micturition reflex that involves a spinobulbospinal pathway that passes through the brainstem.

The unmyelinated afferents contain peptides and most appear to terminate within the lamina propria and within the transitional epithelium itself. Many of these afferents discharge within the higher range of physiological bladder volumes, and are not usually sensitive to detrusor contraction, possibly because only the former causes stretch of the urinary epithelium. The C-fibres in the urothelium and lamina propria contain peptides such as substance P and CGRP, which is a characteristic of one subgroup of afferent C-fibres. These and other C-fibre afferents may mediate the spinal C-fibre micturition reflex seen following cord transection in the cat [87]. It is not

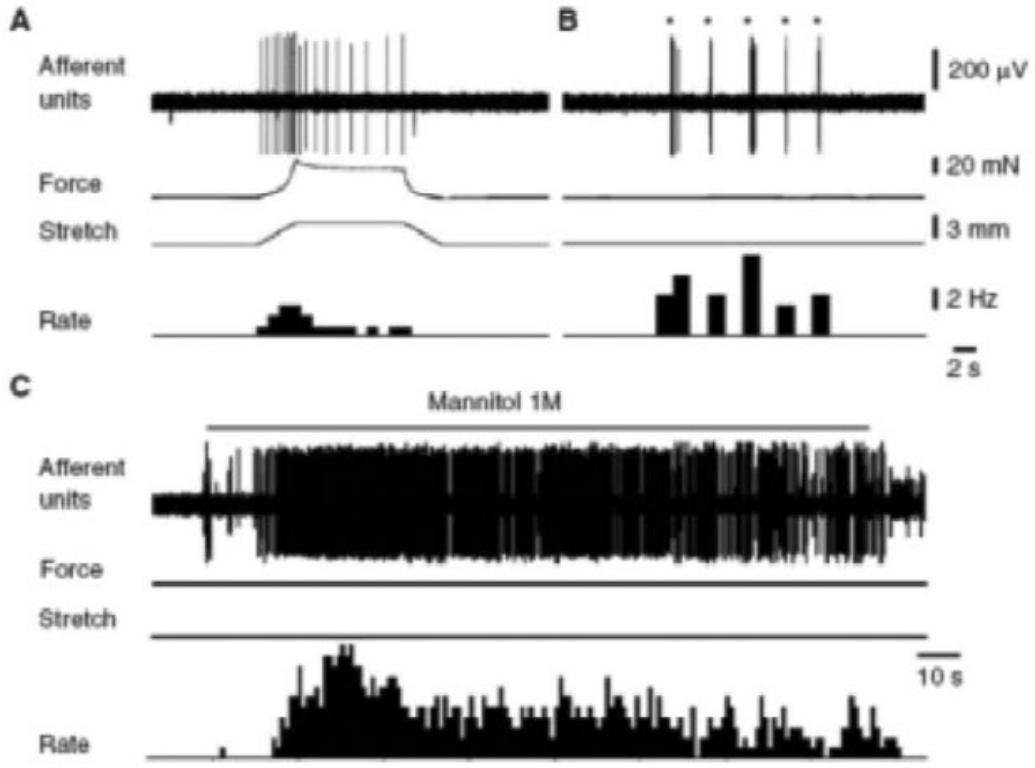


Figure 7 : Tension-mucosal mechanoreceptors. (A) Response of tension-mucosal mechanoreceptors to fast 3 mm stretch at 1000 µm's, held for 10s. (B) Response of the same unit to mucosal stroking with a 0.1 mN von Frey hair (five strokes, indicated by asterisks). (C) Activation of the same tension-mucosal unit by mannitol (1 M) applied to its receptor field in the mucosa.

clear whether they also contribute to normal voiding in this species, but there is increasing evidence that they may be involved in normal bladder control in rats and mice. In rats and mice, these high threshold units respond to a range of intravesical pressures that overlap with the sensitivity of the low threshold units, so that these together cover the spectrum of pressures and volumes seen physiologically, and these may contribute to spinal automatic micturition mediated by the sacral cord.

A third group of unmyelinated bladder afferent axons does not respond to normal distending volumes but only become active during chemical irritation of the bladder, including high osmolality and high potassium solutions and during inflammation, when they behave like the high volume sensing C-fibres. These have been demonstrated in cats and rats, and are usually called 'silent afferents' (meaning that the last group do not respond to normal distensions, but can become mechanosensitive in inflamed or over-distended tissues). Thus it would be unwise to infer function simply on the basis of conduction velocity. This group of afferents also appears to be sensitive to ATP.

Ultrastructural studies of nerves in the human bladder have found only unmyelinated nerves in the urothelial and immediate suburothelial layer, the first small myelinated nerves appearing only close to the smooth muscle layers [88]. Whether or not the suburothelial nerves become myelinated as they pass towards the serosal surface cannot be ascertained from this study but it would be inadvisable to make deductions about the relative number of C and A-delta fibres in the human based on these observations. **Table 1** shows the properties of afferent fibres, classified according to their volume thresholds.

2. UROTHELIAL AFFERENTS

Reference has already been made to the presence of CGRP-containing afferent endings that branch beneath and within the lamina propria, and within the urothelium itself. These axon collaterals can release neurotransmitters on to the various tissues in the lining of the bladder, including blood vessels, smooth muscle, urothelium, connective tissue cells, mast cells and other neurones. In addition there is evidence in the human bladder that intramural neurones receive axonal contacts from axon collaterals that contain the peptides characteristic of primary afferents (see the section on ganglia, and on integrative physiology).

The plexus of afferent nerves is thickest in the neck of the bladder and in the initial portion of the urethra, and it becomes progressively less dense in the adjacent regions. It does not extend beyond the equatorial region, and therefore the lamina propria of the cranial region of the bladder has no afferent axons. In contrast, the afferent innervation of the musculature is more diffuse, and appears uniform throughout the bladder. CGRP-immunofluorescence in urothelial afferent axons is enhanced in the surviving axons 5 days after contralateral denervation, a change which may be an early sign of regeneration of these axons [89]. In the human bladder, CGRP together with Substance P and NKA occur only infrequently in nerves in the muscle but are moderately frequent in the suburothelial layer. Also in the human there appears to be another population of CGRP-containing fibres that co-localize with NPY and galanin and some of these synapse on intramural ganglia within the bladder [90-93]. There is also recent evidence that nerves cross the basal lamina and enter the basal layers of the human urothelium [89]. **Table 2** shows the properties of afferent nerve endings in different locations.

3. SENSITIVITY OF AFFERENT ENDINGS

The term afferent sensitivity refers to the gain of the afferent signal, i.e. the number of impulses that are fired by an afferent ending at any level of distension. Sensitizing mediators are able to increase the size of

Table 1. Properties of low and high threshold afferents from the bladder in cat, rat, mouse and guinea pig

Sensitivity to distension (Cat and Rat)	Natural Stimuli	Conduction Velocity in Cat and Rat	Peptides	Experimental Stimuli	Species	Inflammatory Mediators	P2X3 Agonist	Role of TRPV1	Pathway
Low Threshold (LT)	Distension and Contraction	Mainly A-delta (finely myelinated)	Peptides are relatively sparse	Distension (D) Probing (P) Tension (T) Stroke (S) Chemical (C)	Cat (D) Rat (D,C) Mouse (P,T, S, C) Guinea Pig (P,T,S,C)	Sensitization (M,R)	Increased firing rate	Normal bladder filling	Hypogastric pelvic
High Threshold (HT)	Distension; Some also respond to contraction	Mainly C (unmyelinated)	Many peptides	Distension (D) Probing (P) Tension (T) Stroke (S) Chemical (C)	Cat (D) Rat (D) Mouse (P,T,S,C) Guinea Pig (P,T,S)	Sensitization (M)	Increased firing rate, lower threshold	Nociception	Hypogastric Nerve
Silent (including Nociceptive) (SIL)	Insensitive to Distension unless inflamed	Mainly C (unmyelinated)	Many peptides	Distension (D)	Cat (D) Rat (D) GP (S,C)	Appearance of Mechano-sensitivity (R)	Appearance of Mechano-sensitivity	Nociception	Pelvic

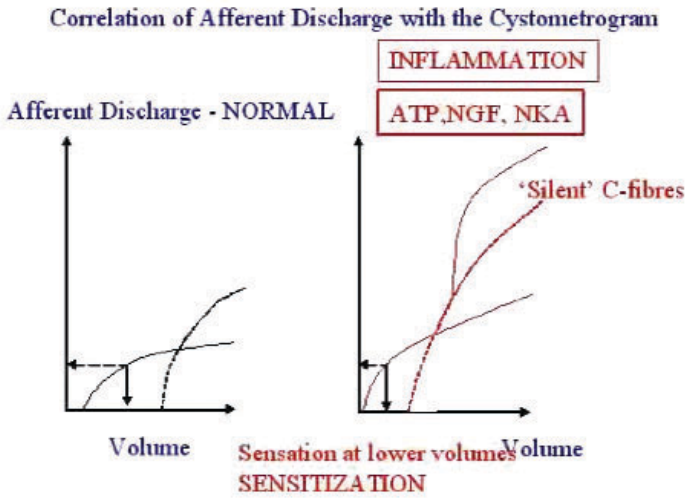


Figure 8 : Correlation of afferent discharge with the cystometrogram. Left: Diagram of afferent discharge at different volumes for A-delta fibres and mechanosensitive C-fibres. Right: Diagram showing the increased responses when the afferent endings are sensitized by inflammation or by administration of sensitizing agents such as ATP, Nerve Growth Factor or Neurokinin A. In addition to the increased discharge rates in the A-delta and C-fibres, a new population of 'Silent C-fibres' is recruited into activity.

Table 2. Location and sensitivity of afferents in the hypogastric (lumbar splanchnic) and pelvic nerves

Location within bladder wall (species)	Stimulus	Threshold	Hypogastric (HN)	Pelvic (PN)	Location within the bladder	Mediators/ inflammation
Muscle (M, GP)	Distension (D) Probing (P) Tension (T) Stroke (S) Chemical (C)	Low (GP) LT & HT (M)	30% (M)	63% (M)	All areas of the bladder	Not dependent on exocytotic synaptic transmission or ATP (GP) Inflammation (M,R)
Serosa (M, C)	D,P	LT & HT (M)	67% (M)	14% (M)	Predominantly at the bladder base	
Urothelial (M, GP)	P,S,T,(M); S,T,C (GP)	LT & HT (M)	3% (M)	9% (M)		Hypertonicity (GP)
Muscular-urothelial (M, GP)	P,S,T	LT & HT (M)	0% (M)	14% (M)		Inflammation (M)

the sensory signal (the frequency of impulse traffic) at a given level of distension, so the sensations that occur at a particular rate of firing in an afferent occur at lower bladder volumes if the afferent endings have been sensitized. (**Figure 8**).

The sensitivity of afferent endings may be influenced by the release of mediators from different cell types, including possibly the urothelium, myofibroblasts, nerve endings, smooth muscle, mast cells and other connective tissue cells. It is likely that many or all of these can release ATP, and some may release other mediators including nitric oxide, tachykinins (Substance P, Neurokinin A, Neurokinin B), growth factors (Nerve Growth Factor [NGF], Brain Derived Neurotrophic Factor [BDNF] and others) and other endogenous mediators such as nociceptin.

The similarity of the properties of the urothelial cells and the C-fibre afferents suggests that the most likely contender for a sensory cell may be a urothelial cell, but it is clear that the afferent endings themselves respond to a variety of stimuli, and that surrounding cells may simply enhance the gain of the transducer. The following paragraphs refer to some of the mediators that can sensitize bladder afferents.

a) ATP and P2X3 Receptors

Recent studies of mice have shown the P2X2/3 receptor, is present in small sensory neurones innervating the bladder, and that the effects of bladder distension on these sensory endings is markedly attenuated if the gene for the P2X3 receptor is deleted. Knockout mice that do not express this receptor exhibit

a marked urinary bladder hyporeflexia, characterized by decreased voiding frequency and increased bladder capacity, but normal bladder pressures [94, 95]. In addition, they have reduced pain-related behaviour in response to injection of ATP or formalin, and lose the rapidly desensitizing ATP-induced currents in their dorsal root ganglion neurons; they also have a reduction in the sustained ATP-induced currents in nodose ganglion neurons. Immunohistochemical studies localize P2X3 to nerve fibres innervating the urinary bladder of wild-type mice, and show that loss of P2X3 does not alter sensory neuron innervation density. Thus, P2X3 is critical for peripheral afferent pathways controlling urinary bladder volume reflexes, which take place at physiological volumes and pressures. Antagonists to P2X3 may therefore have therapeutic potential in the treatment of disorders of urine storage and voiding such as overactive bladder. In one recent study it was observed that humans with detrusor overactivity treated with botulinum toxin showed decreased levels of P2X3 and TRPV1 receptors in the bladder biopsies [96].

Some groups of bladder afferents appear to be sensitive to the release of ATP from the urothelium or other cells. In the last few years, the sensitivity of bladder afferents to ATP and mechanical stimuli has been studied intensively in the rat and mouse using protocols designed to avoid sensitization of the afferents [97-99]. In the rat, 90% of bladder afferent neurons gave persistent electrical responses to the P2X agonist α–β-methylene ATP that were inhibited by the P2X antagonist 2',3'-O-trinitrophenyl-ATP (TNP-ATP) which suggests that pelvic nerve afferents from the rat bladder express predominantly P2X (2/3) heteromeric receptors. In the mouse, Rong et al [99] found that the majority of the low threshold and nearly all the high threshold receptors were sensitized by α–β–methylene ATP, i.e. there was a reduction in the threshold and an increased peak activity during distensions. In addition some of the 'silent' afferents became mechanosensitive. The absence of sensitization in P2X3 knockout mice indicated that the responses were mediated by the P2X3 receptor. However there is a recent *in vitro* study in the guinea-pig that suggests that one group of afferents, the low threshold 'in series' tension receptors in detrusor muscle, are not sensitive to ATP [86].

b) Nitric Oxide

Nitric oxide (NO) is an important mediator that can be released from urothelium and from adjacent neurones. The detrusor however is not very sensitive to nitric oxide in contrast to the urethral outflow region where it effectively relaxes the urethral smooth muscle, suggesting an involvement of nitric oxide in the decrease in urethral pressure at the start of micturition [100].

NO may be involved in the control of afferent sensitivity, and we now know that NO may increase the activity of capsaicin-sensitive nerves within the bladder wall after spinal cord injury [101]. Basal release of nitric oxide has not been detected in the urothelium of the normal cat; however it is released in cats with feline interstitial cystitis [102], and from normal cats after the addition of agonists. Nitric oxide release from neurones depends on the enzyme nitric oxide synthase (NOS) and increased expression of neuronal NOS in bladder afferent and spinal neurones occurs following cord injury [103], and in bladder afferents following chronic bladder irritation with cyclophosphamide. There is also evidence that nitric oxide can inhibit the function of primary afferent neurons [104, 105]. This inhibitory effect may occur in the normal bladder because intravesical administration of a solution of oxyhemoglobin, a nitric oxide scavenger, induced bladder overactivity in the conscious rat [106] . The effect of oxyhemoglobin was reduced by pretreatment with ZD6169, a drug that suppresses capsaicin-sensitive bladder afferents, suggesting that oxyhemoglobin enhances afferent excitability.

Knockout mice that do not have neuronal NOS appear to have normal function in the lower urinary tract [107], and knockout animals that do not have inducible NOS do not show major abnormalities. However, the latter appear to need iNOS in the response to urinary obstruction [108].

c) Tachykinins: Substance P, Neurokinin A and Neurokinin B

The tachykinins are a group of neuropeptides that includes substance P and neurokinin A (which are produced by the same gene), and neurokinin B. They are found in small diameter afferent neurones, particularly within the C-fibre population, and may be released, along with other peptides, by afferent endings when these become active, e.g. during the axon reflex in skin. A similar event occurs in the bladder and is associated with the phenomenon of neurogenic inflammation. These peptides cause vasodilatation and an increase in capillary permeability, and are algesic agents.

In addition some tachykinins can sensitize sensory nerve endings. This view is based on (a) autoradiographic studies that show the disappearance of NK-2 receptors in the lamina propria in capsaicin-treated rats that are deficient in sensory nerves [109], (b) on studies in which afferents or dorsal root ganglia can be made hypersensitive using a NKA- analogue and other intravesical chemical stimuli such as high $[K^+]$ and high osmolality [110-112], and (c) the demonstration that the development of hypersensitivity to a number of sensitizing agents including high $[K^+]$ can be blocked by an NK-2 receptor antagonist [113, 114]. More recently it has been shown that rat dorsal root ganglion neurones are excited by NK2 agonists, but are inhibited by NK-3 agonists [115]. This NK2 action

is on L- and N-type Ca^{2+} channels, whereas the NK-3 action is only on the L-type channels. Both of these effects are blocked by inhibition of protein kinase C.

d) TRP (Transient Receptor Potential) Receptors

Ion channels that act as receptors because they are responsible for transient receptor potentials (TRP) generally work by opening non-specific ion channels. Some are sensitive to capsaicin and other vanilloids (TRPV), while others are associated with other mediators, including cinnamaldehyde (TRPA1) and Melastatin (TRPM). TRP channels are opened by a variety of physical and chemical stimuli including heat, cold, mechanical stress, voltage, hydrogen ion concentration and osmolality, as well as by specific ligands, such as capsaicin, melastatin and trans-cinnamaldehyde. The nerve endings that contain these channels are therefore often polymodal in their properties, one of the characteristics of non-myelinated sensory endings, and also in other cell types, including the urothelium. TRPV1, TRPV2, TRPV4, TRPM8, and TRPA1 have been described in different parts of the urogenital tract, and TRPV1 (the vanilloid receptor) has received special attention, particularly in relation to its role in bladder sensation [116].

The TRPV1 receptor is a cation channel expressed by nociceptive neurones and can also [117, 118] be activated by protons or temperature greater than 43 degrees C [119, 120]. Within the bladder, it may be that it is activated naturally by low pH, but such changes (e.g. in metabolic acidosis) are not usually associated with bladder pain. The expression of the TRPV1 receptor in sensory neurones is regulated by Nerve Growth Factor (NGF), and stimulation of the TRPV1 receptor with capsaicin causes the release of CGRP [121]. Capsaicin and resiniferatoxin act on unmyelinated afferent fibres throughout the body, but it is also clear that capsaicin can act on the urothelium by binding to TRPV1 receptors. Capsezepine is a blocker of this receptor and it has been found that nitric oxide (NO) release and the increase in intracellular Ca^{2+} induced by capsaicin are blocked by this antagonist. In addition to capsaicin and resiniferatoxin, a new agonist, piperine, has been shown to activate TRPV1 receptors and produce bladder hyperactivity and activity of sensory nerves in this organ in the rat [117, 118]. Several groups have searched for endogenous ligands for the TRPV1 receptors, and anandamide, palmitoylethanolamide and nociceptin are three compounds that deserve a mention, although much more work needs to be done to elucidate their exact roles. Anandamide and palmitoylethanolamide (PEA) are endogenous cannabinoids (acting on CB-1 and CB-2 receptors respectively) that also are agonists of TRPV1 receptors [122, 123] and may act on peripheral perivascular sensory terminals in a manner that is antagonized by the capsaicin antagonist capsezepine. These agents can also cause the release of CGRP and Substance P by increasing intracellular Ca^{2+}, and have other actions, such as activation of G-proteins [124]. Both anandamide and PEA have been found to attenuate bladder hyper-reflexia induced by intra-vesical NGF [125-127]. The TRPV1 receptor seems to be important for normal bladder function and for excitation of low threshold distension-sensitive afferents in mice [128, 129] (**Figure 9**).

• Other TRP Receptors

The bladder-cooling reflex (induced by instillation of intravescial cold saline and termed the ice water test) is believed to be triggered by menthol-sensitive cold receptors in the bladder wall. Recently, this test has been used to distinguish sensory symptoms in patients with bladder pain syndrome and overactive disorders [130]. Because pain elicited in BPS patients was not accompanied by reflex detrusor contractions, this test may be of particular interest since the response of afferent nerves to cold (and to menthol) depends on a particular TRP receptor subgroup, TRPM8. In experiments in the guinea-pig bladder, the afferent nerves innervating the organ have been shown to express TRPM8, and the bladder cooling reflex is enhanced in the presence of menthol [131].

The TRPA1 channel is also expressed in dorsal root ganglion cells that innervate the rat bladder. Trans-cinnamaldehyde or allyl isothiocyanate, agonists of this receptor, cause bladder hyperreflexia and appear to act through C fibres that might be mechanoreceptive or nociceptive [132].

e) ORL Receptors

Nociceptin/orphanin FQ, another endogenous ligand that binds with the opioid receptor-like 1 receptor (ORL1 receptor, now also known as the 4th category of opioid receptors, OP4) has been shown to have naloxone resistant inhibitory effects on the micturition reflex. These actions are mediated at several sites including the capsaicin sensitive nerves in the bladder, and a central supraspinal site [133]. Nociceptin produces a long-lasting protection against capsaicin-induced desensitization of TRPV1 in afferent nerves, such that a chemoceptive micturition reflex could be repeatedly evoked by topical capsaicin in nociceptin-pretreated rats. This is in sharp contrast to the effects of nociceptin on the local response to capsaicin, which corresponds to the release of peptides from capsaicin-sensitive afferent neurons. Topical application of nociceptin onto the bladder serosa evokes a tachykinin-mediated contraction [133]. These results suggest that the afferent and 'efferent' functions of capsaicin-sensitive primary afferent neurons in the rat bladder are differentiated by nociceptin, and that nociceptin has a significant action on afferent sensitivity.

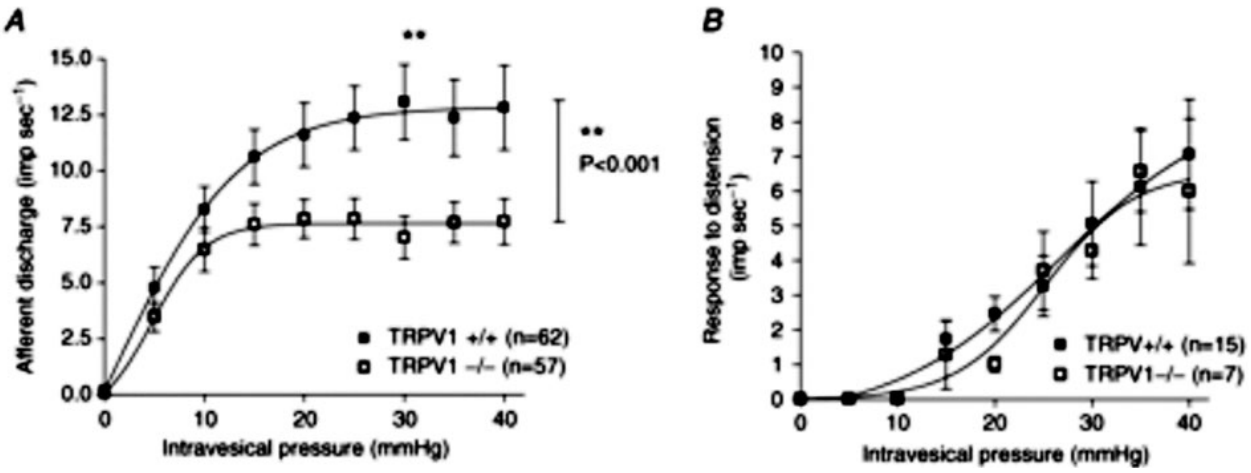


Figure 9 : Stimulus-response profile of low threshold, LT (A) and high threshold, HT (B) bladder afferents. Note that the LT afferents have a blunted response profile in the TRPV1 -/- mice compared to wildtype (P<0.001, 2-way ANOVA and Bonferroni test). The response profile of HT afferents is unchanged (from Daly et al., 2007).

In humans nociceptin elicits a strong acute inhibitory effect on the micturition reflex in patients with a neurogenic bladder [134]). This was in contrast to the placebo, and led to the conclusion that nociceptin and other orphan peptide receptor agonists may be useful in future as drugs for the treatment of neurogenic urinary incontinence.

Local administration of kappa-opioid receptor agonists by intra-arterial injection attenuated the responses of pelvic nerve afferents to high pressure distension of the urinary bladder [135]. These agonists had essentially the same effects whether the bladder was inflamed or not. The conclusion was that the ability of kappa opioid agonists to attenuate the responses of afferents to large bladder distensions indicated a potential use for peripherally acting kappa opioid receptor agonists in the control of urinary bladder pain.

f) Neurotrophins

1. Nerve Growth Factor

(NGF; neurotrophin-1), the first of a group of growth factors called neurotrophins, is produced in larger quantities in humans with detrusor overactivity [136], BPS/IC and bladder cancer [137] , in rats with inflamed bladders [138], spinal cord injury or chemically induced cystitis [139] or bladder outlet obstruction [140], in diabetic rats [141] and a number of other states. This protein is known to sensitize myelinated and unmyelinated afferents from the bladder [142, 143] and it is involved in the production of referred pain in bladder inflammation [144]. It also appears to stimulate the expression of the vanilloid receptor TRPV1 [121], and there is a suggestion that increased NGF levels resulting from intrathecal injection of NGF can induce a decrease in A-type K^+ current density in the afferent pathway that may influence the emergence of bladder overactivity [145].

Recent studies in an animal model for BPS/IC termed feline interstitial cystitis (FIC) in which TRPV1 responses to capsaicin were measured in lumbo-sacral dorsal root ganglion neurones have suggested that affected neurones are increased in size and exhibit exaggerated responses to capsaicin that may be associated with enhanced activity of endogenous protein kinase C [146]. A further study from the same group suggested that the abnormal activity of afferent neurones from cats with feline interstitial cystitis may be due to changes in the behavior of K^+ currents which are restricted to capsaicin-sensitive neurones [147].

2. Brain Derived Neurotrophic Factor (BDNF)

levels in the urinary bladder and some other epithelia are higher than those found in the brain or skin [148]. In situ hybridization experiments showed that BDNF mRNA was made by visceral epithelial cells, in several types of smooth muscle, and in neurons of the myenteric plexus. However the receptors from BDNF (trkB and p75[NTR]) are not present on the urothelium but are present in neurons of the peripheral nervous system. Hence it is thought that in the bladder this neurotrophin is produced by the urothelium and can act on the afferent nerves. The mRNAs for NGF, BDNF and neurotrophin-3 all increase within 2 hours of bladder inflammation in the rat, and this increases expression may contribute to sensory and reflex hyperactivity [138]. Inflammation of the colon also appears to induce up-regulation of CGRP and TrkB (suggesting an involvement of BDNF) in bladder afferent neurons, and suggests that there may be cross sensitization of bladder afferent pathways by colonic inflammation [149]. In spinal cord injury there is also an increase in BDNF and galanin in the dorsal root ganglia and spinal segments below the lesion; interestingly NGF expression was reduced below the level of the lesion [150].

• NGF and TTX-Resistant Na^+ Channels

Sensitization of afferents appears to be an important mechanism that leads to reflex hyperexcitability, and a number of studies have linked the tetrodotoxin (TTX)-resistant sodium channel, sometimes known as Nav1.8 to this process. A number of sensitizing agents including NGF are known to induce increased expression of this membrane channel; this appears to be sufficient to change the properties of afferents so as to lower the threshold for firing of bladder (lower volume threshold for voiding) and induce spontaneous and burst firing (overactive contractions, urgency) [142]. TTX-resistant Na channels ($Na_V1.8$ and $Na_V1.9$) have been found in SP/CGRP immunoreactive small DRG giving rise to C-fibers supplying the bladder [151, 152]; these also express the trkA receptor, which binds NGF and is necessary for its action. Plasticity of TTX-sensitive and TTX-resistant Na^+ channels (Na_V 1.8 and Na_V 1.9) occurs in these neurones after spinal cord injury, and a decreased expression of Na_V 1.8 channel immunoreactivity and a small increase in Na_V 1.9 channel immunoreactivity in bladder DRG neurons can be observed [153, 154].

The dependence of the sensitization of these afferent neurones and the occurrence of overactivity on NGF and its actions on the Na_V 1.8 channels has been shown in experiments using immunoneutralization of NGF or antisense oligonucleotide treatment to reduce the expression of these channels in sensory neurons [151, 155]. More recently studies on ralfinamide, a drug that interferes with TTX-resistant sodium channels, indicate that this drug reduces inflammatory and neuropathic pain as well as bladder overactivity in rats. The ability of ralfinamide to reduce capsaicin-induced hyperexcitability and tonic activity of rat afferent neurones appears to be due to its action as a sodium channel antagonist [156].

In clinical studies the local anaesthetic lidocaine and the oral Na^+ channel blocker, mexiletine, which operate by reducing excitability in sensitized neurones have been used to treat urge incontinence and hyper-reflexic conditions [157-162] with variable degrees of success.

4. SPINAL CORD

This section is concerned with the central projections of the primary afferent neurons. Axonal tracing experiments have been performed in many animal species [163, 164] and have localized the segmental distribution and spinal termination of afferent pathways in the pelvic, hypogastric and pudendal nerves. The primary afferent cell bodies of the pelvic and pudendal nerves are contained in lower lumbar and sacral dorsal root ganglia depending on species; whereas afferent innervation in the hypogastric arises in the rostral lumbar dorsal root ganglia. The central axons of the dorsal root ganglion neurons carry the sensory information from the lower urinary tract to second order neurons in the spinal cord.

Trans-ganglionic transport of axonal tracers has identified the spinal projections and terminal fields of visceral and somatic primary afferent neurons. The dorsal commissure (DCM), superficial dorsal horn and sacral parasympathetic nucleus (SPN) all contain interneurons with rostral projections that are activated during noxious [165, 166] or non-noxious stimulation [167] of the rat bladder and the urethra. These neurons are the site of origin of ascending pathways that project to various structures in the brainstem via spinal pathways that include the dorso-lateral funiculus [168, 169]. In humans spinal tractotomies for intractable pelvic pain provide the only insight available as to the organization of spinal pathways involved in bladder control in man [170].

Visceral afferent fibers of the pelvic [171] and pudendal [163] nerves enter the cord and travel rostrocaudally within Lissauer's tract, and transversely around the dorsal horn via the lateral (LCP) and medial collateral pathways (MCP) to reach the deeper layers of the spinal cord. Within the spinal gray matter, the LCP and MCP provide a dense innervation to laminae I, V, and VII and the dorsal commissure. Muscle and cutaneous afferents in the pudendal nerve terminate in different regions of the cord.

Afferents from the Urethra, Bowel and Genital Organs

Studies have demonstrated that electrical stimulation of urethral afferent fibers when the bladder is full can evoke strong detrusor contractions sufficient for voiding in intact cats [172, 173] as well as acute spinalized cats [174]. Similarly, using minimally invasive methods to apply electrical stimulation within the proximal urethra via a catheter-mounted electrode, it has been shown that reflex bladder contractions can be generated in humans with complete paraplegia but these do not seem to produce efficient voiding can be evoked in chronic SCI cats by stimulation of an excitatory pudendal to bladder spinal reflex [176].

The excitability of the micturition reflex can be influenced by other sacral afferent pathways [177], including facilitatory effects resulting from stimulation of urethral afferents, and inhibition of bladder activity by stimulation of the dorsal nerve of the clitoris in keeping with known interactions from the vagina and colon [178]. Stimulation of urethral afferents by fluid flowing through the urethra can facilitate the micturition reflex; however contraction of the urethral sphincter resulted in inhibition of bladder motility [179].

Excitability of spinal neurons receiving afferent input from the bladder can also be modulated by input from other pelvic structures such as the colon [175, 180, 181].This convergence of sensory information from a number of pelvic organs can occur at the level of the

spinal cord. In addition, the expansion of primary axon terminals within the spinal cord can also play a role in altering bladder reflexes. For example, it has been shown that expression of a number of peptides including CGRP, VIP as well as PACAP is altered in primary afferent terminals and may correlate with changes in bladder function following SCI [182-184].

Glutamate is an important excitatory transmitter in the afferent limb of the micturition reflex, and mediates its effects by means of both NMDA and nonNMDA receptors. This conclusion is based on studies of C-fos expression and the transmission of afferent activity rostrally, and the depressive effects of both NMDA and nonNMDA glutamatergic receptor antagonists [185, 186].

Bladder afferent neurons contain a number of peptidergic neurotransmitters, and the central distribution of bladder afferent terminals and peptidergic immunoreactive fibers is quite similar. There has been considerable interest in the role of tachykinins in the micturition reflex [187] and in nociception. Intrathecal treatment of adult rats with intrathecal capsaicin can result in a reversible block of the micturition reflex [188]. Further, while normal micturition is not altered following ablation of NK1-R expressing SC neurons using SSP-saporin, the response to a nociceptive stimulus was significantly reduced. These and other studies [188, 189] suggest that substance P and its receptors may play a part in transmission of bladder nociceptive responses at the first synapse in the micturition reflex.

5. SPINAL CORD GLIAL CELLS AND MODULATION OF PELVIC AFFERENTS

a) Anatomy and Function of CNS Glia

The nervous systems of animals are generally composed of two cell types: neurons, which propagate electrical currents and function as the signaling moieties of the nervous system, and glia, the functions of which are far less understood [190]. Central nervous system (CNS) glia, (which greatly outnumber the neuronal component [191, 192]), comprise ependymal cells, oligodendrocytes, astrocytes and microglia.

Astrocytes, (similar to neurons and oligodendrocytes) are ectodermal in origin. Two distinct types are described based on their morphology: fibrous astrocytes, which have long thin processes and are commonly found in white matter, and protoplasmic astrocytes, which have short thick processes and are typically found in grey matter. In the reactive state, protoplasmic astrocytic processes become more pronounced [193].

Astrocytes contribute to the regulation of the microenvironment in which neurons develop and function, maintaining a tight control on local ion (notably K^+) and pH homeostasis. In addition, they are involved in the clearance of synaptically-released neurotransmitters, such as glutamate and GABA [194]. These multifunctional cells are key players in the 'tripartite synapse', which is composed of pre- and post-synaptic membranes and extra-synaptic astrocytic contacts; they have the potential to modify synaptic transmission and plasticity [195].

Microglia are considered to have a mesodermal origin and to enter the brain during the neonatal period; they are commonly considered to function as the resident macrophages of the CNS and are more abundant in grey than white matter. In the normal brain and spinal cord, they have a ramified morphology; when activated, such as when there is damage to the CNS, they rapidly retract their processes and migrate toward the site of injury, where they turn into macrophages and remove the debris [193].

While astrocytes and microglia present as two very different types of glial cell, it is now becoming increasingly known that they have functional similarities. Recent evidence has shown that spinal cord activation of either of the two cell types may be involved in both the development and maintenance of central sensitization in various chronic pain conditions. Thus, both astrocytes and microglia are attracting wide interest in the pain field [196].

b) Modulation of Neuronal Signaling by Spinal Cord Glia

At the level of the spinal cord (the first relay site in the transmission of nociceptive information from the periphery to the brain [197], dorsal horn glia may be activated by chemicals released from primary afferent terminals such as the neurotransmitters: SP, CGRP, NO, purinergic agents, glutamate, opioid peptides ,and the chemokines: fractalkine or neuractin. Activation may result in altered cell morphology, changes in receptor expression, or release of factors by astrocytes and microglia, which in turn can lead to changes in neuronal function and ultimately influence pain transmission [198]. There is evidence that microglia may mediate the activation of astrocytes seen in both somatic and visceral pain pathologies-the ' neuropathic pain triad ' [199] . Generally, microglial activation is transient, while astrocytic activation is much longer-lasting. However, activation of either of the two cell types promotes pain [196, 200].

While most reports on the contribution of glia to pain, study somatic rather than visceral forms of chronic pain, there is now increasing evidence pointing to a role for glia as key modulators during inflammatory pain. Microglia probably play a pivotal role in the initiation phase, while astrocytes are likely to contribute to the maintenance of the persistent pain state. There are reports in non- acute [201, 202] and acute [202] models of colonic irritation of altered glial cell morphology, in segments L6-S1 of the spinal cord .

In addition, in the naturally occurring chronic model of bladder pain syndrome/interstitial cystitis (BPS/IC) seen in the cat, a pronounced upregulation in immunointensity of astrocytic intermediate filament glial fibrillary acidic protein (GFAP) in dorsal horn regions which receive pelvic afferent input [202], has been reported. Preliminary findings from functional studies using primary cultures of astrocytes (**Figure 10**) isolated from lumbo-sacral region of cats with BPS/IC and normal cats (Hanna-Mitchell, Buffingtonm and Birder; unpublished data), point to significant differences in the physiology of astrocytes in regions of the spinal cord receiving pelvic afferent input following this pathology.

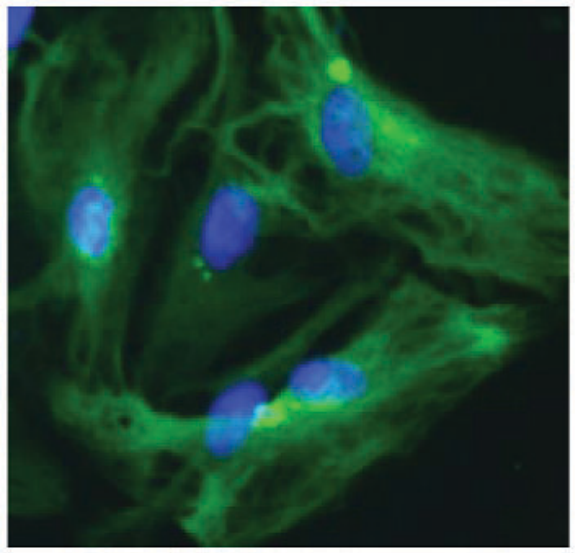


Figure 10: Primary culture of GFAP-immunoreactive astrocytes (8 days in vitro) isolated from cat lower lumbosacral spinal cord.

Together these findings indicate a pronounced activation of spinal cord astrocytes in animal models for BPS, which may plan an important role in the pain syndrome and open up new potential approaches for drug intervention.

III. NEURAL CONTROL OF FEMALE PELVIC FLOOR MUSCLES AND RHABDOSPHINCTERS

1. STRUCTURAL ELEMENTS OF THE PELVIC FLOOR

The pelvic floor [203] in women is a bowl-shaped structure comprised of bone, muscle, and connective tissue. The rim of the bowl is formed by the bones of the pelvic girdle (sacrum, ileum, ischium, and pubis). The “inside and bottom” of the bowl is lined with striated muscle: the iliococcygeus and pubococcygeus (which together comprise the levator ani) as well as the coccygeus, and puborectalis muscles. The muscles are attached to the bone and to each other with various connective tissue supports. These 3 components, bone, muscle, and connective tissue provide support of the pelvic viscera (i.e. rectum, vagina, and bladder) but also allow for excretory and sexual function.

The viscera, as well as striated muscles that serve as true sphincters - urethral and anal rhabdosphincters - are attached to the pelvic floor muscles and each other by connective tissue but do not attach directly to bone. In addition to the urethral and anal rhabdosphincters, striated perineal muscles associated with the viscera include the urethrovaginal sphincter, the compressor urethrae muscle, the ischiocavernosus, and bulbospongiosus muscles [203-205]. In contrast to the levator ani muscles described above, the rhabdosphincter and perineal muscles embryologically develop from the cloaca with a 2 week delay in striated muscular differentiation compared to the levator ani and other skeletal muscles [206, 207]and are completely separated from the levator ani muscles by connective tissue [204].

2. INNERVATION OF THE FEMALE LEVATOR ANI MUSCLES

The levator ani muscle of the pelvic floor is innervated by the levator ani nerve in human (Figure 11) [208], squirrel monkey [209-211], dog [212], cat (Karicheti and Thor, unpublished observations), and rat [213]. The levator ani nerve primarily arises from sacral spinal roots (e.g. S_3-S_5 in humans) and travels along the intrapelvic face of the levator ani muscle with a high degree of variability in branching patterns [208]. In humans, there is some controversy whether or not the pudendal nerve also innervates the levator ani muscle [214, 215]. This is not the case in other species (rat, cat, dog, squirrel monkey) where hodological studies show a marked loss of levator ani muscle mass and a decrease in levator ani myocyte diameter following transection of the levator ani nerve [211, 213] but no change in levator ani muscle mass or myocyte diameter following pudendal neurectomy [211, 213], 2) the existence of only a single motor endplate zone at the point of levator ani nerve insertion into the levator ani muscles [211, 213], 3) absence of contractions of levator ani muscles upon electrical stimulation of pudendal nerve efferent fibers (Thor and Karicheti, unpublished observations) and 4) phenotypically distinctive motor neuron labeling following application of nerve tracers to the pudendal and levator ani nerves [212, 216-221], respectively. All of these divergent techniques support the conclusion that only the levator ani nerve innervates the levator ani muscles with no significant contribution from the pudendal nerve in non-human species.

In consideration of the vast phenotypic differences between rhabdosphincter pudendal motor neurons in Onuf’s nucleus and levator ani motor neurons (**Figure 11** and **Table 3**), the likelihood of pudendal

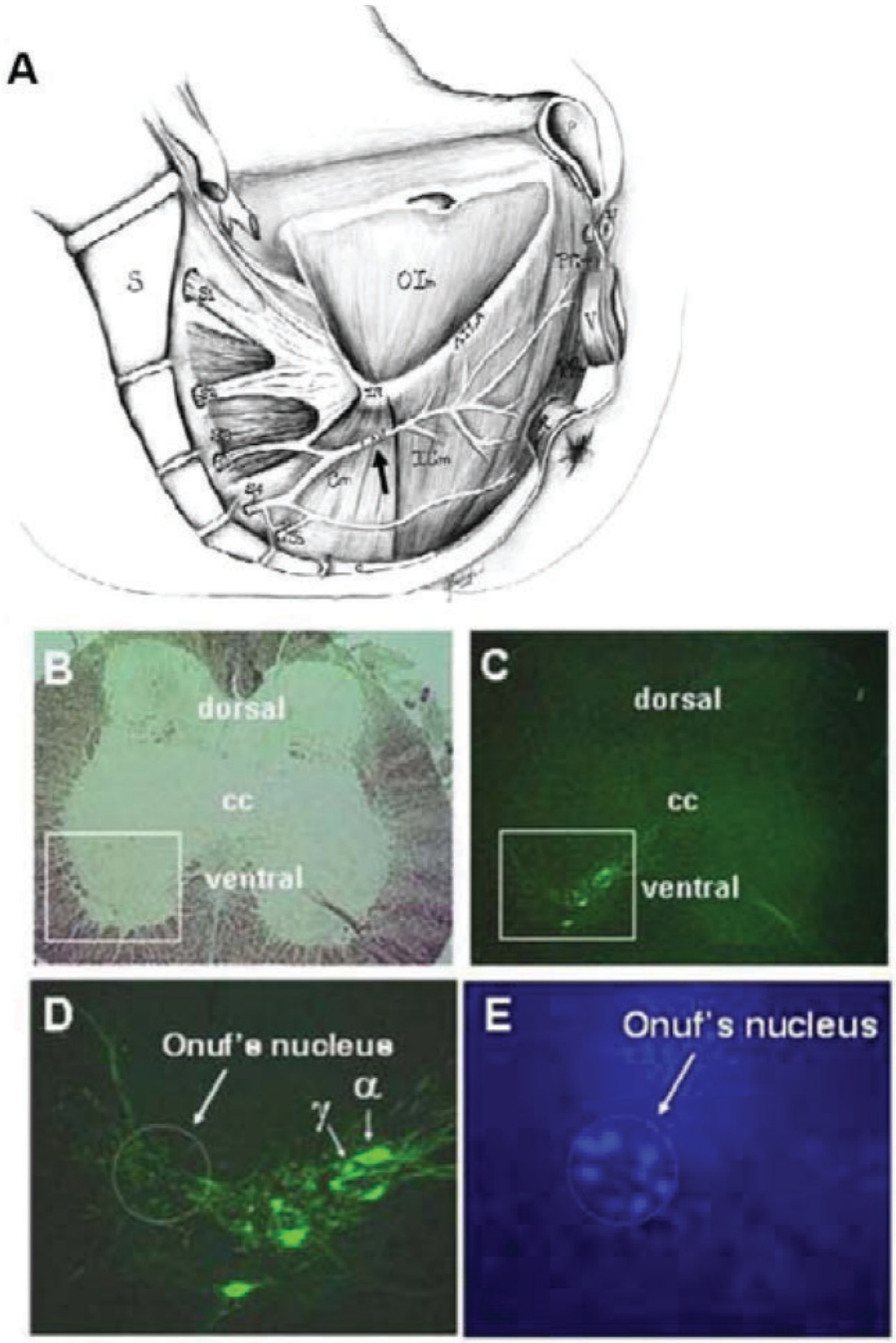


Figure 11 : A) Illustration of the course of the levator ani nerve in a left hemipelvis, sagittal view (arrow points to levator ani nerve). Abbreviations: S, Sacrum; S1-S5, sacral foramina; Cm, coccygeal muscle; LAN, levator ani nerve; IS, ischial spine; ICm, iliococcygeal muscle; OIm, obturator internal muscle; PCm, pubococcygeal muscle; PRm, puborectal muscle; ATLA, arcus tendinous levator ani; C, coccyx; V, vagina; U, urethra; R, rectum (from Barber, 2002). B-E) Photomicrographs of a single transverse section of sacral spinal cord from a squirrel monkey with pubocaudalis muscle injected with cholera toxin B (CTB) and the anal rhabdosphincter injected with fast blue. B) Bright field illumination shows cytoarchitecture of gray and white matter; white box indicates area shown in high power in panels D and E. C) Epifluorescent illumination showing CTB-labeled (bright green) levator ani motor neurons; white box indicates area shown in high power in panels D and E. D) High power photomicrograph of boxed area in panels B and C using epifluorescent illumination to show CTB-labeled levator ani motor neurons. Note large α and small γ CTB-labeled motor neurons; also CTB-labeled processes extending from levator ani motor neurons into Onuf's neucleus (dashed circle) and the ventrolateral funiculus. E) Same area and section as panel D viewed with epifluorescent illumination to show fast blue-labeled (bright blue) anal sphincter motor neurons in Onuf's nucleus (dashed circle). Close apposition between CTB-labeled levator ani motor neuron processes and fast blue-labeled rhabdosphincter motor neurons were observed (from Pierce, 2005).

Table 3 : Neuronal markers preferentially associated with rhabdosphincer motor neurons in Onuf's nucleus.

	Marker	Reference
Transmitters	Enkephalin	(345)
	CGRP	(346)
	Somatostatin	(347)
	Norepinephrine	(289, 292)
	Serotonin	(290-292)
	Dopamine	(348)
	nNOS	(245, 248)
Receptors	NPY_2	(349)
	$5\text{-}HT_{2A}$	(328)
	$5\text{-}HT_{1A}$	(350)
	CRF	(351)
	D_2	(352)
	$5\text{-}HT_{2C}$	(322, 327)
	$5\text{-}HT_{5A}$	(328, 353)
	NK_1	(354)
	TRP-V2	(355)
Ion channel	$CaV_{1.3}$	(356)
Growth-related	P_{75} (growth factor receptor)	(357)
	CNTF receptor α	(358)
	GAP-43	(359)
	trkC	(360)

motor neurons innervating the levator ani muscle in humans seems very small. Similarly, the distinct embryological origins of levator ani muscles versus rhabdosphincter and perineal muscles (the latter originating from the cloaca [206, 207]), as well as a separate "compartmentalization" of the rhabdosphincter and perineal muscles by connective tissue [204], are parsimonious with distinctive "special somatic" motor innervation by the pudendal versus typical skeletal motor innervation by the levator ani nerve. Possibly, the confusing morass of small muscles (puborectalis, compressor urethrae, urethrovaginal sphincter, urethral and anal rhabdosphincter, ischiocavernosus, bulbocavernosus), blood vessels, connective tissues, and nerves in the perineal region makes dissection, identification, and nomenclature of specific nerve branches and muscles difficult in cadavers.

Indeed, this morass has led to confusion regarding even the nomenclature of the muscles themselves [204, 205] and, without extreme care, a contribution of the pudendal nerve to levator ani muscle innervation might be confused. Alternatively, there may be true species differences between humans and other mammals in regards to a minor pudendal nerve contribution to minor levator ani muscles (e.g. puborectalis). However, the ability to conduct precise experimental manipulations in animals provides a clear conclusion that the pudendal nerve does not innervate the major muscles of the pelvic floor, i.e. iliococcygeus, pubococcygeus, or coccygeus muscles to a significant degree.

The positioning of the levator ani nerve on the intrapelvic surface of the muscles may expose it to damage as the fetal head passes through the birth canal [208]. This positioning also puts it in a favorable position to be activated when current is applied with a St. Mark's electrode placed in the rectum. The positioning of the levator ani nerve, close to the ischial spine, also risks entrapment by sutures used for various suspension surgeries for pelvic organ prolapse (POP) and may account for dyspareunia, pelvic pain, and/or recurrent prolapse [222] associated with such surgery. Finally, since the ischial spine is used as a landmark for needle placement when applying a transvaginal "pudendal nerve" block [223], the possibility that this procedure also anesthetizes the levator ani nerve and pelvic floor muscles must be considered. These complicating factors of historically-accepted clinical concepts may explain why a possible innervation of the levator ani muscle by the pudendal nerve in humans remains controversial.

a) Levator Ani Motor Neurons and Sensory Innervation

Retrograde tracing studies involving injection of cholera

toxin B (CTB) into the levator ani muscle and fast blue into the anal rhabdosphincter muscle of squirrel monkeys [217] show that levator ani motor neurons are located in the sacral ventral horn (**Figure 11 B-E**) in a longitudinal column. In contrast to the very dense packing of sphincter motor neurons in Onuf's nucleus [216, 218-221], the levator ani motor neurons are more diffusely distributed. Furthermore in contrast to the uniform intermediate size of pudendal motor neurons, levator ani motor neurons (**Figure 11D**) show a bimodal distribution of large neurons (presumably α motor neurons) and small neurons (presumably γ motor neurons). These 2 sizes of motor neurons are in keeping with the presence of muscle spindles (whose intrafusal muscle fibers are innervated by γ motor neurons) in levator ani muscle [224, 225] and the absence of muscle spindles in rhabdosphincter muscle [224, 226-230]; consequently levator ani may exhibit Ia (muscle spindle) evoked monosynaptic stretch reflexes, whereas the EUS does not [231-234].

Levator ani motor neuron processes (dendrites or axon collaterals) project into two important areas in the sacral spinal cord [217]. The first, medial lamina VI, is a region where primary afferent fibers from muscle spindle and Golgi tendon organs terminate [235, 236]. This again suggests an important role for stretch-activated contraction of levator ani muscles. Importantly, the second projection of levator ani motor neurons is to Onuf's nucleus (**Figure 11D-E**), which contains rhabdosphincter motor neurons. These levator ani motor neuron processes form close appositions with sphincter motor neurons in both monkey [217] and rat (Thor, unpublished observations).

Presumably, these appositions reflect a neuroanatomical substrate for coordination of the rhabdosphincter and the pelvic floor muscles during micturition and defecation. Whether these projections are dendrites designed to receive common afferent input to levator ani and rhabdosphincter motor neurons, or if they are axon collaterals transmitting information from levator ani motor neurons to rhabdosphincter motor neurons to coordinate contractions is not known and will require electron microscopic or electrophysiological analysis to be resolved.

Dual-labeling immunohistochemistry combined with cholera toxin-B (CTB) tracing studies of the levator ani muscles of squirrel monkeys has shown that there are approximately 4 times as many afferent neurons versus motor neurons labeled following injection of tracer into the levator ani muscle [210]. About ? of the neurons were large, myelinated (i.e. RT-97 neurofilament positive) that did not contain the peptide transmitter CGRP, binding sites for isolectin-B4 (IB-4), or the growth factor receptor, TrkA, immunoreactivity. Of the remaining small RT97 negative neurons, approximately 50% contained CGRP, IB-4 binding sites, and TrkA. It is tempting to speculate that the large, myelinated afferent neurons signal proprioceptive information from muscle spindle and Golgi tendon organs, while the small peptidergic, IB-4, TrkA positive neurons signal nociceptive information. A possible role for the large sensory neurons, in addition to control of levator ani contractility, may involve regulation of bladder reflex pathways during on-going levator ani contractile activity; while the small peptidergic fibers may play a role in detrusor overactivity associated with pelvic floor trauma or nerve entrapment by sutures during suspension surgeries, in addition to classical sensation of muscle nociception.

Transganglionic transport of CTB from the primary afferent cell body to their synaptic terminals in the spinal cord was only seen in medial lamina VI of the lumbosacral spinal cord, an area of termination for large, myelinated proprioceptive terminals [235, 236]. Since many of the CTB-labeled primary afferent neurons were positive for CGRP, IB-4, and TrkA, the absence of transganglionic CTB labeling in the superficial dorsal horn is likely due to an inability of small primary afferent neurons to transport CTB rather than a true absence of levator ani nociceptive terminals in the region. Experiments with a tracer (e.g. horseradish Peroxidase, HRP) that is transported to nociceptive spinal terminals should be done to confirm this.

In contrast to the rhabdosphincters [224, 226-230], levator ani muscles have been shown to contain muscle spindles [224] and evidence has been presented that they contain Golgi tendon organs [225].

b) Role of the Levator Ani Innervation in Pelvic Organ Prolapse in Monkeys

Because the pelvic floor is responsible for providing support of the viscera, and because one might expect contraction of pelvic floor muscles to be necessary for adequate support, damage to the levator ani innervation and subsequent muscle flaccidity might be expected to promote pelvic organ prolapse (POP). To test this expectation, the levator ani muscles were bilaterally denervated in 7 squirrel monkeys [209], which is a species that shows age and parity correlated POP similar to humans [237]. Surprisingly, these monkeys showed no POP following this procedure for 2-3 years after surgery, despite showing statistically significant decreases in levator ani muscle mass and myocyte diameter. However, a slight increase in bladder and cervical descent with abdominal pressure was seen on MRI evaluation compared to nulliparous controls. Of possible significance was the finding that, after a single birth, 2 of 4 bilateral levator ani neurectomy animals showed POP, which is unusual. A larger study is needed to confirm whether levator ani nerve damage may accelerate parity-related POP.

Thus, these experiments indicate that, in the absence

of childbirth, the pelvic floor muscle plays a minor role in providing visceral support and suggests that the connective tissue plays the major role. Possibly after stretching of the pelvic connective tissue accompanying childbirth, the muscle plays a compensatory role. In support of this possibility, it was shown that levator ani muscle mass and myocyte diameter in monkeys with naturally occurring POP was equal to or greater than measurements in age-, parity-, and weight-matched monkeys without POP [209, 238], suggesting that the levator ani muscle attempts to prevent visceral descent by working harder, which induces hypertrophy.

3. INNERVATION OF URETHRAL AND ANAL RHABDOSPHINCTERS

At the level of the pelvic floor, the urethra and rectum are surrounded by intimately associated bands of striated muscle fibers; the urethral and anal rhabdosphincters, respectively. The muscles do not have "dedicated" attachments to skeletal structures and thus act as a true sphincters (i.e. contraction produces virtually no movement except constriction of the lumen). In addition, there are small, thin bands of striated muscle (compressor urethra, urethrovaginal sphincter, bulbocavernosus, and ischiocavernosus) that surround the urethra, vagina, and/or rectum and have connective tissue attachments to the perineal body [203].

Extensive studies of the urethral rhabdosphincter, anal rhabdosphincter, bulbocavernosus, and ischiocavernosus muscles have shown that these muscles are innervated by the pudendal nerve [208, 216, 218-221, 239, 240], which originates from the sacral roots and passes along the lateral surface of the internal obturator and coccygeus muscles, through Alcock's canal, to eventually approach these muscles laterally from the extrapelvic surface of the pelvic floor. The specific innervation of the smaller bands of muscles attached to the perineal body has not been characterized.

The nerve fascicles [241], as well as the motor nerve terminals and end plates [242] of the urethral rhabdosphincter, are preferentially located along the lateral aspects of the urethra in rat. Overlap, or crossing of the midline, between the left and right pudendal nerve terminal fields has been described in monkeys [243]. The urethral rhabdosphincter of both men and women contain neuronal nitric oxide synthase (nNOS) which is contained in a subpopulation (43%) of the muscle fibers, as well as nerve fibers, with concentration at the neuromuscular junction [244-246]. Additionally, nNOS has been localized to pudendal motor neurons which innervate the rhabdosphincter [247, 248]. nNOS is responsible for producing the transmitter nitric oxide. While NO is known to increase cGMP levels in many types of smooth muscle; its role in control of striated muscle and neuromuscular junction function is not well established [249]. An NO donor has been shown to reduce urethral pressures at the level of the rhabdosphincter [250], but it is difficult to conclude if the effect is on smooth or striated muscle.

Some evidence suggesting that the rhabdosphincter receives a "triple innervation" from somatic, parasympathetic, and sympathetic nerves [251] was based on anatomical studies that showed these fibers within the portion of the urethra that contains striated muscle fibers. However, this suggestion has been disputed by subsequent studies [252] that showed no physiological effects of autonomic nerve stimulation on striated sphincter function and showed that the autonomic fibers are only "passing through" the outer layer of striated muscle to reach the inner layers of smooth muscle.

a) Urethral and Anal Rhabdosphincter Motor Neurons and Sensory Innervation

Extensive hodological studies locate pudendal motor neurons that innervate the urethral and anal rhabdosphincters (and bulbocavernosus and ischiocavernosus) muscles along the lateral border of the sacral ventral horn in Onuf's nucleus (**Figure 11E**) in man [253] monkey [218, 254], dog [212], cat [219, 220], hamster [255] and guinea pig [256]. Studies in cats [219], monkey [218], and man [253] show that within Onuf's nucleus, urethral rhabdosphincter motor neurons occupy a ventrolateral position and anal rhabdosphincter motor neurons occupy a dorsomedial position within the confines of Onuf's nucleus.

However in other species, urethral and anal sphincter motor neurons are located in separate nuclei. In rat(216), anal sphincter (and bulbospongiosus) motor neurons are located medially in the ventral horn, just ventrolateral to the central canal; while the urethral sphincter (and ischiocavernosus) motor neurons are located in the same region as others species, i.e. along the lateral edge of the ventral horn. In the domestic pig [257] and Mongolian gerbil [258], anal sphincter (and bulbospongiosus) motor neurons are located just dorsolateral to the central canal.

Sphincter motor neurons are exceptionally different from motor neurons that innervate other striated (i.e. skeletal) muscles. They are densely packed within the confines of Onuf's nucleus and exhibit tightly-bundled dendrites that run rostrocaudally within the confines of the nucleus, and transversely into the lateral funiculus, dorsally towards the sacral parasympathetic nucleus, and dorsomedially towards the central canal [219, 259]. This is similar to dendritic projections of bladder preganglionic neurons [260]and very different from that of limb motor neurons, suggesting that EUS motor neurons and preganglionic neurons receive inputs from similar regions of the spinal cord. It has been suggested that the dense

packing and dendritic bundling of sphincter motor neurons may be related to their special sphincteric function and may facilitate simultaneous activation of all sphincter motor units. Recurrent axon collaterals [259] in the absence of recurrent inhibition [232, 261] suggests a recurrent facilitation that may also reinforce simultaneous activation. Because the rhabdosphincters are not attached to bone, efficient closure of the sphincter requires symmetrical contraction of all motor units simultaneously, i.e. partial contraction in one part of the "circle" would be defeated by relaxation in another region, like squeezing a balloon only on one side. It has been suggested that the arrangement of somatic motor nerve terminals bilaterally at dorsolateral and ventrolateral positions in the urethra provide symmetrical activation and force generation [242].

In addition to their unique morphology, rhabdosphincter motor neurons are also physiologically distinctive from skeletal muscle motor neurons in that they do not exhibit significant monosynaptic inputs [232], Renshaw cell inhibition [232], nor crossed disynaptic inhibition [261]. The passive membrane properties (e.g. high input resistance, low rheobase, short after-hyperpolarization, membrane bistability, non-linear responses to depolarizing current injection, which was recently reviewed [262]) are uniquely conducive to simultaneous, prolonged, tonic activity, in keeping with the anatomical and functional properties described above.

While various studies have characterized primary afferent neurons of the pudendal nerve [216, 219, 263], it is difficult to specifically characterize the sensory innervation of the sphincters per se since the pudendal nerve innervates many visceral structures (e.g. urethra, genitalia, rectum, vagina) in addition to rhabdosphincters. However, the absence of labeling of the largest sensory neurons in sacral dorsal root ganglia following application of tracers to the pudendal nerve suggests that the sensory innervation of the rhabdosphincters does not contain large fiber sensory endings such as muscle spindles, Golgi tendon organs, or Pacinian corpuscles. Furthermore, multiple investigators using various techniques have not found muscle spindles or Golgi tendon organs in the rhabdosphincters themselves, nor have they found evidence of the large myelinated nerve fibers (i.e. Type Ia and Ib) associated with muscle spindle and Golgi tendon organs in the pudendal nerve [224, 226-230]. This is consistent with the absence of small γ motor neuron axons (which innervate muscle spindles) in the pudendal nerve [228] and the absence of rhabdosphincter connections to bone by tendons.

Local injection of HRP targeted to the urethral and anal rhabdosphincters [219] produced labeling of the spinal terminals of primary afferent neurons in lateral and medial lamina I, the intermediate gray matter, and the dorsal commissure gray matter. Since the HRP likely spread beyond striated muscle fibers into other tissues of the urethra and rectum, it is possible that this afferent labeling is associated with the viscera and not necessarily the striated muscle fibers. However, since no labeling was seen in large diameter primary afferent neurons nor in terminals in medial lamina VI, an area where large diameter myelinated fibers of muscle spindle and Golgi tendon organs nerves terminate [235, 236] it is again reasonable to conclude that the rhabdosphincters are not significantly innervated by large myelinated nerve fibers typically associated with other striated muscle.

b) Segmental Activation of Urethral and Anal Rhabdosphincters

Rhabdosphincter motor neurons can be activated via segmental [231-233, 264, 265] and descending pathways [232, 266, 267]. The segmental inputs can be activated by stretch receptors and nociceptors in the bladder or urethra or genitalia [268-271]. Electrophysiological studies [231, 233, 234, 264, 265, 272] show that stimulation of either pelvic nerve or pudendal nerve afferent fibers can activate polysynaptic spinal segmental reflexes that can be recorded at a latency of about 10 msec from electrodes placed on pudendal nerve efferent fibers or inserted directly into the urethral or anal rhabdosphincter muscles.

Previously, the afferent inputs from the urinary bladder have been emphasized as being of primary importance for activation of the segmental reflex by pelvic nerve stimulation and is often referred to as the "guarding reflex" or "continence reflex". However, recent studies are placing greater emphasis on urethral afferent fibers [269, 271, 273] mediating spinal reflex activation of the urethral rhabdosphincter. It is tempting to speculate that the guarding reflex is actually activated more vigorously by urethral afferent fibers if urine inadvertently begins to pass through the bladder neck and into the proximal urethra, with a requirement for a rapid closure of the more distal urethral sphincter (i.e. guarding against urine loss) compared to simple bladder distension or increases in intravesical pressure.

The greater importance of urethral afferent fibers is also suggested by experiments where bladder afferent fibers are electrically stimulated. For example, [233] were unable to evoke pudendal nerve firing with electrical stimulation of pelvic nerve fibers close to the bladder in a high number of cats but were able to evoke firing with placement of electrodes more centrally on the pelvic nerve. In our lab (Karicheti and Thor, unpublished observations), we have also found that stimulating nerve bundles close to the bladder is often ineffective in producing a spinal reflex to the urethral rhabdosphincter and/or pudendal nerve, while in the same animals it is possible to consistently evoke a reflex by moving centrally on the pelvic nerve, which would include fibers from the urethra. Since the more

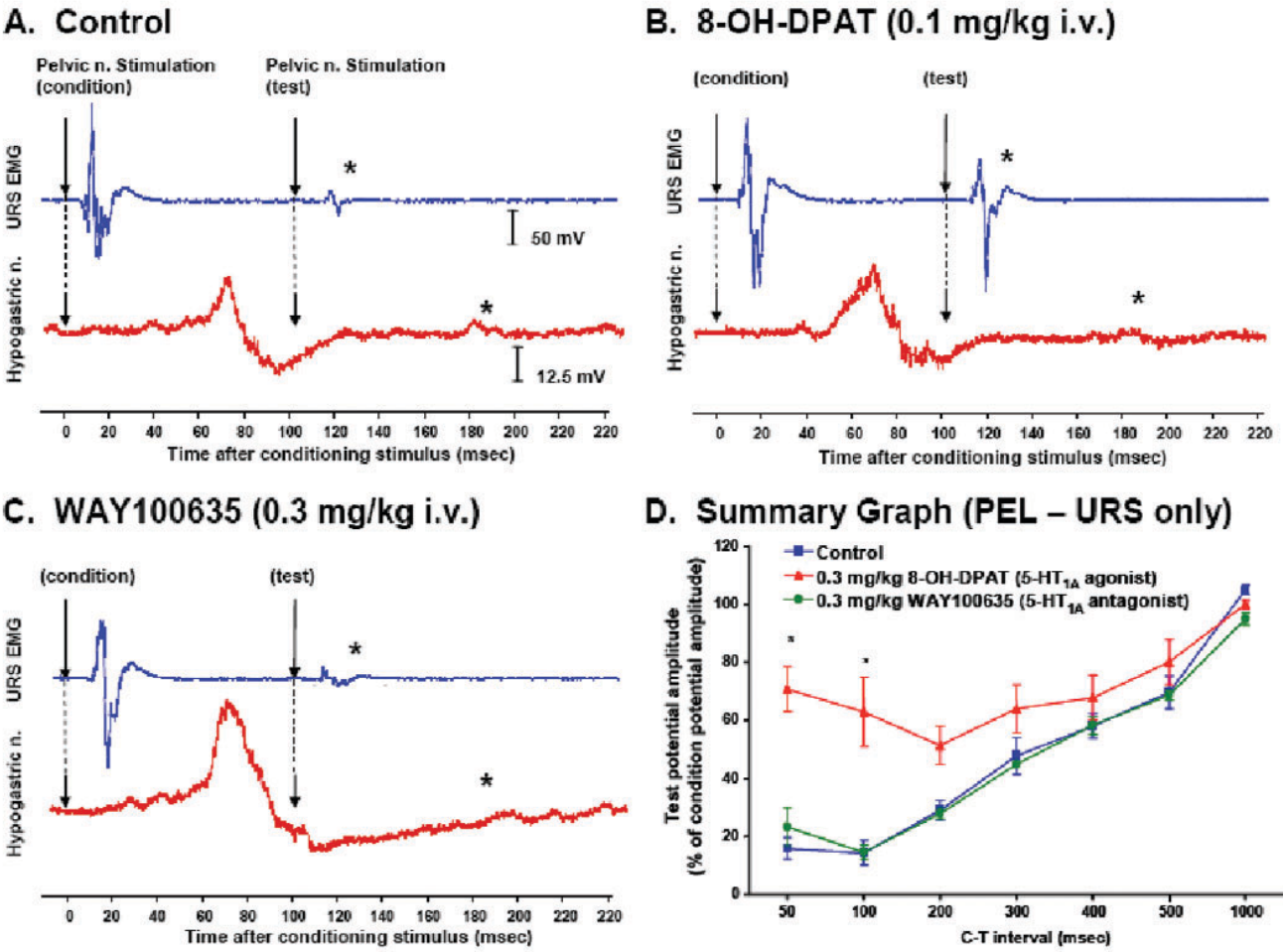


*Figure 12 : Activation of a 'Spinal, Urine-Storage-Reflex, Inhibitory Center' (SUSRIC) and reduction of inhibition by 5-HT1A receptor stimulation. A-C) Averaged tracings (10 sweeps each) of evoked potentials recorded from the urethral rhabdosphincter (URS EMG, top traces) and the hypogastric nerve (HN, bottom traces) resulting from paired pulses of electrical stimulation (1 V, 0.05 msec, 0.5 Hz) of pelvic nerve (PN) afferent fibers (arrows) with a delay of 100 msec between first and second stimuli in each trace, in chloralose-anesthetized female cats. A) Control. Note that the evoked potentials elicited by the first stimulus (i.e. 'condition' stimulus) recorded at 10 msec on the URS and 60 msec on the HN are much larger than the evoked potentials elicited by the second stimulus (i.e. 'test' stimulus). (The evoked potentials resulting from the test stimulus are denoted by an *. B) After administration of the 5-HT1A receptor agonist, 8-OH-DPAT, note that there is a small increase in the condition URS EMG evoked potential but a remarkably larger increase in the test URS EMG evoked potential (), indicating that the inhibition of the URS EMG test potential by the conditioning stimulus is reduced. Also note that 8-OH-DPAT had no influence on the inhibition of the HN evoked potential. C) Subsequent administration of WAY100635, a 5-HT1A receptor antagonist after 8-OH-DPAT restores the inhibition. Note that the inhibition of the test pulse by the conditioning pulse has been restored to control values. D) Graph showing the relationship between the amplitude of the URS EMG test evoked potential (expressed as a percentage of the amplitude of the conditioning evoked potential) versus the condition-test (C-T) interval (ie. time between first and second of the paired pulse stimulations) during the control period (blue squares, n=12), following 8-OH-DPAT (red triangles, n=8), and subsequent WAY 100635 (green circles, n=8). Remarkably, 8-OH-DPAT produced no change in the paired pulse inhibition of the HN evoked potential.*

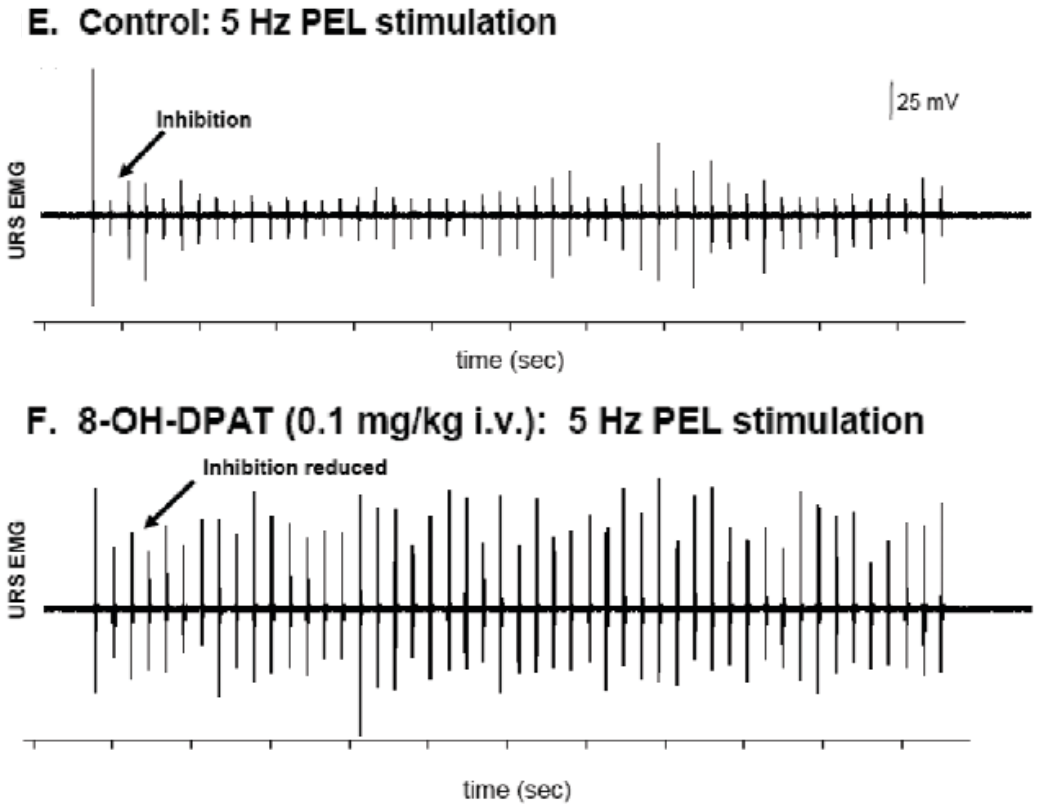


Figure 12 : (Ctd) Importantly, acute spinal transection at the T10 level produced only a small decrease in the paired pulse inhibition, indicating that inhibition is mediated by a spinal network. No effects of 8-OH-DPAT on the paired pulse inhibition were seen in animals following acute T10 spinalization, indicating that the 5HT1A receptors responsible for diminishing the paired pulse inhibition are located supraspinally. E-F) Tracings of URS EMG potentials evoked by repetitive stimulation of the pelvic nerve (PEL) at a frequency of 5-Hz for 10 sec. at 1 V, 0.05 msec pulse. E) Note that during the control period that the amplitude of the second and subsequent PEL-URS evoked potentials are considerably reduced compared to the first potential. F) Note that after 8-OH-DPAT that the amplitude of the second and subsequent potentials are much less reduced (from Thor, 2008).

central electrode placement would also activate colonic and genital afferent fibers, additional experiments are needed to specifically compare urethral versus bladder versus colonic pelvic afferent fibers in evoking the "guarding reflex".

Electrical stimulation of pudendal afferent fibers also evokes a spinal reflex activation of the rhabdosphincter [233, 274]. Since some urethral afferent fibers (as well as rectal, genital, and cutaneous afferent fibers) also travel in the pudendal nerve, it is possible that the spinal urethral rhabdosphincter activation by pudendal afferent stimulation is also a manifestation of the "guarding reflex".

4. SPINAL URINE-STORAGE-REFLEX INHIBITORY CENTER (SUSRIC)

While electrical stimulation of pelvic and pudendal afferent fibers can activate urethral sphincter motor neurons via a spinal reflex, these same stimuli also produce an inhibition of urethral sphincter motor neurons in cats. In early studies of McMahon et al., [233] an inhibition of pudendal nerve spontaneous activity occurred for a period of 50 - 1,000 msec following pelvic nerve stimulation.

Recently, this inhibitory effect has been characterized using electrical stimulation of pelvic (PEL) and pudendal (PUD) nerve afferent fibers and recording of evoked potentials with urethral rhabdosphincter (URS) EMG electrodes (i.e. PEL-URS and PUD-URS reflexes, respectively) in cats [275]. In addition to recording these somatic motor urine storage reflexes, electrodes were also placed on the hypogastric (HgN) nerve (i.e. PEL-HgN and PUD-HgN reflexes, respectively) to record sympathetic urine storage reflexes. Both the somatic and sympathetic urine storage reflexes are reliably evoked when the frequency of PEL or PUD stimulation is below 1 Hz (**Figure 12A-C**). However, when the frequency is raised to 5 Hz or higher, the amplitudes of the reflexes immediately drop (**Figure 12E**). This is not a "rundown" phenomenon because the reflex drop occurs immediately after the first stimulus (i.e. upon application of the second impulse in a given frequency's series of pulses). This suggested that the first stimulus was activating an inhibitory circuit that blocked the second and subsequent stimuli's ability to evoke the reflex (**Figure 13**).

The activation of an inhibitory circuit was shown in anesthetized cats [275] using a paired-pulse or condition-test paradigm, where the first stimulus of a pair (i.e. the condition pulse) precedes the second stimulus of the pair (i.e. the test pulse) by incremental times (i.e. 10, 20, 50, 100, 200, 500, 1,000 msec). This paradigm showed that when a second (i.e. paired or test stimuli) stimulus was applied to the PEL and/or PUD nerve 50 - 500 msec after the first stimulus, the reflexes evoked by the second stimulus were reliably inhibited (**Figure 12A,D**). The paired pulse inhibition was maximal (2^{nd} stimulus evoked potential was < 20% of the 1^{st} stimulus evoked potential) at paired intervals of 50 - 100 msec and gradually disappeared at paired intervals of 1,000 msec or more (**Figure 12B,D**). A possible clinical correlation of SUSRIC

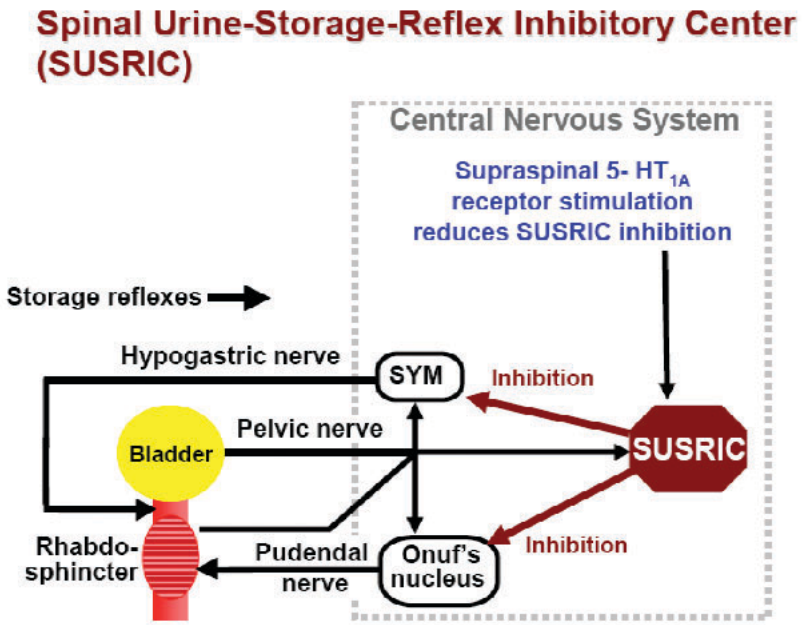


Figure 13 : Diagram showing the somatic and sympathetic urine storage reflexes as previously described (from Thor, 2004). Pelvic and pudendal nerve stimulation, in addition to activating the rhabdosphincter motor neurons in Onuf's nucleus and the sympathetic preganglionic neurons (SYM) to cause contraction of urethral striated and smooth muscle, respectively, also activates a "spinal, urine-storage-reflex, inhibitory center (SUSRIC) that inhibits both storage reflexes for 50-1,000 msec.

activation may be the elegant demonstration that conditioning stimuli applied to the dorsal nerve of the penis (i.e. pudendal nerve afferent fibers) inhibited urethral rhabdosphincter contractions reflexively evoked by magnetic stimulation of the spinal cord at intervals of 20 - 100 msec [276].

The threshold stimulus intensity for evoking inhibition in cats was the same as the threshold stimulus intensity for evoking the reflex, suggesting the same, or similar sized, fibers activate both the reflex and SUSRIC. Inhibition of both the PEL - URS EMG and PEL - HgN evoked potentials showed similar intensity-response characteristics. The inhibition could be evoked by homologous (PEL after PEL and PUD after PUD) or heterologous (PUD after PEL or vice-versa) pulse pairing. Furthermore, stimulation of PEL afferent fibers close to the bladder, which did not evoke a URS reflex, also produced inhibition of the PEL - URS reflex evoked by an electrode placed more centrally on the PEL nerve that did evoke a PEL - URS reflex. This indicates that the inhibition is not due to refractoriness of the rhabdosphincter motor neurons.

The paired pulse inhibition was slightly reduced (10-15%) but otherwise similar after acute spinal transection at T_{10}, indicating that the inhibitory circuit activated by the conditioning stimulus is located in the spinal cord. Inhibition of the PEL - URS reflex also remained after a spinal transection at the L_5 level suggesting that the inhibitory circuit is located within the same segment as the PEL - URS reflex pathway (i.e. sacral cord). (Since L_5 spinalization interrupts the PEL - HgN reflex, which precludes testing for its inhibition, we cannot speculate on the location of the inhibitory circuit for the PEL - HgN reflex except that it is caudal to T_{10}.) Since this inhibitory center is located in the spinal cord, and inhibits both the somatic and sympathetic storage reflexes evoked by either PEL or PUD stimulation, the name, “spinal, urine-storage-reflex, inhibitory center” (SUSRIC), is suggested.

The two nerves that are effective for activating the SUSRIC, the pelvic and pudendal nerves, have spinal terminals that densely project to the dorsal gray commissure region of the sacral spinal cord [219, 277]. This is also an area that contains inhibitory GABAergic and glycinergic neurons that are thought to mediate urethral rhabdosphincter inhibition during micturition [278, 279]. Because pelvic and pudendal nerves densely innervate this same region of the spinal cord, it is tempting to speculate that these GABAergic and glycinergic neurons are also the inhibitory cellular substrate of SUSRIC. Requirement of multiple, parallel inputs to SUSRIC from pelvic nerve axons (e.g. bladder afferent neurons), pudendal nerve axons (urethral afferent neurons), and descending axons from the pontine micturition center might be valuable to prevent sphincter relaxation unless all 3 systems “agreed” that micturition should proceed. On the other hand, redundant inputs might also be valuable to ensure initiation and maintenance urethral sphincter inhibition until micturition is complete and all urine has flowed out of the bladder and completely through the urethra prior to urethral closure. Experiments to test this model are needed.In addition to its physiological role, overactivity of SUSRIC may be involved in the pathology of stress urinary incontinence, while under activity may be involved in bladder-sphincter dyssynergia or retentive urinary dysfunction such as Fowler’s syndrome [280].

• 5-HT_{1A} Receptor Regulation of SUSRIC

Administration of the 5-HT_{1A} receptor agonist, 8-hydroxy-dipropylaminotetraline, 8-OH-DPAT, significantly reduced the paired pulse inhibition of the SUSRIC to 20% of control in spinal intact animals (**Figure 12A-D**) [275]. The 8-OH-DPAT-induced reduction in paired pulse inhibition was reversed by WAY100,635, a highly selective 5-HT_{1A} receptor antagonist, which confirms the role of 5-HT_{1A} receptors in suppressing SUSRIC. 8-OH-DPAT also enhanced the ability of the PEL - URS reflex to follow high frequency (i.e. 2 - 10 Hz) stimulation (**Figure 12E-F**). Importantly, 8-OH-DPAT reduced the inhibition in spinal cord intact cats but not in animals with an acute T_{10} spinal transection, indicating that 5-HT_{1A} receptors are working supraspinally, probably to inhibit a descending excitatory input to SUSRIC (i.e. disfacilitation of the inhibitory center).

Previous urodynamic studies in anesthetized cats [281-283] support the supraspinal location of 5-HT_{1A} receptors that facilitate urethral rhabdosphincter activity. This support stems from urodynamic studies that showed a remarkable enhancement of spontaneous urethral rhabdosphincter EMG activity in the chloralose-anesthetized cat irritated bladder model when the spinal cord is intact [283] but not when the spinal cord is transected, even after spinal bladder reflexes have emerged following spinal shock [281, 282]. In other words, it is proposed that 8-OH-DPAT’s enhancement of urethral rhabdosphincter activity results from stimulation of supraspinal 5-HT_{1A} receptors, which subsequently results in a reduction of SUSRIC-mediated inhibition of rhabdosphincter motor neurons, i.e. excitation results from disinhibition (**Figure 13**).

a) Supraspinal Activation of Rhabdosphincters and Pelvic Floor Muscles

Supraspinal activation of urethral and anal rhabdosphincter motor neurons includes voluntary inputs (i.e. corticospinal [254]), as well as involuntary reflexic inputs (e.g. during coughing, sneezing, vomiting) presumably from nucleus retroambiguus in the caudal medulla [267, 284-286]. Nucleus retroambiguus also innervates the pelvic floor muscle [285, 286], as well as the abdominal muscles; consist

with a role in raising intra-abdominal pressure during Valsalva maneuvers. Generally, the pelvic floor and rhabdosphincter muscles are activated as a functional unit when voluntarily contracted. However, differences in activation between the rhabdosphincter and the pelvic floor muscles have been documented [287], indicating distinct CNS control systems and innervation. Clinical EMG recordings show that even during sleep, activity can be recorded from specific rhabdosphincter motor units [288].

Rhabdosphincter motor neurons are unique among somatic motor neurons in receiving input from the paraventricular hypothalamus[266], although the function of this input has not been determined. In addition, their input from brainstem serotonergic and noradrenergic neurons is among the most dense in the spinal cord [289-292]. Finally, rhabdosphincter motor neurons also receive input from the "L region" of the pons that might be important for maintaining continence, since a lesion in this area produced continuous incontinence in a cat [293].

1. Relative contribution from levator ani nerve, pudendal nerve, and hypogastric nerve in continence mechanisms during sneezing in rats and cats

Analysis of the urethral closure mechanisms during sneeze-induced stress conditions in anesthetized female rats and cats has revealed that pressure increases in the middle portion of the urethra are mediated by reflex contractions of the rhabdosphincter as well as the pelvic floor muscles [294, 295]. Transection of the pudendal nerves reduced sneeze-induced urethral reflex responses by 67% and transecting the nerves to the iliococcygeus and pubococcygeus muscles reduced urethral reflex responses by an additional 25%.

Transecting the hypogastric nerves and visceral branches of the pelvic nerves did not affect the urethral reflexes indicating that sneeze-evoked urethral reflexes in normal rats were not mediated by these autonomic pathways. However, hypogastric nerve transection in conscious, chronic spinal cord injured, female rats reduced urethral baseline pressure, reduced post-void residual urine volumes, reduced maximal voiding pressure, and increased voiding efficiency. This indicates that sympathetic pathways to the bladder neck and proximal urethra contribute to urethral pressure and functional outlet obstruction and voiding dysfunction after spinal cord injury in unanesthetized animals(296).

2. Neurochemical anatomy of urethral rhabdosphincter motor neurons

In addition to their unique morphology, neurophysiology, and supraspinal inputs, rhabdosphincter motor neurons in Onuf's nucleus also exhibit a plethora of unique and highly diverse neurotransmitter inputs, receptors, ion channels, and growth factors (**Table 3**). While many of the markers listed in Table 3 are likely involved in rhabdosphincter control, it is dangerous to assume a role in rhabdosphincter control based strictly on anatomical association with Onuf's nucleus, since some motor neurons in Onuf's nucleus innervate the ischiocavernosus and bulbospongiosus muscles and thus may be involved in control of sexual function. The following section will offer some guidance regarding which transmitter and receptor systems are involved in control of rhabdosphincter function.

5. PHARMACOLOGY OF URETHRAL AND ANAL RHABDOSPHINCTERS

The excitatory amino acid neurotransmitter, glutamate, mediates initiation of action potentials in rhabdosphincter motor neurons (and subsequent rapid contraction of the muscle) by binding to NMDA and AMPA receptors. Both spinal reflex activation and supraspinal activation of the rhabdosphincter are sensitive to NMDA and AMPA receptor antagonists [297-299]. Thus it is useful to think of these transmitters as part of the "hardwired" reflex circuitry that is involved in all or none activation of consistent and reliable storage reflexes, as compared to monoamines and peptide transmitters (see below), that play a role as modulators of the reflexes, increasing or decreasing the gain of the reflexes transmitted by the excitatory amino acids.

The inhibitory amino acids glycine, acting through strychnine-sensitive ionotropic receptors [279, 300], and GABA, acting through both GABA-A (ionotropic) and GABA-B receptors (metabotropic) [257, 301, 302] are thought to be major inhibitory transmitters regulating rhabdosphincter activity. Clinical studies have indicated that systemic [303] and intrathecal [304, 305] administration of the GABA-B agonist, baclofen, may reduce bladder-sphincter dyssynergia in some neurogenic bladder patients. However, because of the ubiquity of glycine and GABA in mediating inhibition of multiple systems, pharmacological studies linking either of these transmitters (or any other inhibitory transmitter) to the inhibition of rhabdosphincter activity during voiding are not definitive.

In addition to amino acid transmitters, the monoamine transmitters (serotonin and norepinephrine) are also important in modulating rhabdosphincter motor neuron activity [306]. It was the preferential association of norepinephrine and serotonin terminals [289-292] that led to extensive studies of noradrenergic and serotonergic control of rhabdosphincter function and eventual clinical studies of duloxetine, a norepinephrine and serotonin reuptake inhibitor, as a treatment for stress urinary incontinence [270, 307-311]. Elegant studies in humans using magnetic stimulation of brain and sacral nerve roots [312] have indicated that duloxetine increases the excitability of rhabdosphincter

motor neurons to both supraspinal and segmental inputs and to double urethral pressure responses to sacral nerve root magnetic stimulation. Importantly, duloxetine's ability to increase urethral rhabdosphincter activity did not interfere with the inhibition of sphincter activity during voiding (i.e. bladder-sphincter synergy was well-maintained). Similar clinical results have been seen with S,S-reboxetine, a selective norepinephrine reuptake inhibitor [313, 314]. This approach of increasing synaptic levels of serotonin and/or norepinephrine is logical since, it has been shown that noradrenergic and serotonergic terminals associated with rhabdosphincter motor neurons show an age-dependent decrease in density in rats [315] that might explain the increased incidence of stress incontinence with aging.

Multiple adrenergic receptor subtypes play a role in control of the rhabdosphincter, and the results with norepinephrine reuptake inhibitors indicate that these receptors can be activated by endogenous norepinephrine in anesthetized cats [272]. Strong evidence exists that α_1 adrenoceptors excite rhabdosphincter motor neurons [272, 316, 317]. Patch clamp studies [318] have shown a direct depolarizing effect of norepinephrine on rhabdosphincter motor neurons (**Figure 14D**) that can be blocked by the α_1 adrenoceptor antagonist, prazosin. Similar conclusions regarding the excitatory effects of α_1 adrenoceptors on rhabdosphincter neurons have been reached in clinical studies [317] where decreases in rhabdosphincter activity were seen after administration of prazosin to human subjects. On the other hand, strong evidence exists that α_2 adrenoceptor stimulation has the opposite effect, i.e. inhibition, of rhabdosphincter activity [272, 319]. Importantly, the innervation of the urethral and anal smooth muscle (i.e. the hypogastric nerve) shows similar adrenergic pharmacology - an enhancement of activity by α_1 adrenoceptors [272, 316, 320] and inhibition of activity by α_2 adrenoceptors [272, 316, 321].

Multiple subtypes of serotonin (5-hydroxytrptamine, 5-HT) receptors are also involved in modulating rhabdosphincter motor neuron excitability. Strong evidence exists that 5-HT_2 receptors can excite sphincter motor neurons [274]. Indeed duloxetine's facilitatory effects on rhabdosphincter activity in anesthetized cats are mediated in part through activation of 5-HT_2 receptors [270]. Both 5-HT_{2A} and 5-HT_{2C} receptor agonists increase rhabdosphincter EMG activity in dogs, guinea pigs, and rats [322, 323]. Recent *in vitro* rat spinal cord slice patch clamp studies show that part of this effect may be directly on rhabdosphincter motor neurons, as opposed to interneurons [318], since 5-HT induces a direct depolarization of rhabdosphincter motor neurons (**Figure 14E**). Interestingly, substance P, a peptide transmitter that is co-localized with 5-HT in raphe spinal nerve terminals, also produces direct depolarization of rhabdosphincter motor neurons in rat spinal cord slices [324], and thyrotropin releasing hormone (TRH), another peptide transmitter co-localized with 5-HT in nerve terminals, induces excitation of rat sphincter activity [325, 326] in vivo.

A peculiar finding regarding 5-HT_2 receptor-induced activity of pelvic nerve-evoked rhabdosphincter reflexes in chloralose-anesthetized cats is that the effect is highly reproducible when the spinal cord is isolated (i.e. acute T_{11} transection), but it is highly variable and statistically insignificant when the spinal cord is intact [274]. This suggests that supraspinal 5-HT_2 receptors have an opposing, inhibitory, effect that counteracts their spinal, excitatory, effect. Whether this phenomenon is an artifact of anesthesia and/or is specific to cats is important to determine.

Immunohistochemical and molecular studies in humans and dogs [322] have shown that 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor subtypes are associated with Onuf's nucleus motor neurons. In addition, 5-HT_{2C} receptor mRNA has been localized to anal sphincter motor neurons in the rat [327]. On the other hand, another immunohistochemistry study with retrograde labeling of urethral rhabdosphincter motor neurons and ischiocavernosus motor neurons in rats indicates that the 5-HT_{5A} receptor is associated with the former, while the 5-HT_{2A} receptor is preferentially associated with the latter [328].

As described in the previous section on the SUSRIC, supraspinal 5-HT_{1A} receptor stimulation enhances rhabdosphincter activity in cats [275, 281-283]. Importantly, the excitatory effects of 5-HT_{1A} receptor agonists on the rhabdosphincter can still be "over ridden" by inhibitory mechanisms during voiding, i.e. bladder-sphincter synergy remains despite 8-OH-DPAT-induced enhancement of rhabdosphincter activity [275, 281-283].

The effect of 5-HT_{1A} receptors on rhabdosphincter activity in rats is more complicated. Firstly, rats do not show an inhibition of rhabdosphincter activity during voiding like cats and humans but, instead, show a high frequency bursting (or "phasic firing") of rhabdosphincter EMG activity during micturition [329]. This bursting is actually necessary for efficient voiding in rats presumably by creating a "pumping" action through the urethra. When the spinal cord is transected, this bursting disappears and rats present with urinary retention. Although the bursting is not seen in anesthetized spinal rats at any time [329], in conscious spinal rats, the rhabdosphincter EMG bursting gradually returns in some animals across a period of 6 weeks [330]. Interestingly, 5-HT_{1A} receptor stimulation can unmask the bursting rhabdosphincter EMG activity in the anesthetized spinal rat [282, 331]. Chang et al [331] further showed that the center responsible for bursting activity in spinal rats was located between the T_{11} and L_4 spinal segments. In

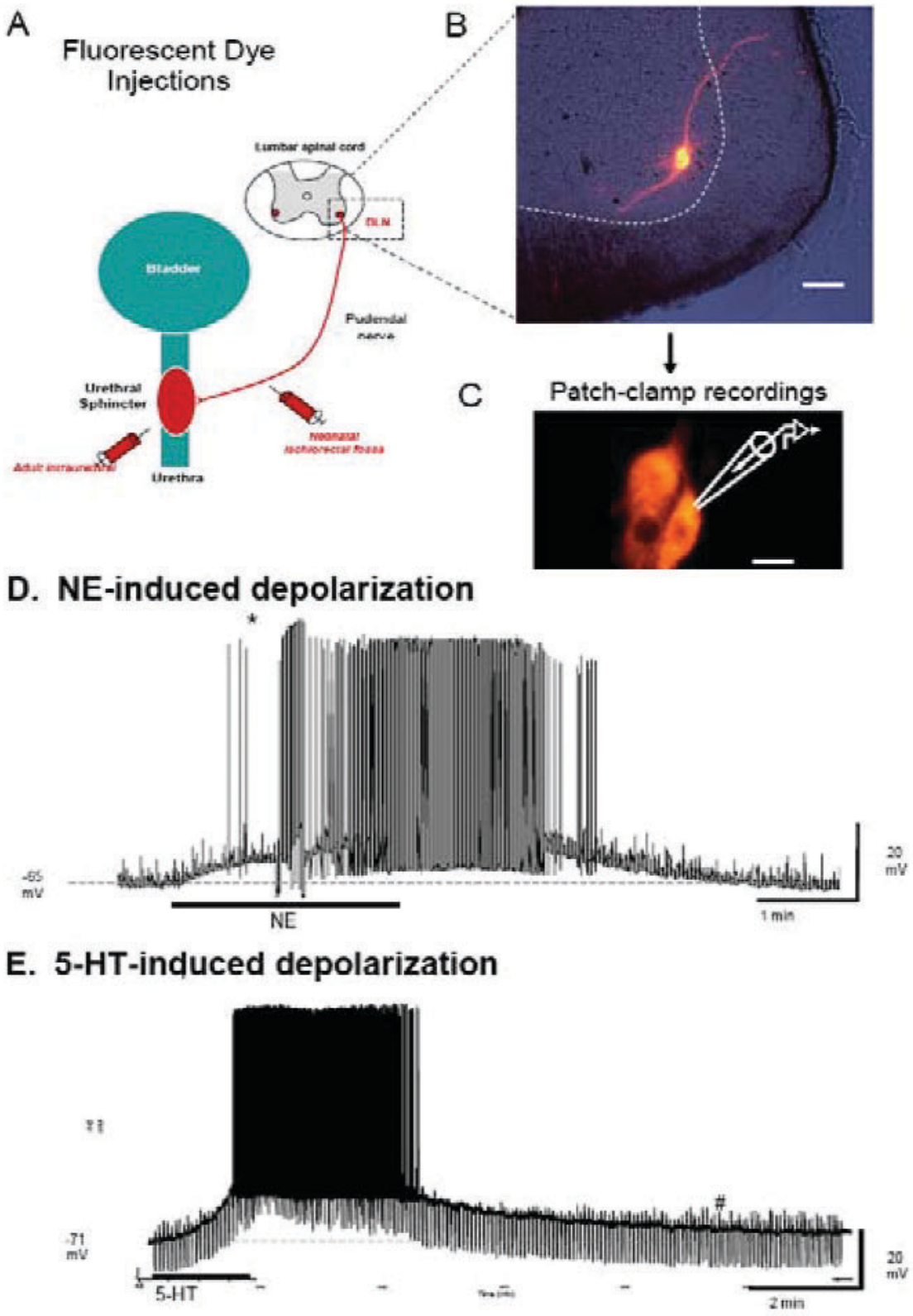


Figure 14 : Monoaminergic modulation of sphincter motor neurons recorded using patch clamp electrophysiology. A-C) Visual identification of rat urethral sphincter motor neurons. A. Retrograde labeling of dorsolateral nucleus (DLN) motor neurons was accomplished by fluorescent dye injections into the urethral sphincter (adult), or the ischiorectal fossa (neonate). Dye was transported through the motor branch of the pudendal nerve to the lumbar spinal cord. B. Composite fluorescence and low-light image showing DiI-labeled DLN neurons in the ventral horn of an adult rat lumbar spinal cord section. The dotted line denotes the grey/white matter boundary, and the dorsal surface is oriented toward the top of the page. C. Enlarged neurons from B with a patch-clamp electrode schematic to demonstrate electrophysiological recordings from DLN neurons. Calibration bars are 80 µm (B) and 20 µm (C). D-E) Either norepinephrine (NE) or serotonin (5-HT) was bath applied to rat lumbar spinal cord slices containing DiI-labeled dorsolateral nucleus neurons. D. NE (1 µM) produced a reversible depolarization and action potential firing. E. 5-HT (10 µM) also produced a reversible depolarization and action potential firing (from Burgard, 2008).

the studies of Gu et al [282], 5-HT_{1A} receptor stimulation had no influence on the asynchronous rhabdosphincter activity that precedes, and follows, micturition-associated bursting, it only re-organized the activity into the bursting or phasic characteristics that accompany micturition.

Another aspect of 5-HT_{1A} receptor function in rats can be seen from studies of pelvic nerve-to-rhabdosphincter reflex potentiation. Early studies showed that this reflex can be potentiated by prolonged, low frequency electrical stimulation of the pelvic nerve and by bladder distention [332-334]. Subsequent studies showed that the potentiation involved a 5-HT_{1A} receptor-based link [335].

An interesting and possibly important comparison between drug effects on rhabdosphincter activity in cats and rats is also shown with κ_2 opioid receptors. In cats, the κ opioid receptor agonist, ethylketocyclazocine, selectively inhibits spinal rhabdosphincter reflexes [234]. In rats, it was found that a κ_2 opioid receptor agonist inhibits the rhabdosphincter bursting pattern associated with micturition, leading to decreased voiding efficiency [336]. In these studies, κ opioid receptor stimulation had no influence on the asynchronous rhabdosphincter activity that precedes and follows micturition-associated bursting.

Thus there may be parallels between excitation of rhabdosphincter reflexes in cats with enhancement of rhabdosphincter bursting activity in rats on one hand (5-HT_{1A} receptor-mediated), and inhibition of rhabdosphincter motor neurons in cats and suppression of bursting activity in rats on the other hand (κ opioid receptor-mediated). The fact that asynchronous rhabdosphincter activity in rat was not affected by either 5-HT_{1A} or κ opioid receptor stimulation highlights differences in the organization of the asynchronous (spinal) and micturition associated-bursting (supraspinal) reflex control in rats.

Another possible parallel for exploration of rhabdosphincter function in rats might lie in comparing the above spinal micturition-associated rhabdosphincter bursting center with the spinal ejaculation center in rats: both centers are located in the mid-lumbar spinal cord [331, 337, 338], both centers exhibit "bursting" [329, 339]; 5-HT_{1A} receptor agonists can facilitate both activities [340-342], κ opioid receptor agonists inhibit both activities [336, 343]. Of course it must be appreciated that differences between the two "bursting centers" exist (i.e. the former activates rhabdosphincter motor neurons while the latter activates bulbospongiosus motor neurons, the former has a period of 6 Hz while the latter has a period of 0.5 Hz).

Future Pelvic Floor Research Needs

The controversy regarding a contribution of the pudendal nerve to the innervation of the levator ani muscles should be addressed. As a first step, agreements regarding nomenclature should be established in regards to which muscles actually comprise the levator ani (i.e. should the definition include only the pubococcygeus, iliococcygeus, and coccygeus muscles or should it include the puborectalis, urethralis, urethrovaginal sphincter, the compressor urethrae; etc.). Careful characterization of these various muscles based on physiological criteria such as embryological origin, skeletal sites of origin and insertion, compartmentalization imposed by connective tissue boundaries, myofiber phenotype, and sensory structures (e.g. Golgi tendon organs and muscle spindles) is likely to provide insight into which types of motor neurons (i.e. alpha, gamma, or "special somatic") innervate each muscle and which nerve carries their motor axons. Resolving this controversy is important for a number of reasons: surgical guidance, understanding iatrogenic pathologies, and understanding physiological and pharmacological control of the individual muscles.

A second important area regards the reflex control of the levator ani muscles and viscerosomatic interactions between pelvic floor muscles and pelvic viscera. While visceral and somatic reflex control of the rhabdosphincters has been extensively studied, studies of levator ani muscle reflexes are few. Understanding whether there are spinal reflex connections between, for example, bladder afferent fibers and levator ani motor neurons would further our understanding of continence control. Similarly, understanding anatomical connections between rhabdosphincter and levator ani muscles are important. Determining whether the cholera toxin B-labeled processes of levator ani motor neurons located in Onuf's nucleus (**Figure 11 D-E**) are dendrites or axonal collaterals may provide important insight into understanding physiological coordination between the pelvic floor and rhabdosphincters. Finally, understanding the role of levator ani primary afferent fibers in controlling lower urinary tract and bowel function might explain indirect bladder symptoms that might be induced by pelvic floor injury or suspension surgeries, as well as physiological controls of levator ani muscles over excretory function and vice-versa. The abundant small diameter peptidergic afferent fibers innervating pelvic floor muscle might also play a role in the etiology of chronic pelvic pain syndrome or interstitial cystitis; two conditions for which a frank pathological cause has not been determined.

A third important area for study is the relative importance of bladder afferent fibers versus urethral / bladder neck afferent fibers in eliciting the "guarding" or "continence" reflex. This is important since there are phenotypic differences between these two groups of afferent neurons in regards to neurotransmitter and ion channel phenotypes(344) that might be exploited to independently control urine storage and voiding dysfunction.

IV. EFFERENT PATHWAYS TO THE BLADDER

The motor arm of the lower urinary tract drives bladder contraction during voiding and the outlet contraction required for urine storage. In this section, spinal and peripheral elements contributing to the motor activity of the bladder is described, while the equivalent structures for the bladder outlet are discussed in section III.

1. PREGANGLIONIC NEURONS

Parasympathetic preganglionic neurons are located in the lateral part of the sacral intermediolateral gray matter in a region termed the sacral parasympathetic nucleus (SPN) and are small, fusiform-shaped cells which send dendrites into lateral lamina I of the dorsal horn, the lateral funiculus and medially into the dorsal grey commissure (DGC). Bladder pre-ganglionic motor neurons are located in the S1-S3 in the cat [361], dog [362] and monkey [363]. In cat these motor neurons are located ventrolaterally in the intermediolateral column, with cells innervating the colon lying dorsomedial. The guinea pig is similar; retrograde labelling following bladder wall injections reveals neurons bilaterally in the ventrolateral part of the intermediolateral column at S1 [256]. The rat is different, as preganglionic motor neurons are located at L6-S1 [364]; unilateral ventral root rhizotomy at the L5 level in the rat decreases peak cystometric pressures [365].

The parasympathetic preganglionic neurones project through the ventral spinal roots to the major pelvic ganglion [366-368], releasing the excitatory transmitter, acetylcholine. They are divided functionally into tonic and phasic types. In some species they also release opioid peptide transmitters and express nitric oxide synthase [369]; there is also evidence of involvement of pituitary adenylate cyclase activating peptide (PACAP), a peptide present in visceral afferent neurones, and of prostaglandins within the spinal cord [370]. The DGC also contains a group of interneurones, which are likely to be active during micturition [371, 372]. These DGC interneurones may influence the function of the parasympathetic preganglionic neurons [279, 371, 372].

At spinal levels L1–L2, both the intermediolateral horn and the DGC contain sympathetic preganglionic neurones whose axons project to the major pelvic ganglion. With ageing, there is selective attrition of preganglionic sympathetic neurones in L1–L2, which project to the pelvic ganglion, with reductions in the extent of the dendritic arbors of remaining cells [366, 367].

In man the preganglionic parasympathetic motor nerves to the bladder (and other pelvic organs, the rectum and descending colon) course through the pelvic nerves from the sacral anterior roots S2-S4. Stimulating these roots with implanted electrodes designed principally for bladder emptying in spinal cord injury [373] elicits two principal responses at S3; at low levels of stimulation, the external urethral sphincter, external anal sphincter and pelvic floor muscles are contracted. At high levels of stimulation, parasympathetic activation contracts the detrusor muscle, leading to efficient emptying of the bladder when the sphincter muscle relaxes [374]. Attempts to use extracorporeal magnetic stimulation to achieve the same effect [375] have shown insufficient power to activate the small parasympathetic neurons at the level of the lumbar-sacral roots [376].

2. GANGLIA

The peripheral ganglia convey the autonomic innervation to the lower urinary tract and reproductive organs, along with a substantial part of the extrinsic motor innervation of the lower bowel. There are substantial species differences in organization and neurochemistry of pelvic ganglion cells and their spinal inputs. Large mammals have a plexus of pelvic and intramural ganglia, containing both sympathetic and parasympathetic neurons. The guinea pig is intermediate in complexity, with separate posterior and anterior plexuses innervating different pelvic organs. In the rat and mouse, the pelvic plexus consists of the major pelvic ganglia (MPG) and a number of small accessory ganglia. In the rat there are two major pelvic ganglia and small accessory ganglia, with less cytological complexity and almost no intramural ganglia.

The preganglionic input releases acetylcholine, which acts on nicotinic receptors. Patients with megacystis-microcolon-intestinal-hypoperistalsis syndrome (MMHIS) [377] have reduced or no alpha-3 nicotinic receptor subunit [378]. Selective gene knockout mice lacking the alpha-3 nicotinic receptor subunit alone or the beta-2 and beta-4 subunits in combination [379], develop severe bladder distension soon after birth, and later overflow incontinence. The detrusor muscle in these animals contracts in response to field stimulation or muscarinic agonists, but not nicotinic agonists [380], indicating the potential importance of alpha-3, beta-2, and beta-4 nicotinic receptor components in control of voiding, but not their functional location.

Within the pelvic plexus there is topographical representation of the pelvic organs. In the female dog, neurons supplying different pelvic organs are located in separate ganglia, which possess a distinctive composition of neurone types and different preganglionic supply [381]. Neurons retrogradely labelled from the urinary bladder mainly occur in ganglia located at the vesico-ureteric junction. They comprise catecholaminergic calbindin neurons and noncatecholaminergic neurons containing calbindin or NOS, with relatively sparse pericellular varicose nerve fibres.

In male mice [382], the major pelvic ganglia are close to the dorsal surface of the prostate gland. Their main inputs are the pelvic nerves, and the hypogastric nerve from the inferior mesenteric ganglion. The major outputs are the penile (cavernous) nerve and the supply to the urogenital organs. Tyrosine hydroxylase (TH) is expressed by one-third of neurons, almost all co-expressing dopamine beta hydroxylase (DBH). Numerous TH axons are present in the hypogastric nerve, but very few in the pelvic nerve, supporting a primarily sympathetic origin. Non-neuronal cells containing TH are also present, resembling small, intensely fluorescent (SIF) cells observed in many other autonomic ganglia [382]. Neurons immuno-stained for choline acetyl transferase (ChAT) have a complementary distribution to noradrenenergic neurons. About half of the cholinergic ganglion cells contain VIP, distributed throughout most of the ganglion, with a cluster near the origin of the penile nerve [382]. Neurones with NPY are numerous and apparently randomly distributed throughout the ganglion, with marked variation between mouse strains. All noradrenergic neurons contain NPY, but many NPY neurons are not noradrenergic. Many of the cholinergic NPY neurons also contain VIP. ChAT is seen in varicose axon terminals closely associated with ganglion neurons. Neither NPY nor VIP are present in preganglionic terminals, except for a small number of individual neurones. The latter may arise from viscerofugal neurons in the myenteric plexus of the lower bowel [383].

Autonomic ganglia are also found in the vicinity of the bladder neck, trigone, proximal urethra and prostate. They receive noradrenergic and cholinergic excitatory innervation and non-cholinergic, non-adrenergic inhibitory innervation [384]. Knowledge of this distribution allows strategic planning for surgical dissection (**Figure 15**). In ureteric reimplantation, several branches of the pelvic plexus travel to the ureterovesical junction and surround the distal ureter as a fine network in the human. The main neural elements are located 1.5 to 2 cm. dorsal and cranial to the bladder trigone, and dorsal and medial to the ureter in both female and male cadavers [385]. In the context of hysterectomy, cadaveric dissections show pelvic autonomic nerves are at greatest risk during sacrouterine dissection [386], corresponding with observed innervation density [387]. In addition pathophysiological processes such as ageing can influence the pelvic ganglia, for example the postganglionic sympathetic neurones in the major pelvic ganglion of rats [388, 389]. In the proximal female urethra, virtually all of the NOS immunoreactive cells also contain the carbon monoxide-synthesising enzyme, haem-oxygenase- (HO-) 2, but of the HO-2 positive cells, 25% did not show NOS immunoreactivity [390].

The bladder wall itself contains intramural ganglia, and small clusters of autonomic ganglion cells are present in the adventitial connective tissue and among the detrusor muscle bundles. There is species variation in the extent of intramural innervation of the bladder; ganglia are present in many species such as the guinea pig [383], while the rat bladder contains the post-synaptic innervation alone [391]. The ganglia are found throughout the bladder wall and vary considerably in size [91, 392]. They show immuno-reactivity to vasoactive intestinal polypeptide (VIP), nitric oxide synthase (NOS), neuropeptide Y (NPY) and galanin (Gal) in varying amounts. However, they do not contain enkephalin (ENK), substance P (SP), calcitonin gene-related peptide (CGRP) or soma-

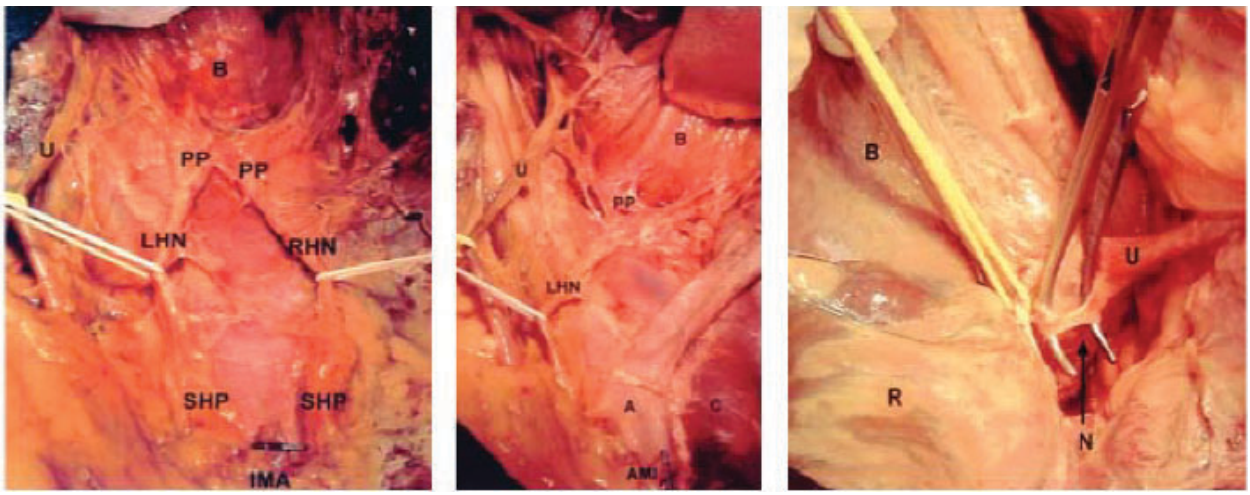


Figure 15 (left panel): Dissection of superior hypogastric plexus, hypogastric nerves and two main portions of pelvic plexus in male cadaver. B, bladder, PP, pelvic plexus, LHN, left hypogastric nerve, RHN, right hypogastric nerve, IMA, inferior mesenteric artery, SHP, superior hypogastric plexus, U, ureter. Middle panel: Left hypogastric nerve with network of pelvic plexus nerve bundles. Branches run to distal ureter (U) and bladder (B) trigone. PP pelvic plexus, A, aorta, C inferior vena cava, LHN, left hypogastric nerve, AMI, inferior mesenteric artery. Right panel: Preparation of distal ureter (U). Clamp is used to dissect nerve bundles of pelvic plexus. B, bladder R, rectum N, pelvic plexus nerve fibre. From Leissner J., et al. 2001.

tostatin (Som) [90], suggesting that cell bodies of sensory neurones are not located in the intramural ganglia. Postganglionic sympathetic nerves, identified with antibodies to TH and NPY, also synapse on these neurones. Nicotinic receptors have been identified on intramural nerve cell bodies within the bladder [380]. α1-adrenergic facilitatory receptors in bladder parasympathetic ganglia (393).

The endothelins (ET-$_1$, ET-$_2$ and ET-$_3$) mediate various biological effects through two receptor subtypes ET-$_A$ and ET-$_B$. Electrostimulation of the sacral roots responsible for bladder contraction in the pig leads to detrusor activation with consecutive rise in bladder pressure, which can be partly inhibited by an ET-$_A$ receptor antagonist, probably by influencing the atropine-resistant component of efferent detrusor activation [394]. Both ET receptors are widely distributed in the urinary tract. ET-$_1$ modulates bladder function [395], eliciting potent and long-lasting contractions of detrusor muscle strips [396, 397]. It is synthesized locally and may be a paracrine mediator of detrusor contraction [397]. The predominant receptor sub-type in the bladder dome is ET-$_A$ [398].

3. TERMINAL NERVE FIBRES

The majority of nerves running in the detrusor stain positively for acetylcholinesterase and for vesicular acetylcholine transferase (VAChT) [392, 399] and are thought to be parasympathetic. Putative postganglionic sympathetic fibres immunoreactive for TH or NPY are rare in the detrusor, although they are moderately frequent in the suburothelium [92]. Nonetheless, presynaptic α1-adrenergic facilitatory receptors are present on efferent parasympathetic nerve terminals in the bladder wall [400, 401]. The parasympathetic efferents release acetylcholine to stimulate muscarinic receptors. However, the presence of additional substances allows immunohistochemical subclassification of nerve fibres, and raises the question as to whether additional transmitters other than ACh have a role in normal micturition function or disease pathophysiology. Many nerve fibres contain NPY and VIP, while some contain NOS or Gal. In the human bladder, markers for sensory nerves (SP, CGRP and neurokinin A) occur infrequently in nerves running in the detrusor, in contrast to the suburothelial layer [90-93]. In the mouse bladder, noradrenergic axons form a sparse supply in the trigone muscle, are quite rare in the detrusor muscle and are absent from the mucosa [382]. Cholinergic axons are prevalent in muscle and mucosa, with similar relative prevalence of co-localised peptides in the two regions. In the muscle of the trigone, the most common axons also contain both VIP and NPY. In the detrusor muscle, the most common axon type varied for the two strains studied. Noradrenergic / NPY axons provide a dense supply to blood vessels, in common with the other pelvic organs. Many vessels also have a sparse supply of VIP axons [382]. Cholinergic nerves are also present in the suburothelium; most of them in addition contain NPY and some contain NOS [92].

Smooth muscle cells in the bladder are grouped into fascicles, several of which make up a muscle bundle. They receive a dense innervation, which runs in line with the axis of the fascicle and is derived from coarse nerve trunks in the connective tissue around the fascicles and bundles. This innervation mediates the widespread co-ordinated detrusor contraction accompanying voiding. The anatomical relationship between the preterminal innervation and the muscle fascicles has been described in a serial sectioning study in the human bladder [402]. The nerve supply is distributed by a series of dichotomous branchings, illustrated schematically in **Figure 16**. Adjacent to the muscle bundles, 1 or 2 primary nerve trunks run parallel to the long axis of the bundle. These give rise to circumferential peribundle branches. Both the longitudinal and circumferential trunks give off transverse interfascicular branches, entering the bundle perpendicular to its long axis, approximately at the midpoint of the bundle. Within the bundle they give axial interfascicular branches running along the long axis within and closely adjacent to individual fascicles, ending in the preterminal and terminal varicose intrafascicular axial innervation.

4. DESCENDING AND SPINAL SEGMENTAL INFLUENCES ON SPINAL AUTONOMIC CENTRES

In the spinal cord, several transmitters mediate the effects of modulatory pathways that influence the onward progression of efferent activity from the SPN. Ascending and descending connections contribute to micturition reflex control, in many cases involving excitatory and inhibitory spinal interneurones between sacral and lumbar spinal segments. In a rat model of neurogenic bladder dysfunction (autoimmune encephalomyelitis), an exaggerated descending excitatory control arises at the spinal segmental level, which gives rise to detrusor overactivity [403].

Some animals with autoimmune encephalomyelitis develop detrusor areflexia rather than overactivity; in these animals, the excitatory control is probably dominated by segmental inhibition, mediated primarily by glycine receptor activation. Spinal shock in rats induces an alteration of glycine/glutamate concentration ratio [404]. A change in the ratio of excitation and inhibition was also observed in humans suffering from spasticity and pain [405]. Ageing affects many synaptic inputs [368]. This balance of inputs, and the potential plasticity of neuronal circuits, is crucial in understanding pathophysiological processes; following injury to spinal roots, plasticity can create new reflex circuits, including a somatic–CNS–bladder reflex, whereby scratching the skin in specific dermatomes may elicit bladder contractions [406-408].

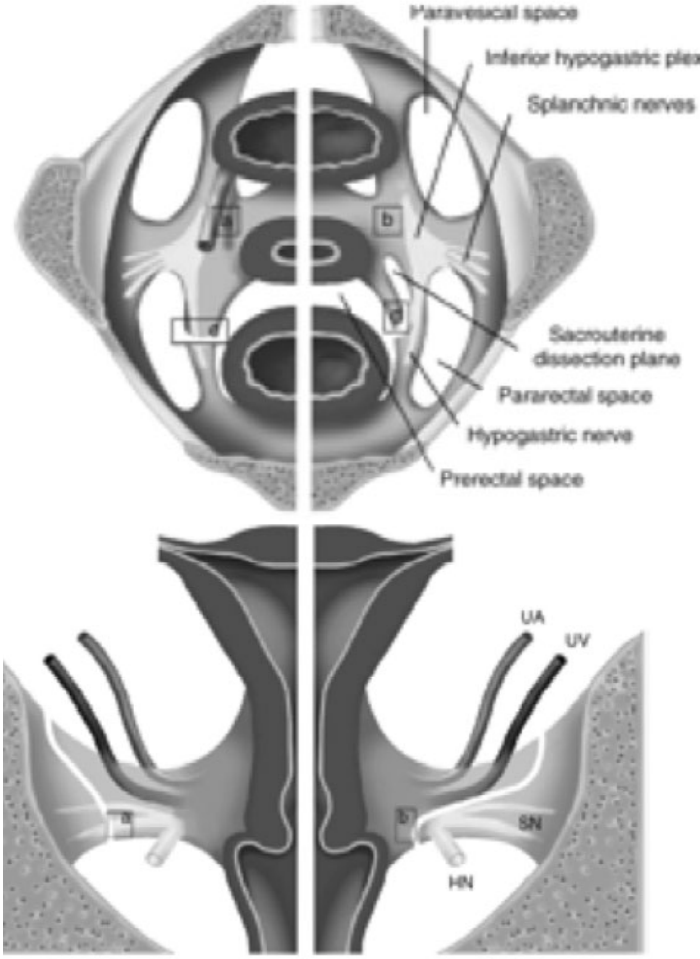


Figure 16 : Top panel: Diagram of the pelvic autonomic nerves in radical hysterectomy. Transverse section through the pelvis showing the bladder, cervix and rectum. Left side represents the conventional technique, right side represents the nerve sparing technique. Scale bar 250 µm. A, Vesicouterine ligament, conventional, B, vesicouterine ligament, nerve sparing; C, Sacrouterine ligament, conventional; D, Sacrouterine ligament, nerve sparing. Lower panel: Diagram of the pelvic autonomic nerves in radical hysterectomy. Frontal sections through the uterus and cardinal ligament. UA, uterine artery, UV, uterine vein, SN, splanchnic nerves, HN, hypogastric nerve. Left side represents the conventional technique, right side represents the nerve sparing technique. Scale bar 250 µm. A, Posterior cardinal ligament, conventional; B, posterior cardinal ligament, nerve sparing. From Maas C.P., et al. 2005

Glutamate Glutamate is present in the terminals of primary afferent neurons in the spinal cord along with interneurons and fibres originating in the medulla oblongata. In general, glutamatergic neurons tend to be excitatory, contrasting with generally inhibitory effects of glycinergic neurons; however, excitatory/ inhibitory effects of transmitters can be reversed by the nature of the post-synaptic neuron. Thus, glutamatergic neurons can indirectly have an inhibitory effect if an inhibitory neuron is interposed before the ultimate target [409]. With ageing, there is a decrease in the density of glutamatergic synaptic inputs, which may influence urinary tract function [368]. Glutamate acts on spinal neurons through a variety of receptor subtypes. These include NMDA receptors, which are important in controlling polysynaptic reflex pathways at the lumbosacral levels. The NMDAR1 glutamatergic receptor sub-unit is present in the spinal cord of male rats, and is expressed in the SPN. Glutamate is present in the dorsal root ganglion cells supplying the bladder [410], and the NMDAR1 sub-unit is also present in L6 dorsal root ganglion cells of the rat [411]. In female rats intrathecal injection of an NMDA receptor antagonist decreases bladder contraction pressure [412]. NMDAR1 receptors may be activated by glutamate released by afferents from peripheral and supraspinal origins to elicit bladder contractions [413].

Glycine/ gamma amine butyric acid Glycinergic and GABAergic interneurones have a major role in neural control processes mediating LUT function [414]. Glycinergic/ GABAergic projections to the lumbosacral cord inhibit the micturition reflex and also inhibit glutamatergic neurons [404]. Rectal distention prolongs the interval, decreases the amplitude and shortens the duration of bladder contractions in rats; this effect is not seen after simultaneous intrathecal injection of low dose strychnine (a selective glycine-receptor antagonist) and bicuculline (GABA-A receptor antagonist), suggesting that the inhibitory rectovesical reflex involves glycinergic and GABAergic mechanisms in the lumbosacral spinal cord, which may be synergistic [415].

Serotonin Spinal reflex circuits involved in voiding function have a dense serotonergic innervation [416] (**Figure 17**). Immunocytochemical studies in rats, cats and primates show that lumbo-sacral sympathetic and parasympathetic autonomic nuclei receive serotonergic inputs from the raphe nuclei [291, 292, 417, 418]. Activation of the central serotonergic system can suppress voiding by inhibiting the parasympathetic excitatory input to the urinary bladder, and 5-HT elicits a prolonged activation of thoracic sympathetic preganglionic neurons. Stimulation of the raphe nuclei in the cat inhibits reflex bladder activity [419-421]. 5-

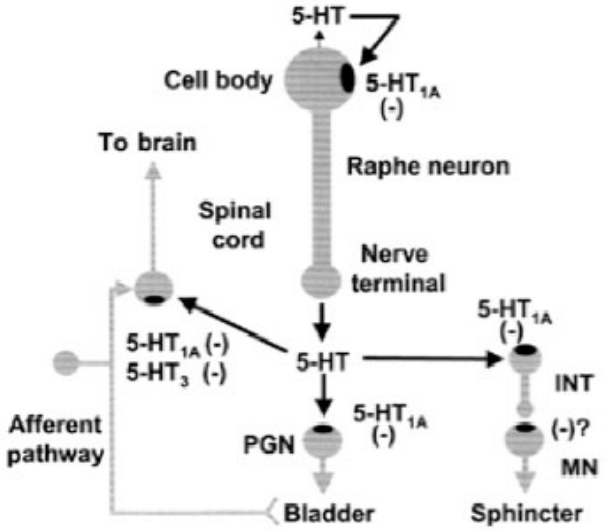


Figure 17 : Serotonergic pathways controlling bladder function in the cat. A neuroanatomic substrate for explaining the effects of serotonergic drugs on the lower urinary tract is shown. Raphe neurons in the brain stem send axons to the spinal cord to control the processing of afferent input from the bladder, the parasympathetic efferent outflow to the urinary bladder and somatic efferent outflow to the striated muscle of the urethral sphincter. 5-HT1A receptors mediate inhibitor effects on 1, parasympathetic preganglionic neurons (PGN), 2, spinal interneurons (INT) that provide an inhibitory input to sphincter multineurons (MN) and 3, raphe neurons in the brain stem. The transmitter released by inhibitory interneurons has not been identified. Activation of 5-HT1A and 5-HT3 receptors also inhibits afferent input passing from the bladder to the brain. Blockade of 5-HT1A autoreceptors in raphe neurons would increase raphe neuron firing and enhance serotoniergic control of spinal reflex mechanisms. This effect would promote urine storage by enhancing sphincter activity and depressing bladder activity. From de Groat W.C., 2002.

HT_{1A} and 5-HT_2 receptors are present in the SPN. However, in different species serotonin (5-hydroxy tryptamine, 5-HT) may have varying functions in the central nervous control of bladder activity [416]. For example, activation of 5-HT1A receptors facilitates reflex bladder activity in rats [416, 422]. It also inhibits excitatory amino acid–induced firing of vesical SPN neurones [423]. Inhibitory effects on bladder activity are most likely mediated primarily by 5-HT_{1A} receptors [283].

5-HT_{1A} and 5-HT_2 receptor agonists depress sacral parasympathetic reflex firing elicited by bladder afferents, and enhance sympathetic and somatic storage reflexes in the cat [283, 424]. Activation of 5-HT_{1A} receptors decreases the amplitude of isovolumetric bladder contractions induced by bladder distension and increases the bladder volume threshold for triggering the C-fiber afferent-mediated spinal micturition reflex in chronic SCI cats, probably at the level of interneuronal pathways in the spinal cord or on the afferent limb of the micturition reflex [425].

Recordings from neurons in the raphe nucleus revealed that the neurons are activated by bladder distention [426], suggesting a possible spino-bulbospinal negative-feedback circuit, in which ascending sensory input from the bladder elicits descending inhibition.

Adrenergic Descending catecholaminergic neurones are primarily located in the upper medulla or pons [315] (**Figure 18**). In clinical use, non-selective α1-adrenergic antagonists influence urine flow and storage phase lower urinary tract symptoms; the two effects probably occur by different mechanisms, and both central or peripheral locations may be responsible [400]. Reflex bladder activity is modulated by at least two spinal α1-adrenergic mechanisms.

Firstly, there is inhibitory control of reflex bladder contractions, probably by modulation of afferent processing. Secondly, there is excitatory modulation of the amplitude of bladder contractions due to regulation of the descending glutamatergic limb of the spinobulbospinal bladder reflex pathway [427, 428]. α_{1A}- adrenoceptors comprise 70% and α_{1B}-adrenoceptor 30% of the α-adrenergic receptors in the rat lumbar spinal cord [429], while α-$_{1D}$-adrenoceptors do not appear to have a significant role [428].

Substance P Substance P- containing terminals are closely apposed to both sympathetic and parasympathetic preganglionic neurones projecting to the major pelvic ganglion [430]. Substance P- containing afferents in the pelvic nerve terminate in the outer laminae of the dorsal horn and in the region of the SPN and DGC[364, 431]. Substance P is also located in intraspinal neurons located in the dorsal horn [432] or DGC [433]. In young adult rats, substance P in the ventral horn is almost exclusively co-localized with serotonin and derived from descending axons of medullary neurones [430, 434, 435].

Notably, substance P is often co-localized with 5-HT in axon terminals in the lumbosacral spinal cord [436]. Functionally, substance P affects micturition reflex activity [437]; intrathecal administration of Substance P at spinal levels L5–S1 induces bladder contraction [438]. Substance P also increases the firing rate of sympathetic preganglionic neurons [439]. Studies in the rat show that substance P levels decline with ageing in both the dorsal and ventral regions of the lumbosacral cord [440, 441]. Substance P-immunoreactive innervation of the dorsolateral nucleus (supplying the EUS) is not obviously altered with ageing [442].

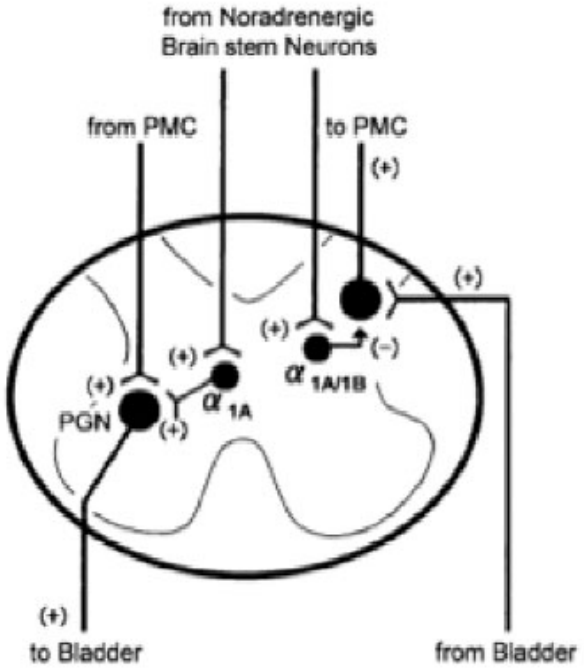


Figure 18: Scheme showing putative mechanisms using alpha 1 adrenoceptor subtypes in the L6-S1 spinal cord that contribute to the control of reflex activity of bladder in the rat. PGN preganglionic neurons, PMC pontine micturition centre. Descending noradrenergic pathways from the brainstem may excite inhibitory ineterneurons to regulate the sensory pathways from the bladder and excitatory interneurons to regulate the efferent pathway to the bladder. From Yoshiyama M. and de Groat W.C., 2001

5. NEURAL TRAFFIC

Low frequency (10 Hz) stimulation of the pudendal nerve elicits a continence-like response, by decreasing parasympathetic outflow in the pelvic nerve and increasing sympathetic outflow in the hypogastric nerve, thus inhibiting the bladder and activating the external urethral sphincter. Stimulation of both genital [443] and anal [444] sensory branches in the pudendal nerve results in bladder inhibition. If low frequency stimulation is applied during a bladder contraction the bladder pressure decreases and the activity of the external sphincter increases. When the pudendal nerve is stimulated at higher frequency in the cat (intact or after spinal transection), the response is fundamentally different [444]. Following spinal transection, pudendal nerve stimulation at 33 Hz elicits bladder contraction synergically with reduction in external urinary sphincter activity, provided the bladder contains more than a threshold volume. Indeed, efficient post stimulus voiding can be induced by intermittent high frequency pudendal nerve stimulation [425]. Such activity may be influenced by various peripheral receptors, exemplified by menthol (TRPM8) [445].

6. PELVIC ORGAN INTERACTIONS AT THE EFFERENT NEURAL LEVEL

a) Bladder and Outlet

Neural coordination of physiological and behavioral functions depends on convergence within the nervous system of information from relevant areas, and convergence could result in collateral effects of pathology in one organ affecting function elsewhere. There is extensive convergence of pelvic organ input [446, 447] at the levels of the spinal cord, dorsal column nuclei, solitary nucleus, medullary reticular formation, and thalamus [448]. Convergent processing underpins the coherent functioning of systems controlled by efferent outflows diverging from a common starting point, as exemplified by the synergic co-ordination of bladder and urethra required for normal voiding. The fundamental role of supraspinal mechanisms in lower urinary tract synergy is well recognized. However, synergic lower urinary tract function may also be a feature of the peripheral innervation, independent of CNS co-ordination. In the female minipig, pre-ganglionic pelvic nerve stimulation evokes a pressure increase in the bladder and a pressure decrease in the urethra [449]. It remains to be determined whether this observation reflects coherent activation of separate motorneurones (excitatory to the bladder, inhibitory to the outlet), or whether postganglionic motorneurones send branches which supply both bladder and urethra. In the latter arrangement, release of different neuromuscular transmitters from branches of the same motorneurone, or interposition of an additional intermediary cell would be required. The former is circumstantially supported by the observed co-localization of acetylcholine- and nitric oxide-related enzymes [450].

b) Bladder and Bowel / Uterus

Clinicians are familiar with the detrimental effect of bowel disorders on lower urinary tract activity. Physiologically, the efferent limb of the micturition reflex is inhibited by afferent input from the rectum [451]; thus, rectal distention inhibits bladder activity via glycinergic and GABAergic mechanisms in rats [415]. Dual labeling studies show that many neurones in Barrington's nucleus supply both colon and bladder, with smaller populations supplying the two organs separately (**Figure 19; 20**). At the level of the major pelvic ganglion, double-labeled cells are relatively infrequent, but processes of colonic-retrograde-labeled cells often surround cell bodies of equivalent cells for the bladder. Dual-labeled cells in the spinal cord are rare [452].

The pelvic organs are supplied by sensory and autonomic fibers in the hypogastric nerve [453]. Inflammation of the uterine horn or colon gives rise to inflammation in the bladder, an effect that can be eliminated by sectioning the hypogastric nerve {454]. Bladder overactivity induced by inflammation is

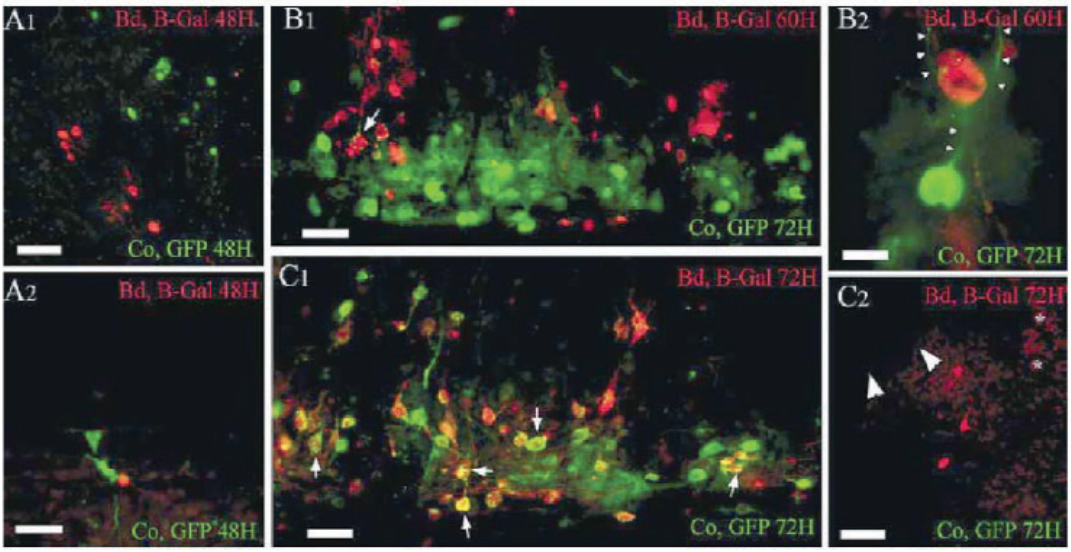
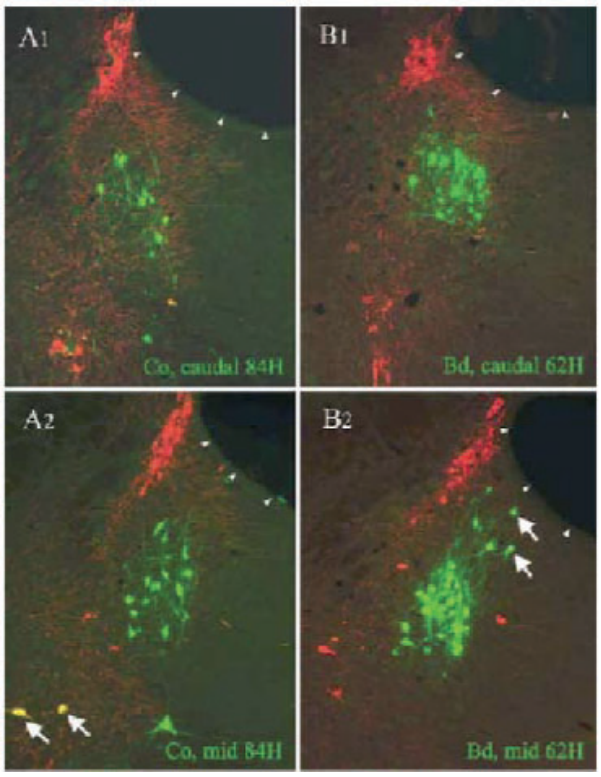


Figure 19: A, Fluorescent micrographs of sections at the level of (A1) the major pelvic ganglion, (A2, B1, B2 and C1) the lumbosacral spinal cord and (C2) Barrington's nucleus of rats injected with PRV-Beta-GAL in the bladder and PRV-GFP in the colon and having different survival times. The viscera, tracer and survival time are indicated in each photomicrograph. BD, bladder. CO, colon. A1. Separate labeling from both viruses is apparent in the MPG at 48 hours. A2. In the same case, only occasional cells are labeled in the spinal cord. B1. Substantial labeling from both organs is visible in the preganglionic parasympathetic column of the spinal cord and most cells are singly labeled from either the colon or the bladder. The arrow points to a rare double-labelled neuron. B2. Bladder- and colon-related neurons in close proximity. Processes from the colon-related neuron (arrow heads) are apposed to the bladder-related neuron. C1. Increasing survival time results in greater number of double-labelled cells (arrows). In most double labeled cells PRV-Beta-GAL label from the bladder is surrounded by PRV-GFP label from the colon. C2. Section from Barrington's nucleus from the same case as C1 indicating that only a few cells are transsynaptically labeled from the bladder and none from the colon at the survival time. Arrow heads point to the surface of the fourth ventricle and stars indicate the location of trigeminal mesencephalic neurons. Calibration bars: 50 µm A1 and A2, 30 µm B2 and C1, 50 µm B2, 50 µm C2. From Rouzade-Dominguez, M., et al. 2003.



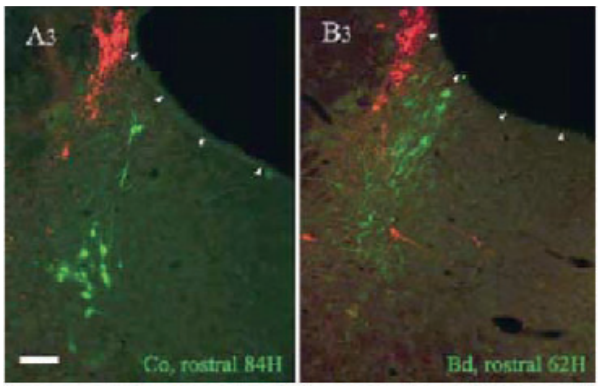


Figure 20: Fluorescent photomicrograph of 30 µm thick coronal sections through dorsal pons, proceeding caudal to rostral. A, Sections from rats injected with PRV in the distal colon. B, Rats injected with PRV in bladder. PRV-labelled neurons are green and TH immuno-labelled neurons are red. The arrows in A2 point to double-labelled neurons, which appear yellow. The arrows in B2 point to bladder-related neurons near the ventricular surface. Dorsal is at the top, medial is to the right. BD, bladder. CO, colon. Arrowheads show the ventricular surface. Calibration bar 60 µm. From Rouzade-Dominguez, M., et al. 2003.

influenced by estradiol, probably mediated through effects on the sympathetic nervous control of the bladder [455]. There are several potential mechanisms by which neural input could contribute to emergence of inflammation in neighbouring organs [454] (**Figure 21**);

1. Axon reflexes occurring in hypogastric sensory nerves that branch to supply more than one pelvic organ. Though such branching has not yet been specifically identified, a small proportion of single afferent fibres may branch to supply the colon and bladder [456].
2. A dorsal root reflex; hypogastric afferents from the inflamed organ could, via a spinal interneuron, sensitize and antidromically activate other hypogastric afferents from an uninflamed organ, exemplified by sensitization of a population of spinal neurons responding only to bladder input with chronic colonic inflammation [457].
3. Input from the inflamed organ (via the hypogastric nerve) activates neurons in the dorsal horn that activate postganglionic neurons in the pelvic ganglion via thoracolumbar preganglionic neurons.
4. A spinal mechanism could be mediated by intraspinal connections to lumbosacral pre-ganglionic neurons (as seen for gynaecological organs [458]).
5. A suprasacral mechanism could be mediated through the brain stem [452, 459, 460].

7. EFFERENT INHIBITION

The possibility of bladder inhibition by the CNS can be inferred from various experimental observations [461]. The sympathetic nervous system mediates inhibition of ganglionic transmission to the bladder. Isolated whole bladders manifest spontaneous contractile activity [118, 461-465], suggesting the possibility of active neural inhibition of the bladder during urine storage. In the decentralised bladder, following transection of sympathetic input, addition of the ganglion blocking agent hexamethonium leads to an increase in spontaneous bladder activity, likewise suggesting that a peripheral reflex inhibits the bladder during urine storage [461]. Rat brainstem/ spinal cord/ bladder preparations or neonatal spinal cord/ bladder preparations show tonic inhibition, arising at L6-S1 and involving a peripheral ganglionic synapse [465-467]. Clearly, efferent inhibition of the bladder will facilitate urine storage.

In the neonatal rat, considerable activity arises in the bladder wall when inputs from the lumbosacral spinal cord are disrupted [465]. Selective spinal cord and root lesions indicate that intrinsic bladder activity of the neonatal rat is tonically inhibited by parasympathetic efferent outflow [465]. This path is additional to the predominant cholinergic preganglionic efferents mediating the main voiding reflexes. The functional difference in the two sets of cholinergic ventral root efferents may result from differing synaptic targets, since both are blocked by the nicotinic antagonist hexamethonium [465]. Thus, inhibitory efferents must synapse with noncholinergic inhibitory neurons in the major pelvic ganglia, in contrast to excitatory efferents synapsing with the cholinergic detrusor innervation.

In addition to efferent input, local reflexes may contribute to the inhibition of detrusor activity, probably driven by interstitial cells [468], so that peripheral autonomous activity increases as a result of bladder distension [462, 469]. This has been proposed to signify the presence of a regional regulatory influence [470] and a peripheral “pacemaker” [471] and various mechanisms for the propagation of activity within the bladder wall [464]. In the clinical context, processes affecting peripheral innervation would then predispose to emergence of inappropriate detrusor activity during urine storage (through loss of inhibitory fibres), associated with inefficient bladder emptying (through loss of excitatory fibres).

8. PERIPHERAL EXCITATORY MECHANISMS

Agonist exposure appears to elicit contraction by two different mechanisms, comprising a component derived from direct stimulation of the muscle cell (‘classical’ efferent), and a separate component which is more phasic, responsible for the obvious pressure fluctuations. The latter ‘intrinsic’ mechanism may involve an intermediary cell type [472]. Optical imaging and calcium-/ voltage-sensitive dyes in whole rat bladder preparations have detected electrical activity moving in a co-ordinated manner from localized regions over the entire bladder [473, 474]. The isolated whole bladder shows regionalized responses when exposed to cholinergic/ muscarinic agonists [463, 465, 472, 475, 476]. Dynamic migrating localities of contraction and elongation, give rise to a complex mix of micromotion phenomena, including micro-contractions, microstretches and propagating waves. Several species show differences in contractile activity according to the region of the bladder from which a muscle strip is taken. Different effects are seen according to stage of development- spontaneous activity is not apparent in bladder strips from neonatal rats [401], but subsequently emerges, so that at one month, high-frequency spontaneous contractions occur in conjunction with high-amplitude, low frequency contractions.

The likely functional significance of peripheral excitatory mechanisms is exemplified by the rodent neonate voiding reflex, which is induced by parental stimulation of the perineum, prior to establishment of mature control by the higher micturition centres [401, 465]. The physiological role of such activity in the adult is not known, but could include; 1. Optimization of the bladder wall configuration for volume contained,

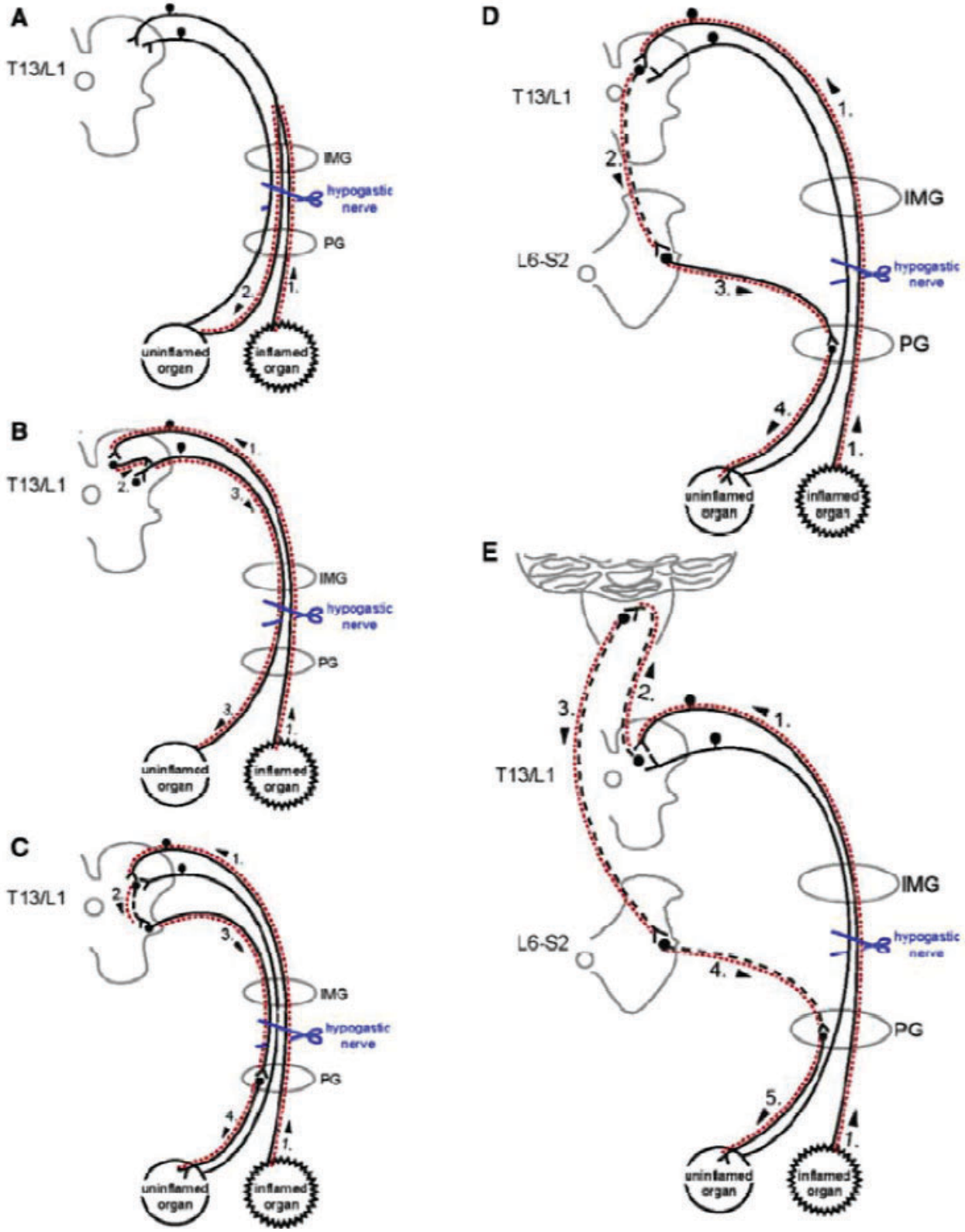


Figure 21: Five compatible mechanisms by which hypogastric nerve fibres can contribute to the process of inflammatory induction between organs. A.Branching sensory afferents. B. Dorsal root reflex. C. Multisynaptic route involving sensory afferents from the inflamed organ to the T-13/L1 segment of the cord followed by output from preganglionic fibres in the T13/L1 segment to postganglionic in the pelvic ganglion that innervate the uninflamed organ. D. Multisynaptic route involving sensory afferents from the inflamed organ to the T13/L1 segment of the cord followed by output from preganglionic fibres in the L6-S2 segments to postganglionic fibres in the pelvic ganglion that innervate the uninflamed organ. E. Multisynaptic route involving sensory afferents from the inflamed organ to the T-13/L1 segment of the cord followed by output from preganglionic fibres in the L6-S2 segments to postganglionic fibres in the pelvic ganglion that innervate the uninflamed organ. In this case the multisynaptic route includes ascending connections from spinal cord to brain and descending connections from brain to L6-S2. From Winnard, K.P. et al. 2006.

to ensure efficient voiding regardless of volume [477], 2. Stimulation of 'in series' receptors for signaling bladder volume [476], 3. A mechanistic component of accommodation during filling, a counterintuitive suggestion supported by the observation that accommodation in the colon involves synchronous contraction and relaxation [478], 4. Servoassistance of voiding for maintenance of contraction [479]. The potential importance of peripheral functional structures may explain the recognition that nerve growth factor can contribute to overactive bladder problems [480].

V. MIDBRAIN-BRAINSTEM CONTROL OF BLADDER FUNCTION

With the direct role of both limbs of the autonomic nervous system in regulating filling and voiding of the bladder, the involvement of brainstem circuitry is paramount in normal bladder function. The brainstem is involved in reflexes controlling filling, storing and emptying of the bladder. Clinical cases of lesions within specific brainstem regions, most notably the pons, can result in either bladder continence or incontinence problems. This section will review the current understanding of brainstem neuronal networks that influence the parasympathetic and sympathetic motor outflows destined for the detrusor and smooth muscle of the urethral sphincter.

1. AFFERENT PATHWAYS TO THE BRAINSTEM

Sensations of bladder fullness are conveyed to the spinal cord by the pelvic and hypogastric nerves, while input from the bladder neck and urethra is carried in the pudendal and hypogastric nerves. Afferents arising from the bladder and urethra are mechanoreceptive (Aδ fibres) and nociceptive (C fibres). The most important afferents for initiating micturition are those passing in the pelvic nerves, whose fibres terminate in discrete regions of the lateral aspect of the dorsal horn of the lumbar and sacral spinal cord (see [481, 482, 483] for reviews). Many of these dorsal horn neurones make spinal connections that mediate segmentally organised reflex responses. However, a proportion of the spinal interneurones send ascending projections to the brain. These afferents are involved in transmitting sensory information to the cerebral cortex and can trigger micturition mediated by neural circuitry within the brainstem.

Ascending fibres from dorsal horn neurones receiving afferent input from the bladder terminate in the gracile nucleus and the central aspect of the periaquaductal grey (PAG; **Figure 22**); the latter has been described as a most dense spinal innervation [483, 484], perhaps emphasizing its essential role. The gracile nucleus relays information regarding nociception to the thalamus and cortex. Those PAG neurones receiving direct spinal inputs project to multiple sites including the pontine micturition centre (PMC) and the thalamus for onward relay to the cerebral cortex [483]. Although these data are based on animal species, the pivotal role of the PAG and PMC has been confirmed in man using positron emission tomography [485, 486] and functional magnetic imaging [487] with and without a full bladder (see **Figure 23**). Indeed, the PAG projections to the PMC originate from its lateral aspects [488]. Since the spinal projections relaying bladder distension target the central PAG there is the possibility of some integration prior to onward transmission to the PMC. This is likely to include integration of descending information from higher brain regions such as the limbic system and cerebral cortex [483], which provide contextual information concerning the appropriateness for micturition. Recently, the parabrachial nucleus appears activated during bladder contraction and may also receive afferent inputs directly from the spinal cord or via other central structures (**Fig 23**).

2. DEFINING BRAINSTEM CIRCUITRY REGULATING BLADDER FUNCTION

It is evident from recent reviews [481, 482] that most regions of the brainstem involved in controlling bladder function are known. However, as is evident in figure 4 of de Groat's (2006) review, it remains unclear how these central nervous structure are connected up (**see Figures 23 & 24**). Are there, for example, brainstem pathways running in parallel and/or series that drive the preganglionic motoneurones? A method of revealing key brainstem regions regulating end organ function that has the ability to address this is transneuronal retrograde labelling using pseudorabies virus (PRV). Both the bladder wall and urethra of the rat have been injected with PRV. This has resulted in similar regions of the brainstem being labelled, which supports the close coordination of detrusor and urethral muscle function as suggested previously [481]. The brainstem regions labelled included Barrington's nucleus (the pontine micturition centre), midline raphe (magnus and obscurus), locus coeruleus (A6), subcoeruleus, A5, reticularis gigantocellularis and nucleus paragigantocellularis [489, 490] but also in neonatal rats only the prepositus hypoglossal nucleus and lateral vestibular nucleus [489]. In addition, the PAG was labelled (**Figure 22**). Recent work has also indicated a role for the medial parabrachial nucleus (**Figure 22**). Whilst this provides information concerning the major players within the brainstem, the PRV technique does not imply precise information about connectivity. For example, are all these brainstem regions connected in parallel to the spinal interneurones and / or pre-ganglionic neurones or are some positioned in series? Are reciprocal connections between these nuclei present? Much of this information remains unknown but seems important if one is to understand the way in which the brain controls bladder storage and emptying and how one obtains reversible switching between these essential

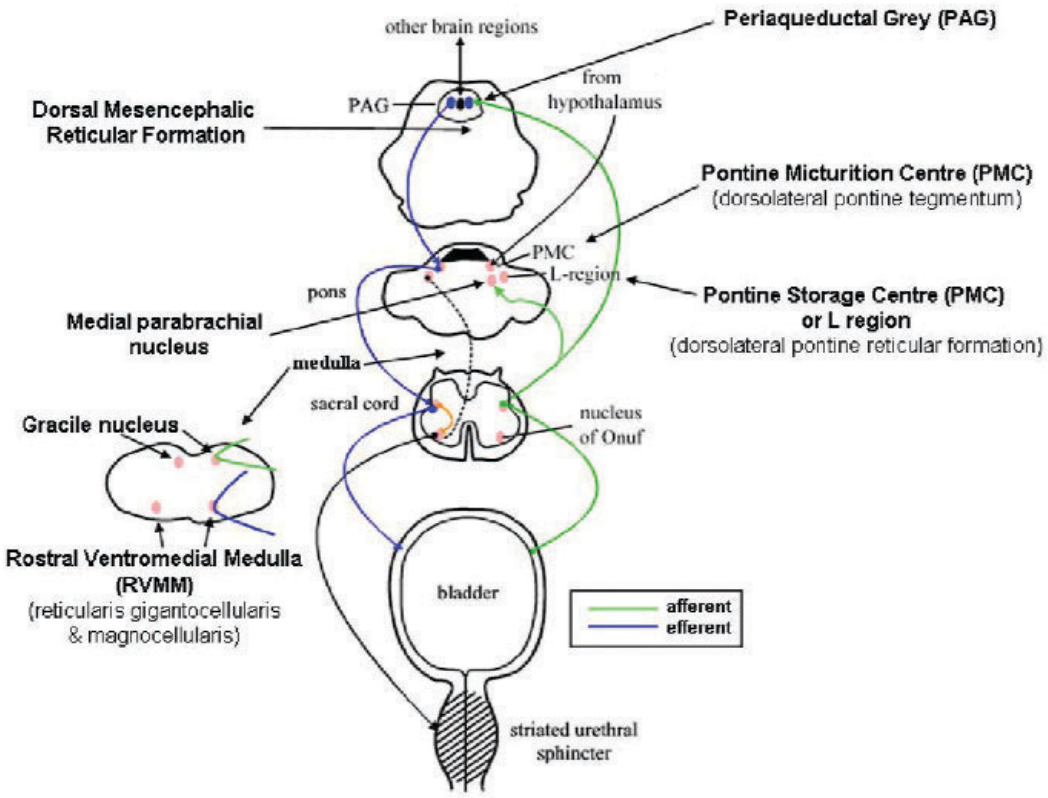


Figure 22: Schematic representation of the described sensory and motor pathways regulating bladder function in animals. Of note are roles for gracile nuclei in transmitting afferent information from the spinal cord to the thalamus and cortex; the pontine storage centre activated during bladder filling, the lateral parabrachial nucleus (as a sensory integration centre) and the rostral ventromedial medulla as a centre important during voiding. For further details and all references see text.

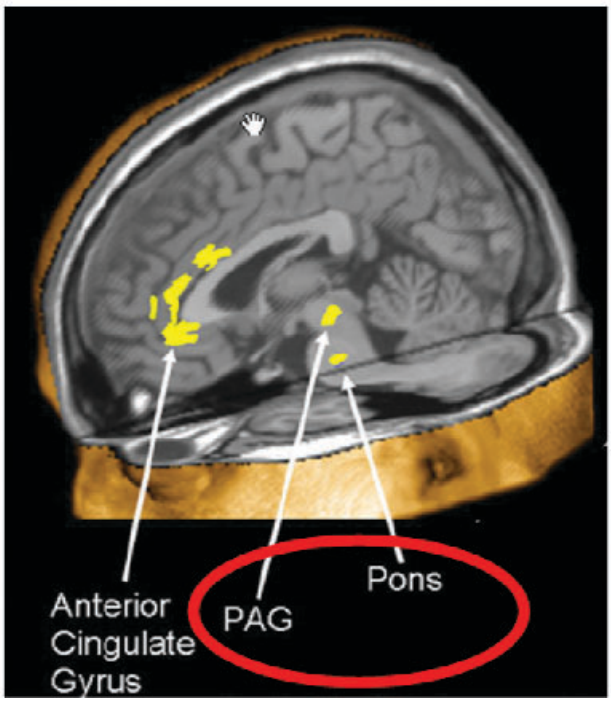


Figure 23: Consistent with animal studies, regions of the human brain, imaged using positron emission tomography including aspects of the midbrain (periacqaductal gray, the dorsal mesencephalic reticular formation) and brainstem (dorsolateral pontine tegmentum-pontine micturition center and dorsolateral pontine reticular formation-pontine storage centre) are evident when the bladder is filled. (From Kavis et al., 2005).

functions. Most anatomical, electrophysiological and imaging studies to date have concentrated on the PMC as this appears pivotal regarding bladder function in both animals and man.

3. THE PONTINE MICTURITION CENTER (PMC)

In the rat, the PMC is just dorsomedial to A6 [293, 491] whereas in the cat it appears to be within A6 extending ventromedial into the mesencephalic tract of the trigeminal nerve [266]. It is these regions that are termed Barrington's nucleus- a region named after Barrington, who in 1925 was the first to describe this pontine control centre for micturition in the cat (**Figure 22**). In man, comparable regions in the pons can be imaged and found activated when the bladder is full (**see Figure 23**; [486]).

a) Barrington's nucleus: is it the master of ceremonies for bladder emptying

Based on the finding that its activation (electrical or chemical stimulation) in rats and cats relaxes urethral sphincters and initiates bladder contractions [293, 492, 493], it seems reasonable to propose a dominant role for the PMC in micturition. Moreover, lesions localised to this pontine region in animals abolish micturition and cause urine retention [293]. Similarly, a PMC lesion (on the right hand side) resulted in urinary retention in man [494]. Thus, the PMC would appear to play a major point of convergence of numerous pro- and anti micturition drivers. Thus, it may be a major integrating centre and, indeed, possible 'command' centre for initiating and orchestrating the act of bladder emptying.

b) How is the PMC connected up?

The inputs to, and outputs from, the PMC are considered below:

1. Inputs: As described above, the lateral aspects of the PAG send information regarding the degree of bladder distension. These inputs are powerful and can trigger micturition as evidenced by the finding that chemical stimulation of ventrolateral PAG can cause voiding [488]. Recently, Kuipers et al. [495] have examined the afferent inputs to the PMC using conventional anterograde and retrograde tracing techniques. In addition to the PAG (ventrolateral and dorsomedial aspects) inputs arose from: ventromedial pontomedullary tegmental field (**Figure 22**); medial preoptic area, posterior hypothalamus (perifornical region). It is unclear as to the exact function of the pontomedullary tegmental field, although Holstege believes this is a diffuse input providing a 'general level-setting' of PMC neuronal activity [496]. This might be interpreted as a potential mechanism for establishing a neuronal set-point or threshold beyond which micturition occurs. The medial pre-optic region may well provide inhibition and this may be important during sleep and/or sexual activity to suppress micturition [266, 483, 497], although the previous work by Gjone demonstrated that micturition could be evoked following activation of this region [498], which is inconsistent with an exclusive inhibitory role. The posterior hypothalamic region is involved in fight or flight (defense response). Its connection to the PAG may account for the urination reported in conscious animals during a defense response, since regions of the PAG can also evoke the defense response [499] and, via PMC, could also initiate urination [500].

The study by Kuipers et al [495] in the cat failed to confirm the numerous central nervous afferent inputs to the PMC described by Valentino et al. in the rat [501]; these included the Kolliker Fuse nucleus, raphe, nucleus tractus solitarius, parabrachial nucleus, nucleus paragigantocellularis and the cuneiform nucleus. Whether a species difference can explain this seems unlikely. Certainly the regions labelled by Valentino et al. [501] map more closely to data obtained using PRV injected into the bladder wall or urethra (see [489, 490, 502]) as described above. With this said, it is likely that many of the nuclei that project to the PMC also connect to each other but this needs confirming using localised injections of neuronal retrograde and anterograde tracers.

2. Outputs*:* During micturition the PMC exerts simultaneous excitatory influences on parasympathetic outflows that cause bladder contraction and complete emptying but inhibition of sympathetic preganglionics to relax the urethral sphincter. It is, therefore, entirely appropriate that within the PMC there are bulbospinal neurones (**Figure 24**): PMC sends direct projections to parasympathetic preganglionic motoneurones innervating the bladder [503]. The latter authors provided ultrastructural evidence of asymmetric (excitatory) synaptic contacts on sacral para-sympathetic preganglionic neurones originating from the PMC. Moreover, single neurones have been recorded extracellularly from Barrington's nucleus in cats (i.e. medial to the mesencephalic tract of the trigeminal nerve) and found to have spinally projecting axons based on antidromic invasion testing from the dorsolateral funiculus of the first sacral segment [504-506]. An involvement of glutamate as the main neurotransmitter in this pathway has been described [507]. Such a pathway would explain bladder contraction. Urethral relaxation requires an inhibition of somatic motoneurones and this appears to be mediated from PMC projections onto inhibitory interneurones located in the intermediolateral cell column at the sacral segmental level [278]. Both glycine and gamma amino butyric acid are thought to play a role here [279, 508].

c) On-switching micturition: a role for the PMC

With the PMC being essentially a pre-motor (parasympathetic/sympathetic) micturition nucleus, it should fulfil certain criteria if it is involved in the initiation

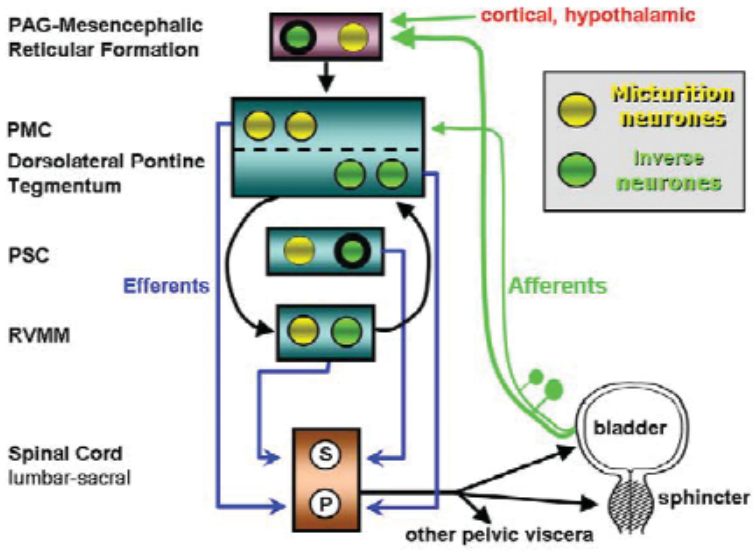


Figure 24: Schematic depicting neuronal types (micturition or bladder contraction) versus inverse or bladder filling within the major midbrain and ponto-meduallary regions involved in central neural regulation of bladder function. A major future aim must be to understand both the intra-and inter- nuclear connectivity of neurons involved in bladder contraction and filling. Who drives who and what terminates firing of neurones during bladder filling and contraction. Additionally, how are the volitional drives integrated with the automatic control circuitry of the lower brain. Abbreviations: P, parasympathetic neuron; PAG, periacqueductal grey; PMC, pontine micturition centre; PSC, pontine storage (continence) centre; RVMM, rostral ventromedial medulla; S, sympathetic neuron.

of bladder contraction and sphincter relaxation. For example, neurones should project to the spinal cord, fire ahead of a bladder contraction and fire continuously with each bladder contraction. Previous studies have found pontine units that fired in phase with bladder contraction [509, 510], but whether these connected to the spinal cord directly was not tested. However, subsequent single unit recordings by Sasaki [504, 505] and Sugaya et al. [506] provide convincing evidence that many PMC neurones do project to the spinal cord. Studies have shown that feline spinally projecting neurones (as demonstrated using the antridromic collision test) located in Barrington's nucleus discharged in phase with bladder contractions (**Figure 25**). Many had firing patterns not different to those recorded from bladder afferents. But this is not always the case. Indeed, some of these units fired prior to the onset of bladder contraction consistent with a role in initiating bladder contractions (**Figure 25**). Whilst the proof of causality is always a difficult one, these neurones do make excellent candidates for driving/initiating bladder contraction. Indeed, many were also shown to be synaptically activated (orthodromically) following electrical stimulation of the spinal cord [506] raising the possibility of bladder afferent input, which may assist in providing positive-feedback and additional excitatory drive to both sustain and possibly amplify firing during micturition. The latter of course should be taken cautiously as it is not clear whether the spinal input demonstrated originated from bladder mechanoceptors. Physiological stimulation of the bladder mechanoreceptors themselves, rather than electrical stimulation of the spinal descending afferents, would be necessary to confirm this.

The issue of whether the PMC is the only pre-motor micturition initiating region is addressed by Sugaya et al. [506]. From their recordings, units localised to the rostral medial medulla (reticularis gigantocellularis and magnocellularis) could also be activated antidromically from the spinal cord (L1) and also fired in or out of phase with bladder contractions (**Figures 22, 23 & 25**). These units included those that fired just before and/or with bladder contractions as well as firing in between bladder contractions. Interestingly, many of these rostral medial medullary units responded at short latency (2 to 3 ms) following stimulation of the PMC, supporting direct orthodromic activation. Thus, Sugaya et al. [506] proposed two descending pathways from the PMC for initiating micturition: one direct to the parasympathetic preganglionic neurones and the other via the medial reticular formation. Incidentally, the study also showed that these medullary units could be orthodromically activated from L1 indicating the potential for bladder mechanosensitive afferent drive to these neurones, although no direct proof for this was given that this spinal input originated from the bladder wall.

A question arises as to whether the spinally projecting PMC and rostral medial medullary neurones are actually defined pre-parasympathetic versus pre-sympathetic neurones? Said differently, does a single unit project to both autonomic preganglionic neuronal types in the spinal cord (see [508, 511]). This relates to the current understanding that during micturition the bladder contracts (parasympathetic activation) but relaxes the urethral sphincter (somatic inhibition). No data exist for this presently. However, in the nucleus tractus solitarius located in the dorsomedial medulla, a similar issue exists. Here, neurones receive mechanoreceptor sensory inputs from arterial baroreceptors activated by increases in blood pressure, which when activated mediate simultaneous excitation of cardiac parasympathetic motor outflow but inhibition of vasomotor sympathetic activity, thereby reducing cardiac output and peripheral resistance respectively that together lower arterial pressure to normal levels. Simms et al. [512] recently reported that the cardiac parasympathetic limb was activated at a

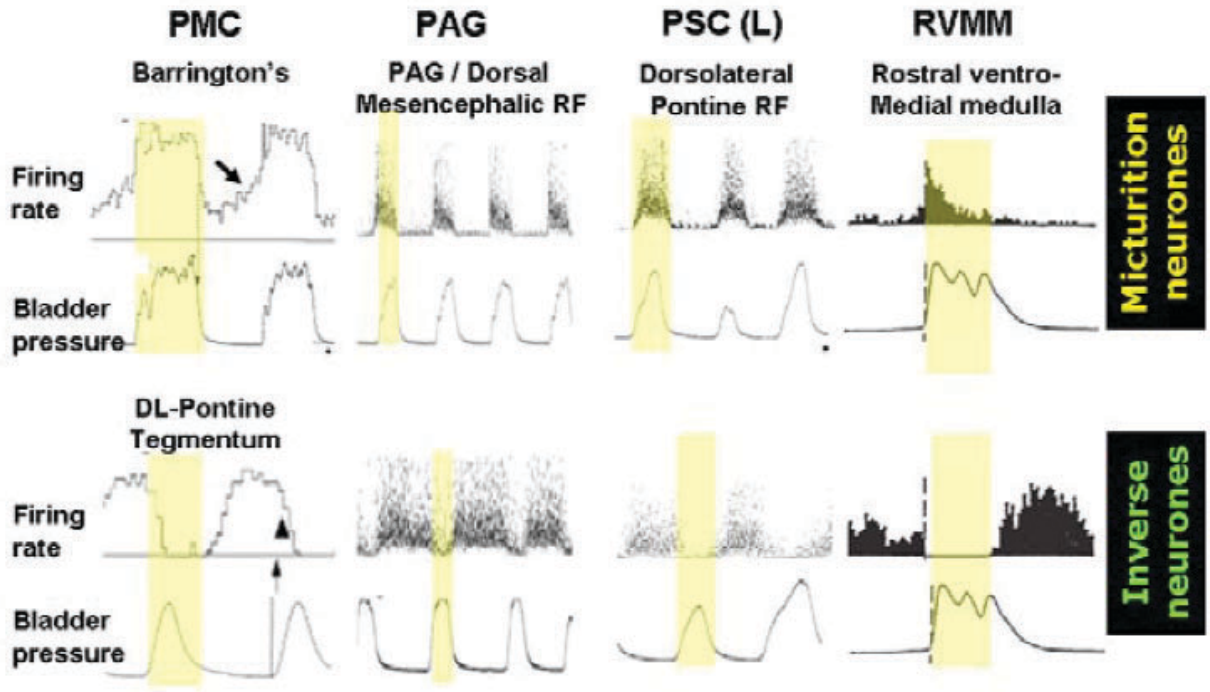


Figure 25: A montage of neuronal activities during bladder contraction and filling located in major regulatory brainstem and midbrain regions. Note that not all neurons showed firing to a single phase; phase-spanning (i.e. from bladder contraction to relaxation) exists. A challenge for future studies is to confirm these neuronal activities as causal to bladder control (and not reactive to simultaneously occurring responses such as those in arterial pressure, breathing, heart rate that accompany micturition). Data compiled from Liu et al (2004), Sakakibara et al (2002), Sasaki (2004; 2005) and Sugaya et al (2003).

higher pressure threshold compared to the sympathoinhibition. It was surmised that output neurones within NTS are dedicated and already destined for either the parasympathetic or sympathetic networks [512] Thus, a similar organization may exist within the PMC and could be teased apart by differences in the bladder pressures threshold necessary for bladder contraction versus urethral relaxation. Moreover, each autonomic limb of the baroreceptor reflex could be modulated differentially (e.g. [513-515].

4. BLADDER 'FILLING' NEURONES IN THE PMC AND MEDIAL RETICULAR FORMATION: WHAT'S THEIR ROLE?

Within both the vicinity of the PMC and medial reticular formation, neurones were encountered that fired during the interval between bladder contractions but were silent during contractions. These were termed type 2 neurones by Sugaya et al. [506] and 'inverse neurones' by Sasaki et al. [516]. Many of these neuronal types had spinal projections (e.g. 26/35 type 2 neurones of Sugaya et al. [506]) but some could not be antidromically back fired. Although the latter is not definitive proof that these cells do not project spinally, the data are suggestive that they form a group of interneurones.

So, what is the function of these spinally and non-spinally projecting inverse neurones? Considering those with spinal projections, these cells might provide excitatory drive to sympathetic pre-ganglionics destined for the detrusor muscle to bring about active relaxation, via $\beta_{2/3}$-adrenoceptor stimulation. In regard to the non-spinally projecting neurones that fire out of phase with bladder contractions, these may be involved in ensuring that the neurones which are active during bladder contractions that project spinally to the parasympathetic preganglionic neurones remain silent during filling via local inhibitory connections. If so, one might predict that they contain GABA or glycine as an inhibitory neurotransmitter.

Future studies could consider difference in neuronal phenotype spinally and non-spinally projecting neurones by juxtacellular labelling and *post-hoc* immunocytochemistry or *in situ* hybridisation to identify their neurochemical content (glutamatergic versus GABAergic, for example) as elegantly performed by others in the cardio respiratory regions of the medulla [517, 518]. Equally, the non-spinally projecting PMC neurones that fired in phase with bladder contractions could provide a source of synaptic inhibition to prevent neurones involved in bladder relaxation from firing prematurely.

5. OFF-SWITCHING MICTURITION – THE PONTINE CONTINENCE CENTRE (PCC)

As Sasaki (2004) points out [505], since the majority of units recorded from Barrington's nucleus (60/76) continued to fire after the onset of bladder relaxation and that almost all inverse neurones do not start to fire until after relaxation is well underway (Fig 25), there must be a separate region for 'off-switching' bladder contractions and for relaxing the detrusor muscle. Simultaneously the urethral sphincter should constrict to allow filling and prevent incontinence. For these functions, the pontine continence centre or PCC is considered. This region was also termed the L region being distinct from the M region designated to be the PMC (see [519]).

Where is the PCC, what types of neurones exist and what are their connections?

The PCC is located in the dorsolateral pontine tegmentum (**Figure 22**). Neuroanatomical evidence indicates that PCC neurones do not project to spinal regions influencing detrusor muscle. Rather, projections appear to be limited to Onuf's nucleus in the sacral cord. This nucleus contains the urethral sphincter motoneurones, suggesting that PCC drives their contraction. Perhaps some of the PCC neurones therefore have a defined pre-motor autonomic function as discussed above in the context of the arterial baroreceptor reflex. However, activation of PCC can stop micturition contractions [520]. Importantly, 78% of PCC neurones fire during the relaxation phase of bladder contractions (**Figure 25**) and the onset of their firing can be prior to the initiation of bladder relaxation [521].

Indeed, this would make sense if their prime function is to close the urethral sphincter. Another potential role is in off-switching micturition. One argument against this is that there appear to be no connections between the PCC and PMC, although a small number of PCC units could be either ortho- or anti- dromically activated from the PMC using electrical stimulation [506], which does not necessarily indicate the origin of the input or axon terminal. Others believe that the PMC and PCC act independently [520].

However, evidence supporting a role in off-switching micturition is that lesions within PCC cause incontinence, excessive detrusor activity, an inability to store urine and relaxation of the urethral sphincter. Further, activation of the PCC stops micturition, excites the pelvic floor musculature and contracts the urethral sphincter [278, 520]. Whether the PCC is actively involved in off-switching micturition to allow filling and storage and how it does this are questions requiring future experimental consideration. An area that may play a role in regulating micturition is the periaqueductal grey or PAG.

6. THE PERIAQUEDUCTAL GREY (PAG): IS THIS AN ESSENTIAL REGION FOR SUPPRESSING THE MICTURITION REFLEX?

The PAG is often referred to as a relay between ascending bladder afferent information and PMC activation of sacral parasympathetic preganglionic motoneurones. The present evidence suggests that the PAG is not just a simple 'relay'. As mentioned above, afferents of sacral origin terminate in central regions of the PAG but that onward transmission to the PMC originates from more lateral aspects [484, 488, 490, 502]. Therefore, some form of 'neural integration' within the PAG is very likely. Chemical stimulation of the ventrolateral PAG can trigger micturition together with defensive behaviours (see [522]) but equally inhibition of micturition can be evoked by stimulation of rostral dorsal and caudal ventral PAG regions as well as areas ventral to the PAG (e.g. dorsal mesencephalic reticular formation and nucleus reticularis pontis oralis; [523]). Lesions within the PAG abolish the micturition reflex in the pre-collicular decerebrate cat [524] but studies in human also report bladder overactivity [525]. Matsuura, Downie et al. (2000) have shown that micturition evoked by chemical stimulation of the ventrolateral PAG is mediated by Barrington's nucleus in the urethane anaesthetized rat. Moreover, these same authors were able to obtain coordinated sphincter activation from the PAG (with bladder contraction) as well as sphincter activation without bladder contraction at different sites within the PAG. This suggests the PAG is involved in both voiding and storage functions. This proposal is consistent with single unit recording from the PAG: Liu et al. [523] recorded from 84 micturition-related PAG neurones of which the majority were storage neurones (i.e. fired during bladder filling; 58%; **Figure 25**). Further, PET imaging revealed PAG regions becoming active during urinary retention in man [485]. However, close examination of the recording sites in Liu et al. study [523] reveals that the majority of neurones (68 of the 84) were located ventral to the lateral/ventrolateral PAG (in which 16 were recorded) in the dorsal mesencephalic reticular formation and nucleus reticularis pontis oralis. Nevertheless, based on the prominence of storage neurones in this region the idea that it plays a role in both urinary filling *and* suppression of the micturition reflex was advanced by Liu et al. [523]. They also proposed that micturition neurones may reciprocally depress the storage neurones. If true, this makes this part of the midbrain important in terms of providing a command locus for on- and off- switching micturition. In support of this was the observation of diverse firing patterns of the neurones including augmenting and decrementing [523]. Such firing patterns have been ascribed roles as 'phase-switching neurones' in the respiratory network [526], and could well play a role

in the transition between storage and voiding. Some of the midbrain cells recorded by Liu et al. [523] also fired across voiding/filling or filling/voiding transitions (**Figure 25**); this again supports the possible role in switching again akin to the brainstem respiratory network [526] between filling and emptying of the bladder. With the well established connections of the PAG that include orbital and pre-frontal cortex, amygdale, pre-optic region of the hypothalamus [500], it is proposed that the PAG may form a site for the integration of information from both higher centres, such as cortex, and bladder distension afferent inputs. With this said, the PAG is a clear contender for decision making about when to void or not, which it could execute via connections to PMC and PCC respectively.

7. NEUROTRANSMITTERS & MODULATORS WITHIN BRAINSTEM NETWORKS CONTROLLING BLADDER FUNCTION

There is not the scope in this review to provide a thorough detailed coverage of all transmitters and modulators affecting brainstem regulation of the urinary bladder. For recent reviews see: [481, 482]. These latter reviews highlight the importance of classical transmitters (glutamate, GABA and glycine) and non-NMDA, NMDA, $GABA_A$ and glycine receptors within the brainstem (and PMC) for regulating bladder capacity and micturition (see also: [297, 492]). Important observations include that both NMDA and non-NMDA receptors within the brainstem are important for micturition [527] and that the PMC is under tonic inhibitory tone mediated by $GABA_A$ receptors [492]. This prompts the question as to where and how this inhibitory tone is generated. Possibilities could be the PAG or higher centres, such as pre-optic hypothalamic structures, for example. Below is a brief account of two important and clinically relevant transmitter systems affecting bladder function via effects within the brainstem.

a) Serotonin

Many transmitter systems have been investigated by drug applications into the brain ventricles. Whilst this determines whether or not a modulating system has an effect on micturition it is difficult to assess where this might be exerted. In their review [528], Burgard et al. emphasise that serotonin appears to affect nervous control of bladder function at multiple levels including sensory processing of bladder wall afferents within the dorsal horn of the spinal cord and at the level of the spinal motoneurones. In all cases this appeared to be inhibitory on detrusor muscle activity but excitatory on urethral sphincter. A model proposed, was that 5-HT_{1a} receptors were located on the terminals of sensory afferent fibres to depress neurotransmitter release. Similarly, Ito et al. [529] have described a predominance of an inhibitory effect evoked from the midline raphe system extending from the pons to medulla on micturition in cats. Where this effect is mediated (i.e. supra-brainstem, pons or spinal cord) is unclear. Moreover, 79 raphe neurones were recorded in which their firing was related to bladder pressure with 66% related to storage [529]. These data support a role of the raphe system in suppressing micturition and facilitating external urethral sphincter activity in cats, which is consistent with earlier studies identifying a central inhibitory role for 5-HT1a receptors [283]. In stark contrast, this does not seem to be the case in the rat where 5-HT1a system facilitates micturition [530].

b) Dopamine

In Parkinson's disease, where there is a reduction in dopamine availability, the bladder is hyperactive (409). Yoshimura et al. [531] discovered that D_1 and D_5 receptors acting within supraspinal sites are inhibitory to micturition whereas other dopamine receptor subtypes were facilitatory. This is an important finding given the major clinical and psychological problems of incontinence.

• Looking towards the horizon

A number of pertinent questions regarding brainstem mechanisms regulating detrusor and urethral muscle have evolved. First, the issue of the neuronal mechanisms underpinning the switching from storage to voiding but also off-switching detrusor activity at the end of voiding remain unclear. Most brainstem neurones relating to bladder voiding/filling appear to show firing that is either in or out of phase with bladder contractions (as indexed by increases in bladder pressure). Whether this neuronal firing is a cause or consequence of the bladder contractions is unclear. Therefore, future studies could address the issue of the origin of neuronal activity (whether coincident with bladder contraction or relaxation) in terms of that generated centrally versus that which is dependent on sensory afferent mechanoreceptor feedback for midbrain and brainstem regions. Second, and a related issue, is following the volitional command to void, which lower brain structures execute this demand? Is this in the brainstem or midbrain (PAG) or higher centres? Based on the evidence presented here, a most likely candidate appears to be the PAG/dorsal mesencephalic reticular formation. This structure appears to have the right neuronal connections in terms of both ascending spinal afferent inputs and outputs to pontine micturition centres. In addition, it receives inputs from higher centres. Third, switching between voiding-filling-voiding has to be coordinated with the corresponding control of the urethral sphincters. This means there has to be cross talk between detrusor and sphincter neural circuits. Whilst evidence was discussed for a spinal inhibitory mechanism within the sacral cord, no such cross connectivity was found between PMC and PCC regions but there remains a possibility that some cross talk may exist in the PAG/dorsal mesencephalic reticular formation. Fourth, is whether there are within

the PMC/PCC (or PAG) neurones that are dedicated to the sympathetic or parasympathetic nervous system that exist as pre-motor autonomic neurones. It was discussed above that such organisation exists for brainstem control of the baroreceptor reflex in regulating arterial pressure. Finally, some technical issues. Most studies inflate the bladder and work on spontaneous isovolumetric contractions. This is clearly not physiological as the bladder does not void. This will affect detrusor muscle mechanoreceptor afferent feedback as well as afferent feed back from the urinary tract. The bladder volume/pressure always remains above the micturition threshold, again non-physiological. Many of the early experimental studies were based on the cat. Typically this was decerebrate raising the issue of how this relates to the conscious intact animal where higher centres are intact and known to regulate micturition. It is clear that more recent studies have ventured to the rat and most recently man. One wonders how compatible the regions regulating bladder function is between species and whether the connectivity and neuronal mechanisms are preserved or different. A good example is the opposing effects of 5-HT1a receptors in cats (inhibit micturition) versus rat (pro-micturition; for references see above). This is an important issue in terms of translating work from cat/rat to man. Whilst serotonin seems to be inhibitory to detrusor muscle activity in cat this is not the case in rat. Thus, the idea that modulation of the descending serotonergic system as a potential treatment strategy for incontinence in human [528] will need further validation. However, it is notable that areas identified as key brainstem sites for micturition in animal models do relate to regions identified in man using fMRI [486]. Finally, a universally agreed consensus needs to be defined as to how to characterize a neuron involved in central control of the bladder. Basing it entirely upon its firing response to inflation or contraction of the bladder could give false positive information. Since bladder afferents can affect other autonomic variables (breathing, blood pressure, heart rate) one has to be sure that the neuronal response obtained is not a consequence of alterations in these other variables. A proposal for stricter criteria to enable a more accurate characterization of neurons involved in urinary storage and voiding function, and sphincter control.

VI. CORTEX AND BRAINSTEM CONTROL OF BLADDER FUNCTION

1. BACKGROUND

The aim of this section is to present a synthesis of the human literature and review what this tells us about brain regions mediating specific aspects of human bladder behaviour.

Prior to the advent of functional brain imaging techniques, our knowledge of the cerebral and brainstem areas involved in the control of micturition was based on a small number of carefully observed clinical cases: patients presenting with specific bladder symptoms who were found by investigation to have lesions at particular brain sites. However this “lesion” literature is really quite sparse, presumably mainly because if bladder symptoms occurred as a result of a cerebral lesion the clinical picture was usually dominated by a variety of other neurological deficits and furthermore such bladder symptoms that there were would not have been of interest to the majority of neurologists. Initially the lesion studies were based on observations made in life correlated with post mortem or pathological specimens, but with increasingly better means of imaging it was possible to correlate symptoms with smaller, more discrete abnormalities.

From what was written, the importance of the frontal cortex and pons was clearly recognized and there are a number of case histories of patients reported with lesions at either site. Subsequent functional brain imaging studies confirmed the clinically based findings and then greatly extended our understanding of the complex cortical and subcortical networks involved in sensing bladder filling and activating appropriate voiding. In fact the few further cases of “cortical bladders” described since the emergence of functional imaging findings have reported lesions at sites that previously would probably not have been recognized as being important in “lesion” evidence presented here and summarized in **Table 4**. In fact functional imaging and lesion studies should be regarded as complementary because, functional brain imaging provides information about regional activation of grey matter, whereas lesions may give information about damage to white-matter connecting pathways. The lesion literature also improves our understanding of patients’ complaints. Most of the functional imaging studies to be reviewed were carried out using either positron emission tomography (PET) or functional magnetic resonance imaging (fMRI). Both provide indirect measures of regional blood flow, assumed to be related to local neuronal activity, but their temporal resolutions are quite different: several minutes for PET versus a few seconds for fMRI. For this reason PET is good for measuring long-lasting states of a system, while fMRI is better for following relatively fast events. Experimental paradigms are therefore quite different for the 2 techniques.

2. ROLE AND IMPORTANCE OF CEREBRAL CONTROL OF VOIDING

In order to understand the cerebral control of voiding it is helpful first to examine what would happen if there were no such control. Provided brainstem and midbrain are intact, micturition is organized in 2 phases, storage

Table 4. Effect on bladder behavior of pathological changes in key brain regions.

Key brain regions	Pathology	Number of cases	Effect on bladder behaviour
Frontal			
	Various causes of frontal lobe pathology (1)	36	Altered bladder sensation and incontinence in absence of intellectual deterioration or retention 3 cases had retention
	Frontal lobe tumours (537)	7	Frequency, urgency incontinence
	Frontal abscess (540)	1	Retention
	Frontal abscess/haematoma (539)	2	Retention
	Anterior cerebral vascular lesions (538)		Various bladder disorders and hemiparaesis
ACG			
	Bilateral infarction of anterior cingulate gyri (553)	1	Complex behavioural changes and incontinence
	Glioma of ACG and supplementary motor cortex (533)	1	Urgency incontinence with loss of sensation
Insula			
	Glioma of insula and inferior frontal gyrus (533)	1	Incontinence without loss of bladder sensation
Hypothalamus			
	Pituitary tumours extending into the hypothalamus (534)	3	Urgency incontinence, weight loss, psychiatric symptoms
	Cystic lesion of hypothalamus (551)	1	
	Ruptured anterior cerebral aneurysm (556)	5	
PAG			
	Presumed inflammatory lesion (532)	1	Urinary retention
Pons	Posterior fossa tumours (558)		Voiding difficulty
	Brain stem tumours{Ueki, 1960 #2994		
	Brainstem vascular lesions (564)		
	Brainstem gliomas in children (563)	24	Voiding difficulty
	Developmental malformation (559)	1	Urinary retention and disordered eye movements
	Low grade glioma (560)	1	Paraparesis, urinary retention and disordered eye movements
	R pontine lesion, unknown (494)	1	Urinary retention and disordered eye movements
	Herpes encephalitis (561)	1	Urinary retention and disordered eye movements

and voiding, governed by reflexes involving the brainstem and spinal cord. During storage the urethral sphincter mechanism contracts tonically, preventing urine leakage, while the detrusor remains relaxed, so as to avoid developing a pressure that would expel urine. Urethral contraction is maintained by sacral reflexes known collectively as the 'guarding reflex'; detrusor relaxation is ensured by absence of excitatory parasympathetic input as well as active sympathetic inhibition provided by spinal reflexes (2).

During voiding the urethral sphincter relaxes, facilitating urine flow, and the detrusor contracts so as to expel urine. This coordinated relaxation and contraction of urethra and bladder respectively is driven by a long-loop spinobulbospinal reflex (2), shown schematically in **Figure 26**. As the bladder fills, increasingly strong bladder afferents travel via synapses in the sacral cord to the brainstem and midbrain, where they synapse in the central periaqueductal gray (PAG). If the afferent signals exceed a trigger level, efferent fibres in the more lateral PAG are excited and they in turn excite the pontine micturition centre (PMC). Efferent signals from the PMC descend to the sacral cord, where they excite an indirect inhibitory pathway via the nucleus of Onuf that leads to sphincter relaxation [535] and an excitatory pathway to the bladder that leads to detrusor contraction; thus voiding occurs. In the absence of higher control, the reflex system just described would lead to involuntary bladder emptying (i.e. incontinence) whenever the bladder volume reached a critical level. However human behaviour relating to bladder function is fundamentally different from that of other species because in modern-day society we void in a controlled fashion, preferably in privacy. Embarrassment with inappropriate voiding and feelings of shame about incontinence are deeply embedded in human behaviour. Voiding at a socially acceptable time and place is only achieved by maintaining strict voluntary control of the voiding reflex.

The decision to void is based on a combination of factors, including one's emotional state, an appreciation of the social environment and the afferent (sensory)

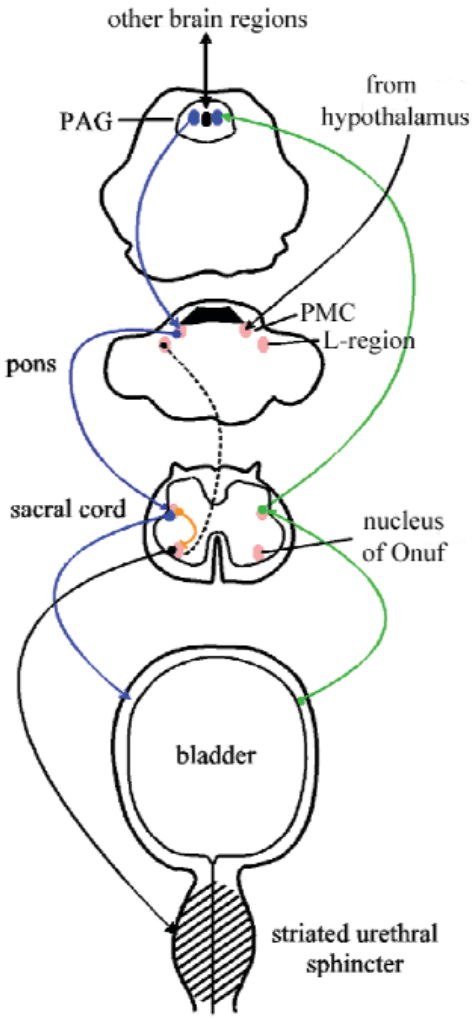


Figure 26 : The voiding reflex. The lines on the right (green) show bladder afferent activity ascending to the PAG during storage. Note there is no direct afferent input into the PMC.
On the left (blue), lines show activation of the PMC by the PAG, with descending activity to the sacral cord from where the parasympathetic innervation to the detrusor arises. An inhibitory synapse onto the motor innervation of the sphincter (Onuf's nucleus) results in relaxation of the striated sphincter muscle and thus synergistic detrusor sphincter activity. Note that higher brain regions control the voiding reflex almost exclusively via the periaqueductal grey (PAG), apart from a connection via the hypothalamus.

signals arising from the bladder. Knowledge of the extent to which one's bladder content is comfortable and 'safe' is central in this process. Thus, voluntary control of the bladder and urethra has two important aspects, namely registration of bladder filling sensations and manipulation of the firing of the voiding reflex. The PAG – the most rostral part of the reflex pathway shown in **Figure 26** – appears to have a pivotal role in both aspects. On the one hand it receives bladder afferents [535] and transmits them to higher brain centres and into the realm of conscious sensation. On the other hand it receives projections from many higher centres and controls the primary input to the PMC [536]. During bladder filling, such higher brain centres can suppress any excitatory signal from the PAG to the PMC and prevent voiding or incontinence. Then, when voiding is consciously desired, they can allow the PAG, and thus the PMC, to be excited.

Thus, during the storage phase, the net effect of higher (voluntary) control is tonic suppression of the voiding reflex. This suppression can be interrupted to allow voiding to occur if appropriate: i.e. if it is *necessary* (bladder volume is adequate), *socially acceptable*, and judged to be *safe*.

3. CORTICAL AND SUBCORTICAL CENTRES INVOLVED IN BLADDER CONTROL. EVIDENCE FROM OBSERVATIONS OF LESIONS AND FROM FUNCTIONAL BRAIN IMAGING IN HUMANS

a) Frontal Lobes

Although Andrew and Nathan (1) were not the first to describe disturbances of micturition resulting from a variety of causes of frontal lobe pathology, their celebrated paper reporting the syndrome of frequency, urgency (and in some patients faecal) incontinence causing distress to the patient, is regarded as seminal in the field. Their description of these patients cannot be improved upon.

"The patients described here were not demented, indifferent or lacking in social awareness; they were much upset and embarrassed by these symptoms. The acts of micturition and defecation occur in a normal manner; what is disturbed by this frontal lesion is the higher control of these acts. The lesion causes frequency and extreme urgency of micturition when the patient is awake, incontinence when asleep. The sensation of gradual awareness of increasing fullness of the bladder and the sensation that micturition is imminent, are impaired. When the syndrome is less pronounced, the sensation underlying the desire to micturate is absent, whereas the sensation that micturition is imminent still occurs. Then the patient is waylaid by a sudden awareness that he is about to pass urine; when neither sensation is experienced, the patient is amazed to find that he has passed urine. The threshold of the micturition reflex is much lowered. In the most complete form of the syndrome, the patient cannot inhibit the detrusor contraction of the micturition reflex; he is thus forced to empty his bladder as soon as the reflex occurs. When the syndrome is less pronounced, the patient can make a conscious effort to stop the act of micturition, and he may or he may not succeed. The lowering of the micturition reflex threshold may account for the fact that the patient

does not feel the normal gradual filling of the bladder that underlies the desire to micturate; but it cannot account for the unawareness of the sensations arising from the activity of pelvic and perineal muscles or of the sensation that urine is passing."

The cases of leucotomy in Andrew and Nathan's series were regarded as most useful for localizing the lesion causing the syndrome. The significant plane of the lesion was that lying immediately anterior to the tips of the ventricles and the genu of the corpus callosum. Such lesions involved grey matter, in particular the superomedial part of the frontal lobe (see **Figure 27**); but they caused a permanent disorder of the control of micturition and of defecation only when they involved some of the white matter lateral to the anterior horns of the lateral ventricle. Lesions that did not affect this white matter caused only transient disordered control of micturition. Correspondingly, Andrew and Nathan's Figure 10A suggests that most of the critical lesion area was in this white matter (**Figure 27**).

Subsequently the same features were observed in 7 patients out of a series of 50 consecutive frontal tumours [537]. In an analysis of patients with acute hemispheric strokes the occurrence of disturbance of micturition was found to be more common in frontal than occipital lobe lesions and there was an association with hemiparesis [538]. In 3 cases, Andrew and Nathan observed that lesions in similar areas to those discussed above led to urinary retention rather than incontinence (**Table 4**). Three further cases of frontal lesions with urinary retention have been reported. Successful treatment brought recovery of bladder function [539, 540].

Imaging studies, both PET [485, 541-544], and fMRI [545-548], are in agreement that, during bladder filling, storage and withholding of urine, there is activity in the right inferior frontal or dorsolateral prefrontal cortex, perhaps extending into the lateral part of the superior frontal cortex (**Figure 28A,B**). There is some right-sided predominance. In contrast there is little evidence for activation of the medial parts of the frontal cortex during storage. One PET study showed medial frontal activity during sacral nerve stimulation [548], but this is difficult to interpret; another study that showed medial frontal activity during filling [549] employed SPECT imaging, which has poor spatial resolution. An fMRI study showed abnormally *weak* activation in medial prefrontal cortex in subjects with urge incontinence [547], and further analysis revealed that bladder filling tends to provoke *de*activation in this region [550]. Regardless, there is little overlap with the superomedial frontal region described by Andrew and Nathan (1), except in part of the anterior cingulate gyrus (ACG; see **Figure 29**). These observations are consistent with the concept that functional imaging reveals *grey-matter* activation or deactivation, while lesions may damage critical links in *white-matter* connecting pathways as well as grey-matter regions.

Neuroanatomical observations indicate that there is a strong and direct connection from medial prefrontal cortex to PAG [536]. It is tempting to postulate that this pathway is responsible for tonic PAG suppression of the voiding reflex, thus maintaining the storage phase. White-matter lesions in this pathway would then allow involuntary excitation of the voiding reflex, with resulting incontinence. It would appear from this account that bilateral lesions would be required to cause incontinence, yet in practice a lesion on one side seems to be sufficient. Andrew and Nathan's suggestion – that this is because a unilateral lesion is likely to involve the fibres connecting one side with the other [551] – is difficult to follow.

Voiding has not been studied directly by fMRI, but there are 3 PET studies of voiding [535, 542, 543] and one fMRI study of simulated voiding [552]. All identify activation of the right inferior frontal gyrus (dorsolateral prefrontal cortex) and in the medial prefrontal cortex or nearby ACG (see next section).

b) Anterior Cingulate Gyrus (ACG)

The anterior cingulate is an extensive area with parts serving varied functions. The cases of aneurysm in Andrew and Nathan's series of frontal lobe pathologies were thought to have involved the anterior end of the cingulate gyrus (1) (**Figure 29**, cyan circle). Incontinence was observed as part of a complex behavioural disorder following bilateral infarction of the anterior cingulate gyri [553]. A recent report of a patient in whom a glioma in the right posterior ACG and supplementary motor area was resected described how she experienced urgency incontinence and loss of bladder sensation following surgery [533].

Many functional imaging studies have observed ACG responses – activation or occasionally deactivation – to bladder filling, storage or withholding [485, 541, 542, 544, 547, 549]. The reported locations form a trail extending from dorsal to ventral ACG, suggesting that different parts of the ACG respond to the very varied experimental paradigms that were used (**Figure 29**). There is a cluster of activations near the anterior area identified from lesion studies by Dr Nathan (personal communication), confirming that this part of the brain is indeed critical for control of micturition. Response of the dorsal ACG to bladder filling is abnormally pronounced in patients with urge incontinence, even in the absence of any detrusor contraction [547, 550], suggesting that it is involved both in recruitment of accessory pathways (to help avoid loss of control of the bladder) and in the sensation of urgency that accompanies this situation. Imaging studies of voiding [541-543] and simulated voiding [552] have all revealed ACG involvement.

c) Insula

Given what we now know from functional imaging

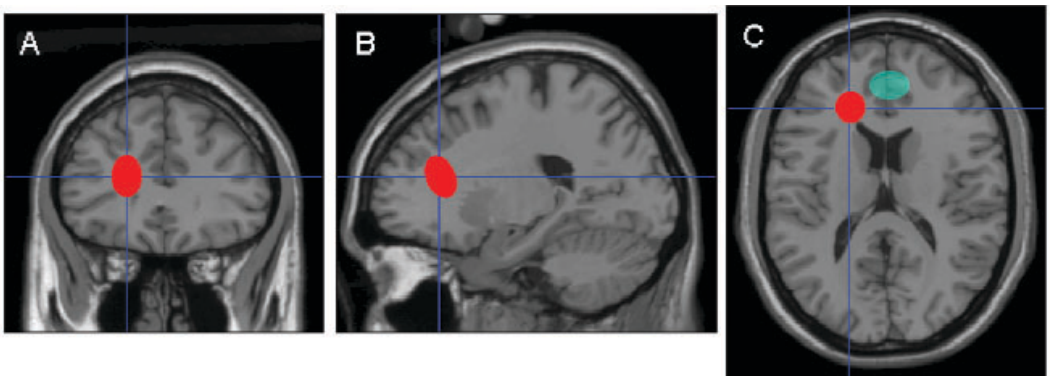


Figure 27 A, B and C : Location of white matter lesions causing persisting incontinence (red: based on Andrew and Nathan's Figures 10 A, B and C (1)); and of grey matter lesions causing incontinence (cyan: personal communication to CJF from Dr Nathan).

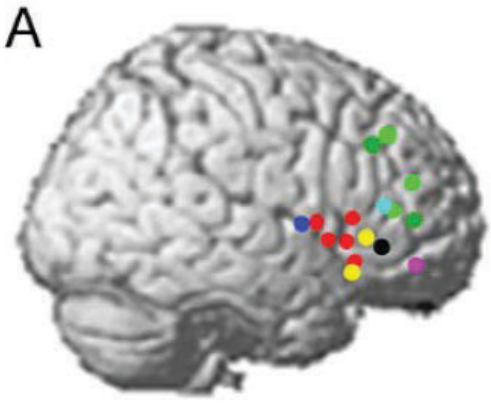


Figure 28A : Locations of peaks of activation reported as right inferior frontal or lateral prefrontal cortex, during bladder filling, storage and withholding of urine. Locations of peaks more than 20 mm from the midline are projected onto right lateral surface of brain. Note some overlap with insula shown in Figure 30.

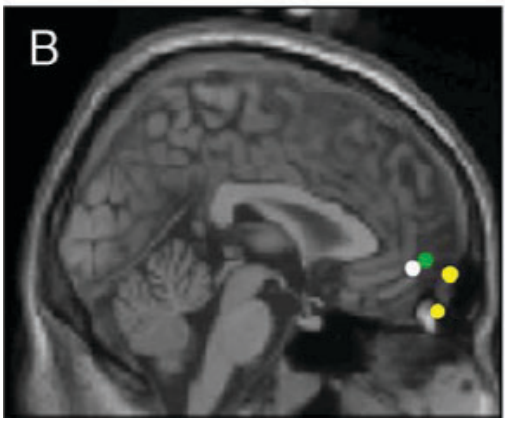


Figure 28B : Medial prefrontal area activated in a few studies during withholding of urine. Locations of peak activation less than 20 mm from the midline are projected onto the midline plane.

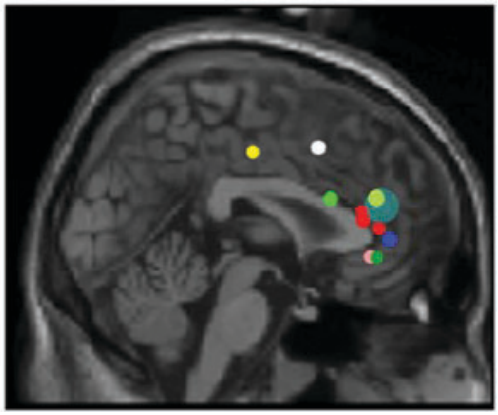


Figure 29: Cingulate gyrus areas activated on withholding of urine or full bladder, projected onto midline plane (dots). The larger cyan circle is the projection of the superomedial grey matter area shown in Fig 27 onto the midline plane.

experiments about the importance of the insula in processing afferent activity from the bladder (see **Figure 30**) it is remarkable that there have not been more reported cases of bladder dysfunction from lesions at that site. Possibly the bilateral nature of insular connections, even though there is some right-sided predominance, protects against a drastic effect of insular lesions. One patient in whom a glioma affecting the inferior frontal gyrus and the insula was excised experienced incontinence without loss of bladder sensation [533].

Imaging studies during storage or withholding of urine show that regions reported as insula form a cluster near the expected location of that structure (**Figure 30**) [541-544, 547, 549, 554]. There is some overlap with the lateral frontal activations reported above and shown in **Figure 28A**. In the figure right and left sides are projected on the same brain surface, masking any right-sided predominance, and the insula is about 20 mm deeper than the brain surface shown. In healthy subjects, insular activation becomes stronger with increasing filling of the bladder, consistent with its postulated role in bladder sensation [555]. In normal elderly, this insular response to bladder filling decreases with age, consistent with age-associated loss of sensation. However, insula activity cannot by itself be responsible for conscious desire to void or urgency, because these sensations are lost following extensive frontal lesions [551], suggesting that integrity of connecting pathways between insula and frontal cortex is essential for conscious sensation.

The insula was activated in only one [543] out of four imaging studies of real or imagined voiding, suggesting that it is not strongly involved in this phase of micturition.

d) Periaqueductal Grey (PAG)

A single case history describes a young man presenting with urinary retention in whom the only abnormality found was a small, presumed inflammatory lesion in the PAG [532]. Presumably in other cases the clinical picture was dominated by other symptoms and deficits that were more striking to a neurologist.

Given the spatial resolution of PET and fMRI (a few mm), it might be expected to be difficult to distinguish small but functionally different areas in the brainstem and midbrain. In fact however the brainstem/midbrain activations reported during the storage and voiding phases seem to cluster in 3 distinct regions (**Figure 31**), one of them being the PAG. PAG response to bladder filling is reported in 3 studies [485, 544, 547]. This response may reflect increased afferent signals arriving at the PAG (**Figure 26**) or increased inhibitory activity from the medial prefrontal cortex (MPFC), needed to prevent triggering of the voiding reflex (see **Figure 32**). The PAG responded to imagined voiding in one fMRI study [552], but not to real voiding.

e) Hypothalamus

Lesions at this site as a cause of bladder symptoms are rare but 3 cases of pituitary tumours, extending upwards into the hypothalamus have been described with urgency incontinence or retention, weight loss, psychiatric disturbance and bitemporal field restriction [534]. Other instances include gliomas involving the hypothalamus [551] or vascular disturbances of the anterior hypothalamus. Andrew and Nathan also reported five patients with bladder symptoms appearing after a ruptured cerebral aneurysm and speculated that the site of lesion responsible was the anterior hypothalamus [556].

Animal observations suggest that the anterior and caudal hypothalamus have monosynaptic connections with the PAG and PMC [536]. Correspondingly, two human brain imaging reports suggest response to bladder filling in a region near the caudal hypothalamus [485, 555], and one near the preoptic area [547]. It has

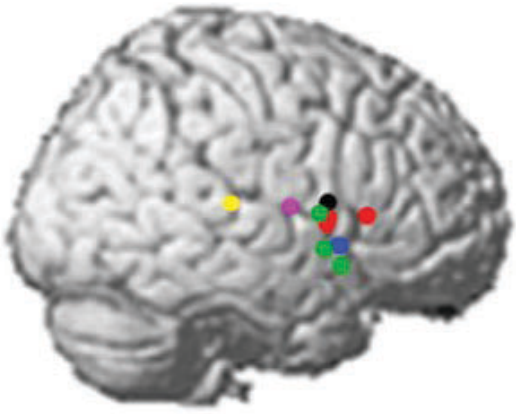


Figure 30: Locations of activation peaks reported as insula, during bladder filling, storage and withholding of urine, projected onto right lateral surface of brain. Note overlap with areas shown in Fig 28A.

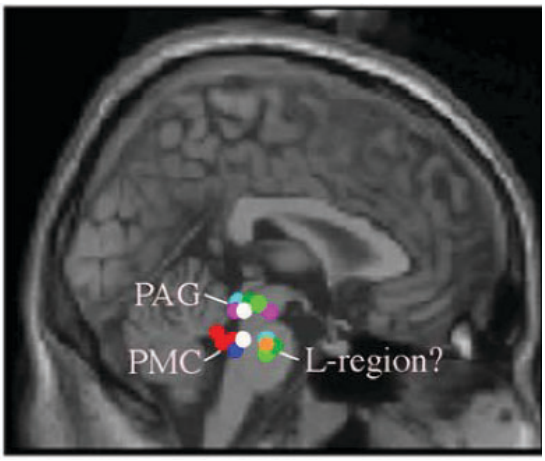


Figure 31: Brainstem areas activated during storage or voiding, projected onto the midline plane.

been suggested that these connections allow the hypothalamus to inhibit voiding unless the situation is judged to be safe.

f) Pons

The demonstration by Barrington [557] in the cat that a centre existed at the level of the pons necessary for activation of micturition, provided the background for recognizing a comparable centre in humans and the early report of the association of difficulty with micturition with posterior fossa tumours [558]. Later histories of individual cases of discrete pontine lesions [559, 560, 561], [494] and reports of difficulties with micturition or retention as a feature of brainstem gliomas in children [562, 563] or vascular lesions [564], confirmed the likely existence of a comparable centre in humans. Studies using MRI to visualize the precise location of the responsible lesions, sited this in the dorsolateral pons, including the pontine reticular nucleus and the reticular formation, adjacent to the medial parabrachial nucleus and locus coeruleus [564]. Lesions in this location are frequently associated with disturbances of consciousness and respiration and bladder symptoms may therefore be overlooked. The commonest clinical association of urinary retention arising from a pontine lesion is an internuclear opthalmoplegia.or disorder of eye movements.

Functional imaging experiments have shown a cluster of activations near the postulated location of this pontine micturition centre (PMC), **Figure 31**. There are 3 reports of PMC activation during voiding [541-543] and – surprisingly – one in response to bladder filling [555]. However, excitation during bladder filling might be inhibitory rather than excitatory, a distinction that cannot be made by functional imaging.

A few studies suggest activation of the postulated pontine L-region or continence centre (**Figure 31**), somewhat ventral, lateral and/or caudal to the PMC (**Figure 26**): during storage [485], during failed attempt to void [541, 542], during imagined voiding [554], and during imaginary inhibition of voiding [552]. However, not all these studies recognized the L-region as such.

g) Other Regions

The regions discussed in sections 3.1 to 3.6 are reasonably well established since they are observed in lesion, imaging and (in some cases) animal studies. In particular, the triad insula/ACG/prefrontal cortex is well known from studies of other aspects of brain function [565]. Other regions relevant to bladder control however have been revealed only by functional imaging. They include parts of parietal and frontoparietal cortices, posterior cortex (precuneus, posterior cingulate cortex), putamen, caudate, parts of the limbic system (hippocampal complex, amygdala), and various parts of the cerebellum. Rarely seen in functional imaging experiments is activity in the basal ganglia, yet dopamine pathways are thought to have a profound inhibitory effect on the PMC in health, which is lost in Parkinson's Disease.

4. WORKING MODEL OF BRAIN/BLADDER CONTROL

The diagram in **Figure 32** shows what are currently thought to be the principal areas and connections involved in bladder control, with the more important connections identified by thicker lines. This scheme is hypothetical but offers, at this point in time, a framework based on evidence from the clinical reports of the effects of "lesions", the regions shown to be activated in the PET imaging experiments (**Figure 33**) and the results currently emerging from functional MRI and connectivity studies.

The PMC is the final efferent brain nucleus involved in bladder control – when activated there follows co-ordinated sphincter relaxation and detrusor contraction, resulting in voiding. However the PMC has been show to have only a few central connections (**Figures 26 and 32**) namely, from the hypothalamus and from the PAG. The PAG is now recognized as having a central role in mediating the influences of higher function on the PMC and so achieving voiding at brain- rather than bladder-determined volumes. To perform this role the PAG receives afferent input via the sacral roots conveying "bladder fullness" information, as well multiple inputs from higher centres including the

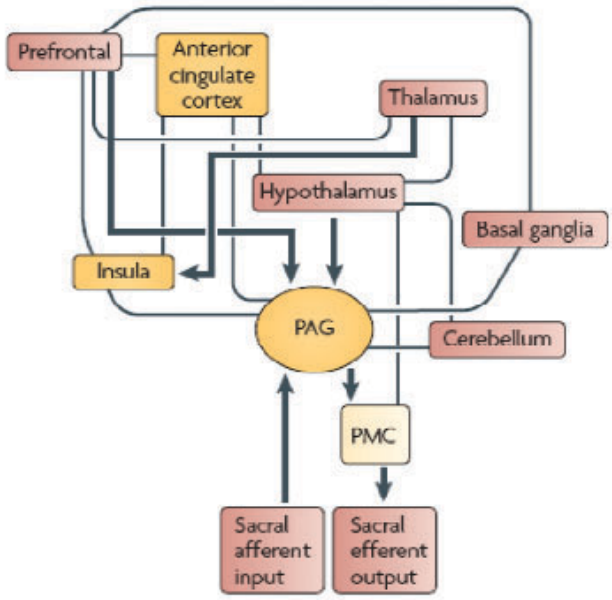


Figure 32 A: preliminary conceptual framework, based on current evidence, suggesting a scheme for the connections between various forebrain and brainstem structures that are involved in the control of the bladder and the sphincter in humans. Arrows show probable directions of connectivity but do not preclude connections in the opposite direction. PAG = periaqueductal grey; PMC = pontine micturition centre. Reproduced, with permission (2), based on the work of (4).

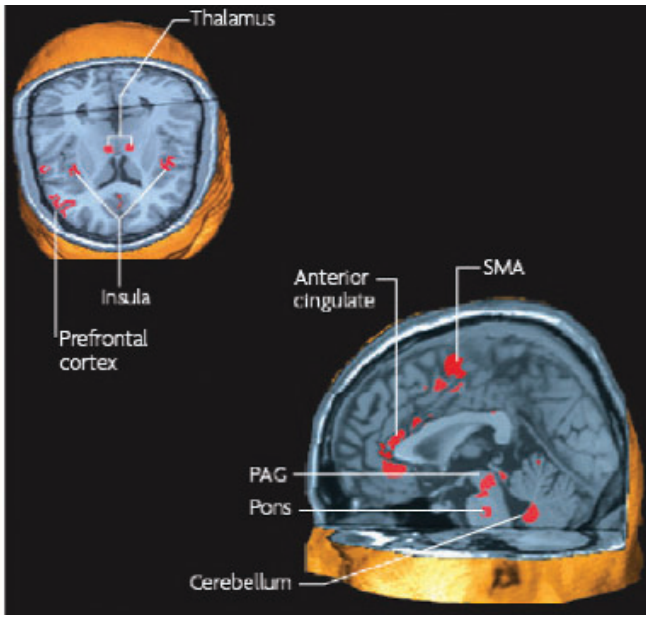


Figure 33 : Brain areas involved in the regulation of urine storage. A meta-analysis of positron-emission tomography and functional MRI studies that investigated which brain areas are involved in the regulation of micturition reveals that the thalamus, the insula, the prefrontal cortex, the anterior cingulate, the periaqueductal grey (PAG), the pons, the medulla and the supplementary motor area (SMA) are activated during the urinary storage. Reproduced with permission from (3)|

prefrontal cortex, ACG, insula and hypothalamus and also probably the basal ganglia and cerebellum. The insula (the right in particular) has been shown to respond in a way that suggests it is responsible for mapping and processing bladder as well as other visceral sensations and is sometimes referred to as the "sensory cortex of the autonomic nervous system".

The ACG monitors stress and conflict and generates appropriate autonomic arousal. It may also determine how much attention is paid to signals from the bladder and how one reacts to them. The medial prefrontal cortex (MPFC) has a role in response selection and so may be involved in evaluating afferent activity. The MPFC is crucial for decision-making in an emotional and social context and is thus evidentially important in controlling micturition. It is close to the anterior cingulate region revealed by lesion studies and functional imaging (**Figure 29**) and has strong and direct connections with the PAG, suggesting that it may be responsible for maintaining the tonic suppression of the voiding reflex during the storage phase, which normally is only relaxed when the decision to void has been made. This decision is conveyed back to the PAG, which correspondingly excites or inhibits the PMC (**Figure 34**).

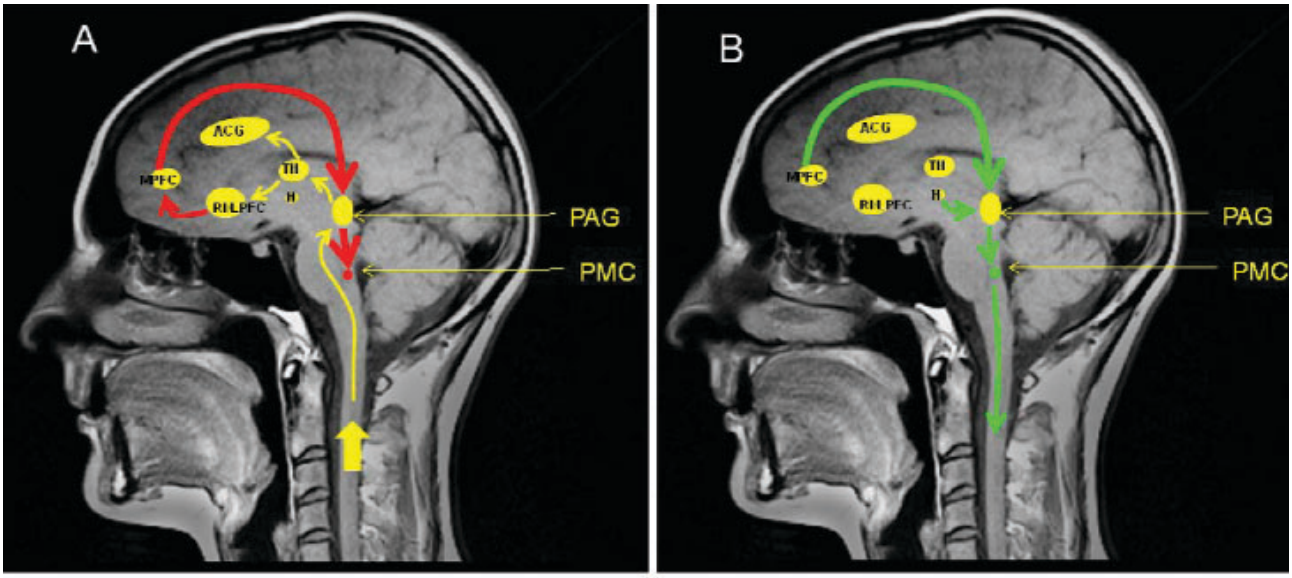


Figure 34: A preliminary working model of lower urinary tract control by higher brain centres. A: During storage, ascending afferents (yellow) synapse on the midbrain periaqueductal grey (PAG); they are relayed via the thalamus (TH) to the dorsal anterior cingulate gyrus (ACG: responsible for monitoring and warning) and to the right insula (RI: responsible for sensations such as desire to void) and lateral prefrontal cortex (LPFC); in the storage phase they pass to the medial prefrontal cortex (MPFC, red arrow) where the decision to void or not is made; in this phase the decision is not to void, and this situation is maintained by chronic inhibition of the PAG via a long pathway (red arrows) from the MPFC; consequently the pontine micturition centre (PMC) is also suppressed, and voiding does not occur. B: When the decision to void is made, the MPFC relaxes its inhibition of the PAG (green arrow) and the hypothalamus (H) also provides a 'safe' signal; consequently the PAG excites the PMC which in turn sends descending motor output (green arrow) to the sacral spinal cord that ultimately relaxes the urethral sphincter and contracts the detrusor, so that voiding occurs.

VII. ABNORMAL LOWER URINARY TRACT FUNCTION

Dysfunction of neural control may underpin a wide range of clinical urinary tract problems. On the afferent side, neural dysfunction will alter reflex activity and influence sensation, which can either be enhanced, reduced or altered (e.g. with the emergence of pain instead of usual bladder filling sensations). On the efferent side, motor activity within the components of the lower urinary tract (bladder and outlet) can be increased, reduced or uncoordinated. The following is not a comprehensive description of the entire scope of this complex arena, but focuses on key issues relevant in the clinical context.

1. ABNORMALITIES INVOLVING INFLAMMATION

• Bladder Pain Syndrome / Interstitial Cystitis (BPS/IC)

Although no consensus has been reached on the fundamental causes of Bladder pain syndrome/ Interstitial Cystitis (BPS/IC), existing data suggest 3 pathophysiological mechanisms: epithelial dysfunction, mast cell activation, and neurogenic inflammation [566].

1. Mast Cell Activation – Mast cells may be activated by a number of mechanisms within the bladder wall. Increased permeability with influx of potassium ions may lead to sensory nerve up-regulation resulting in mast cell activation. Vasoactive, nociceptive and pro-inflammatory molecules released from mast cells can produce neuronal sensitisation and secretion of neurotransmitters that further stimulate mast cells. Mast cell – neuronal interactions may therefore provoke a vicious cycle in BPS/IC, contributing to the painful symptoms of the disease [567].

2. Neurogenic Inflammation – The close physical relationship between the C fibres and mast cells is of particular importance, as substance P (SP) released from the nerve fibres degranulates mast cells. During degranulation, the mast cells release a multitude of proinflammatory agents including nerve growth factor (NGF), tumour necrosis factor-α (TNFα), histamine, heparin, proteases, interleukins, serotonin and others. This induces an inflammatory response in the form of vasodilatation (redness) and plasma protein extravasation (oedema). Additionally, newly released nerve growth factor (NGF) stimulates a further increase in the number of afferent fibres, thus increasing the potential for further releases of SP and the transmission of nociceptive impulses. Inflammatory models of cystitis result in increased levels of TNF-alpha, SP and NGF production in the bladder, paralleling the hypothesized neuro-inflammatory etiology of IC [568].

During mammalian development, NGF is required for the survival and growth of several populations of neurons. There is evidence to suggest that it also plays a role in the ongoing regulation of neural function, as well as inflammation and pain [569].

In the urinary tract, NGF is produced by the bladder, smooth muscle and urothelium, and levels of NGF increase in response to interstitial cystitis. In one study, patients with BPS/IC were found to have elevated levels of neurotrophic factors in their urine, including neurotrophin-3, nerve growth factor and glial cell line-derived neurotrophic factor [570]. This finding is consistent with the observation of increased NGF mRNA and protein in bladder biopsies from patients with BPS/IC compared with controls [571].

Intravesical NGF is known to sensitize bladder afferent fibres [142], and sequestration of NGF can reduce inflammation associated with chemical cystitis in a rat model [572]. Blockade of NGF using either endogenous antibody or antibody against the NGF receptor, or a fusion protein that prevents interaction between NGF and its receptor, prevents neural plasticity and bladder overactivity in experimental models of these conditions [143, 573].

Other substances including neurotrophins, prostaglandins, and tachykinins may also contribute to altered afferent excitability [574]. The potential relevance of these changes has been demonstrated in cats diagnosed with feline interstitial cystitis (FIC) (which demonstrates nearly all the characteristics and symptoms of human BPS/IC), in which capsaicin sensitive neurons are larger, with dorsal root ganglia that exhibit increased excitability and slower desensitisation to capsaicin, suggesting changes in the properties of the primary afferent neurons [146, 147], a finding that may be associated with tendency of NGF to stimulate TRPV1 expression.

2. INVOLVING ABNORMAL URINE STORAGE

a) Overactive Bladder / Detrusor Overactivity

Detrusor overactivity can arise in neuropathic conditions, secondary to bladder outlet obstruction or idiopathically. Several observations on structural and functional properties of the bladder have been made in individuals with detrusor overactivity:

- Patchy denervation is present within the bladder wall, while sensory neurones and parasympathetic ganglion cells are enlarged
- Exaggerated spontaneous myogenic activity can be seen in isolated detrusor muscle strips, with increased incidence of fused tetanic contractions
- Muscle strips also show altered responsiveness to nervous and pharmacological stimuli

- Characteristic changes in smooth muscle ultrastructure have been described.

Smooth muscle strips dissected from the bladder in detrusor overactivity often show altered responses to nerve stimulation and to various agonists. For example, in obstructed overactive bladders there is reduced contractile response to intrinsic nerve stimulation, along with supersensitivity to muscarinic agonists and potassium solutions [575-578]. Among neuropathic conditions, spina bifida is associated with supersensitivity to cholinergic agonists and potassium solutions, but there is no change in the sensitivity to intrinsic nerve stimulation [579]. In spinal cord injury, there is no reduction in sensitivity to electrical field stimulation, but the maximum force generated by each milligram of bladder tissue is significantly reduced [92]. In idiopathic detrusor overactivity, bladder strips show supersensitivity to potassium, but not to muscarinic agonists, and there is a reduced contractile response to intrinsic nerve stimulation [580]. Where functional denervation is present, there appears to be an increase in spontaneous contractile activity and presence of fused tetanic contractions, a feature more typical of well-coupled smooth muscles [581, 582]. A common ultra-structural feature of the overactive detrusor is the emergence of protrusion junctions and ultra-close abutments between the smooth muscle cells [583]. Overall, the cells may be better coupled electrically in detrusor overactivity, perhaps allowing spontaneous activity to propagate over a wider area. Bladder biopsies from overactive detrusor show a patchy denervation; some muscle bundles may be completely denervated, whilst neighbouring ones appear normal and in other areas sparser innervation is also seen [92, 579, 580, 584]. A similar pattern is seen in animal models [576, 585]. Overall, these observations suggest that response to loss of local innervation by the smooth muscle cell may explain the altered behaviour of the bladder in detrusor overactivity [575, 586].

Preclinical studies in animal models of OAB / detrusor overactivity contribute further to our understanding of the importance of neural control in this condition. The main models relevant to OAB include (i) instillation of irritative agents into the bladder during cystometry, (ii) partial bladder outflow obstruction, (iii) the spontaneously hypertensive rat, (iv) spinal cord injury, and other CNS lesions similar to those responsible for bladder dysfunction in humans [587].

Irritative agents are employed in animals to evoke a painful or irritant response, in particular through C-fibres. Both acetic acid and citric acid have been used, although the latter probably represents a better model since it is less irritant and therefore less likely to invoke an acute inflammatory response. Both agents have been shown to increase bladder contractile activity, decrease bladder capacity and reduce bladder compliance, while micturition pressure remains normal or is increased, suggesting that this model may contribute to understanding of increased bladder sensory activity, as may occur during the symptom of urgency. These effects are due to stimulation of nociceptive afferent fibres, confirmed through demonstration of increased c-fos expression in rat spinal cord and in regions of the periaqueductal grey [372, 588], an effect that can be eradicated by desensitization of TRPV1 receptors on the sensory neurones following pre-treatment with resiniferatoxin [589].

Partial bladder outflow obstruction has been achieved through application of urethral ligatures or a constricting ring that results in partial urethral occlusion, mimicking the obstruction seen in men with BPH who often develop secondary OAB [577]. The animals develop cystometric features such as increased bladder capacity and non-voiding bladder contractions, with associated histological features (muscle hypertrophy, patchy denervation and enlarged sensory neurones and parasympathetic ganglia) and detrusor functional features (spontaneous myogenic activity and altered response to stimuli) reminiscent of the human condition [586]. Afferent plasticity in animals with bladder outflow obstruction involves NGF, the content of which is increased in obstructed bladders prior to the enlargement of bladder neurons and the development of urinary frequency [590]. The relevance of NGF in the response to obstruction in animals is suggested by the finding that rats immunized with mouse NGF in order to develop autoantibodies do not develop neural plasticity and urinary frequency in response to obstruction [591]. While neuronal changes are evident in the obstructed model, further observations appear to indicate that the overactive phenotype in obstructed rats derives from a combination of increased propensity to localized muscle contraction, together with a tendency towards wider propagation of this activity in an organ that may have developed greater autonomy from central control as a result of the neuronal changes [592]. In this context, the relevance of peripheral contractile modules appear to emerge as the functional units of detrusor activity [477].

The spontaneously hypertensive rat (SHR) is a genetic model of hypertension, which is also known to exhibit abnormal bladder function; in particular, SHRs have been shown to have reduced bladder capacity and voided volume, increased urinary frequency and increased occurrence of non-voiding contractions, associated with altered detrusor innervation and physiological response [450]. Again, increased bladder smooth muscle NGF levels appear to be associated with these changes [593]. The bladders of SHRs also show increased levels of calcitonin gene-related peptide immunoreactive fibres (presumably afferent) with increased size of neuronal cross-sectional area profiles for bladder afferents in the L6–S1 dorsal root

ganglia as well as the major pelvic ganglia [594]. The potential importance of the afferent innervation in this model can be highlighted by the finding that intrathecal application of antisense oligonucleotide against the tetrodotoxin- resistant sodium channel (Nav1.8) reduces bladder hyperactivity [595].

Spinal cord injury (SCI) in animals has been shown to result in changes in lower urinary tract function similar to those seen in humans [596]. Recovery of bladder function from the initial areflexia after SCI in animal models is dependent in part on plasticity of bladder afferent pathways and the unmasking of reflexes triggered by the normally silent capsaicin-sensitive C-fibre bladder afferent neurons, resulting in cystometric changes akin to those seen in neurogenic detrusor overactivity [596]. Studies in rats indicate that the increased excitability in the C-fibres is associated with an increase in the expression of sodium channels from a high-threshold TTX-resistant type to a low-threshold TTX-sensitive type [597].

Other animal models of CNS lesions associated with bladder dysfunction have also been developed. For example, a model of Parkinsonism secondary to 6-hydroxydopamine injection into the substantia nigra pars compacta in rats has been developed, confirming the relevance of D1/D5 dopaminergic stimulation in improving bladder capacity [531]. Furthermore, a model of bladder overactivity associated with cerebral infarction due to occlusion of the middle cerebral artery in rats has been shown to lead to significant ischaemia within the putamen and cerebral cortex, confirming the importance of these areas in the control of micturition [598]. In this latter model, a role for glutamatergic and dopaminergic stimulation in the development of bladder dysfunction has been demonstrated [598, 599]. These findings are consistent with the observation that anterior brain lesions in humans are more likely to be associated with incontinence than posterior, occipital lesions [600].

Additional evidence for the importance of emergent C-fibre contribution to afferent signalling in OAB and detrusor overactivity can be found in experimental clinical studies. For example, patients with various idiopathic and neurogenic bladder dysfunctions have reduced current perception threshold to direct bladder stimulation [601] and urethral stimulation [602] at frequencies that selectively stimulate C-fibres, indicating increased excitability in the C-fibre afferents in these patients. Similarly, ice water instillation (which excites C-fibres) into the bladder can trigger involuntary detrusor contraction in patients with neurogenic detrusor overactivity [603]. In addition, the efficacy of capsaicin and resiniferatoxin in the treatment of patients with both idiopathic and neurogenic detrusor overactivity confirms the role of C-fibres and the afferent limb of the micturition reflex in bladder storage conditions [604]. While the hyper-excitable C-fibres appear to be the conduit for the abnormal sensory signals from the overactive bladder, the origin of the sensations probably lies in the bladder wall itself, either in uncoordinated asynchronous localized detrusor contractile activity [476, 605] which may lead to urgency with negligible associated pressure rise within the bladder, or in a more co-ordinated propagation of autonomous contractile activity via emergent gap junctions providing more syncytial characteristics to the overactive detrusor [583].

b) Stress Urinary Incontinence

Stress urinary incontinence (SUI) is characterized by reduced outflow resistance during urinary storage due to weakness in the urethral sphincter mechanism. It is often associated with weakness of the pelvic floor and urethral musculature, but peripheral nerve dysfunction is also implicated, in particular pudendal nerve damage following childbirth in women [606]. Animal models have contributed to understanding of the importance of the peripheral innervation of the urethra in SUI, with development of disease models association with pudendal nerve crush and vaginal distension initiating neuropraxic nerve injury in rats [607] and mice [608]. The role of pharmacological neuromodulation in the treatment of SUI has shown that urethral function and therefore incontinence can be improved by augmenting somatic neuronal discharge using the serotonergic (5HT) and noradrenergic (NE) reuptake inhibitor duloxetine, which is thought to act in the sacral spinal cord at Onuf's nucleus, the pudendal somatic motor nucleus of the spinal cord which is densely innervated by 5HT and NE terminals [307, 609]. More recent data on the effect of the selective noradrenergic reuptake inhibitor, [S,S]-reboxetine in the treatment of SUI, indicate that the serotonergic activity of duloxetine may be redundant and that effects are dependent solely on noradrenergic reuptake inhibition [610, 611].

3. INVOLVING ABNORMAL VOIDING

• *Bladder Outflow Obstruction*

Bladder outflow obstruction (BOO) is typically associated with prostatic enlargement in men, although it is also seen in women following surgery for stress urinary incontinence and in children secondary to proximal urethral valves. Bladder overactivity is seen in many patients with BOO, and this storage dysfunction together with the associated neuronal changes has been described above. The voiding dysfunction per se seen in patients with BOO is caused by a combination of both passive and dynamic obstruction of the proximal urethra and, in some patients, by detrusor decompensation resulting in a deterioration in the contractile function of the bladder during voiding.

During obstructed voiding, the bladder is subject to high pressures required to expel urine through a region of high resistance. These pressures compromise detrusor

blood flow during voiding leading to periods of ischaemia and hypoxia, followed by reperfusion [612]. Recurrent cycles of ischaemia and reperfusion result in progressive neuronal damage in the bladder wall, an effect that has been seen in animal models of obstruction and bladder distension, as well as in an in vitro model in which detrusor muscle is intermittently exposed to hypoxic glycopenic perfusate followed by oxygenated glucose-containing perfusate [384, 613]. This neuronal damage initially has a patchy appearance, when it seems to be related to the development of an overactive phenotype in pigs [586]. With time, however, bladder decompensation with incomplete voiding and chronic retention can develop, and this is associated with a more generalized detrusor denervation and reduced contractile response to neuronal stimulation in organ bath studies [614].

4. CO-MORBID DISORDERS

The significance of neural control mechanism in lower urinary tract dysfunction is further implied by a number of comorbid conditions that have been associated with BPS/IC and incontinence.

Patients with BPS/IC and SUI are more likely to report depressive symptoms. Findings on two validated depression measures indicate that patients with interstitial cystitis report significantly greater depressive symptomatology than healthy controls, although less than 20% notice moderate or severe depressive symptoms. This finding is consistent with the observation that patients with chronic pain experience a higher level of depressive symptoms than healthy controls and other chronically ill populations. Patients report low mood (56%), fatigue (63%), difficulty concentrating (49%), insomnia or excessive daytime sleepiness (49%) and are 3 to 4 times more likely to report suicidal thoughts than the general population [615]. The association of IC/PBS [616] and incontinence [617] with depression has been corroborated in other studies, although a more recent study suggests that, after multivariate adjustment for the influence of other urogenital symptoms, only the symptom of nocturia remains significantly associated with depression [618].

BPS/IC has also been associated with other chronic pain conditions, especially fibromyalgia [616, 619-621]. An association has also been shown for child abuse [616, 622]. Based on recent improvements in understanding of pain processing pathways in the central nervous system, and in particular the role of limbic structures, especially the anterior cingulate cortex, hippocampus and amygdala, in chronic and affective pain perception, a condition termed limbic associated pelvic pain has been proposed to explain the concurrence of these various chronic pain conditions. This limbic dysfunction is manifest both as an increased sensitivity to nociceptive afferents from pelvic organs, and as an abnormal efferent innervation of pelvic musculature, which undergoes tonic contraction as a result of limbic efferent stimulation, generating a further sensation of pain. The nociceptive afferents from these pelvic organs then follow the medial pain pathway back to the sensitized, hypervigilant limbic system. Chronic stimulation of the limbic system by pelvic pain afferents again produces an efferent contraction of the pelvic muscles, thus perpetuating the cycle [622].

VIII. BRAIN-GUT INTERACTIONS

The bidirectional interactions between different hindgut organs (urinary bladder, rectum) show both similarities and differences. The primary function of both organs is to store waste products, and to empty them in accordance with a) the state of filling as well as b) the overall state of the organism, and environmental and social constraints. While the former is accomplished by a series of hierarchal reflexes, the latter is accomplished by modulatory input from the brain to change the gain of these reflexes. While spinal and supraspinal reflexes are involved in both the regulation of bladder and rectal emptying, peripheral reflexes contained within the enteric nervous system are unique to the colorectum, while supraspinal reflexes involving the periaqueductal grey and Barrington's nucleus (or pontine micturition center) appear to play a more prominent role in bladder emptying. Conscious perception of the state of these organs is the end result of various afferent inputs to the insular cortex, including visceral afferents, and inputs from cognitive and emotional arousal circuits. Based on this model, modulation of both emptying and perception of peripheral events in both health and disease can occur at multiple levels, and by various mechanisms. It is proposed that while peripheral changes in the end organ by inflammatory processes may play the primary role in organic disorders, central changes in modulatory circuits may play the primary role in so called functional disorders, like irritable bowel syndrome and bladder pain syndrome/interstitial cystitis (BPS/IC).

1. BACKGROUND

Functions required for the elimination of waste products are coordinated with each other, with the body's needs and with appropriate behaviors. This integration is accomplished at different levels of the neuroaxis, from the lumbar spinal cord (reflex functions; bladder – rectal integration) to pontine nuclei such as Barrington's nucleus (integration of bladder and rectal function with other information about the state of the organism) to the periaqueductal grey (PAG) and to cortical regions (insula, anterior cingulate cortex, ACC). The PAG and BN receive inputs from cortical and emotional brain circuits, integrating pelvic visceral function with voluntary behavior and with the emotional state of

the organism. Symptoms affecting several pelvic viscera, such as incontinence and chronic pain and discomfort related to the distal colon/ rectum (irritable bowel syndrome, IBS) and to the urinary bladder (BPS/IC) often co-exist.

This section on bidirectional brain distal gut interactions tries to put rectal sensations and continence mechanisms into the larger context of regulation of homeostatic function, and highlights the differences and similarities in neural mechanisms underlying fecal and urinary continence mechanisms. The approach of providing a general framework to understand how the nervous system, in particular the human brain, processes and modulates visceral afferent input arising from two viscera derived from the embryological hindgut is useful to understand the comorbidity of fecal and urinary incontinence, and of BPS/IC and IBS.

a) The concept of homeostatic emotions

We humans uniquely experience feelings from each of the tissues of our bodies, including the viscera concerned with storage and evacuation of bodily waste (anorectum and the urinary bladder), because evolution has produced brain mechanisms in humans for the conscious perception of an image of the physiological condition of our bodies.

The classical term *interoception*, formerly used only to refer to visceral sensation, has been re-defined, to refer to the sense of the physiological condition of the body. Recent neuroanatomical findings indicate that all of the feelings from our bodies reflect its physiological condition and can be viewed as *homeostatic emotions*.

This includes not only visceral sensations such as hunger and thirst, but also a range of other sensations including rectal or urinary fullness, urge and pain. Like all emotions, these comprise both a (conscious) feeling and a motivation and motor response. While the anterior insula has been identified as an important neurobiological substrate involved in the conscious perception of feelings and sensations from our viscera, a set of brain circuits referred to as the emotional motor system [623] is involved in the mediation of the motor response to such visceral input.

b) Homeostatic emotions drive behavior

Sensory inputs that relate the body's physiological condition, or homeostatic afferents, drive the homeostatic mechanisms that promote survival. The primordial means of regulating the evacuation of stool and urine in all vertebrates is motivated behavior, so the pathways that guide homeostatic sensory input to motivational processes must be ancient. Such behavioral motivation (and the accompanying autonomic and somatic motor changes required for the evacuation of waste products) is generated in non-humanoid mammals by a signal to the forebrain from the main homeostatic afferent integration site in the brainstem, the parabrachial nucleus (PBN).

As proposed by Craig, [624] evolution produced a new direct (spino-thalamo cortical) pathway in humans to the forebrain that surmounts the primal pathway and which generates both an affective motivation and a sensation in the limbic motor and sensory cortices (**Figure 35A**). The basic homeostatic (interoceptive) feelings or modalities include abdominal fullness as well as fecal and urinary urge. These feelings are the human percepts of distinct "homeostatic emotions" that directly relate to the body's needs.

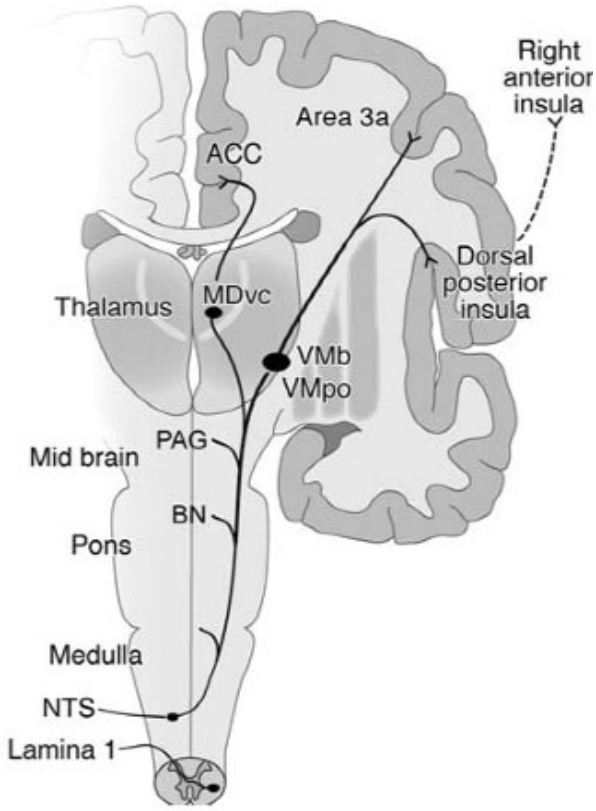


Figure 35A : Ascending projections of homeostatic afferents: Ofganization of interoceptive pathways. Small diameter afferents that travel with sympathetic and with parasympathetic efferent's provide input to lamina I and NTS, respectively.
In mammals, the activity of both types of afferents is integrated in the PBN, which projects to insular cortex. In non-human and human primates, a direct projection from lamina I and from the NTS exist to ventromedial thalamic nuclei (Vmpo and VMb, respectively).
Neurons in these nuclei project in a topographical fashion to the mid/posterior insula. In humans, this cortical image of the homeostatic state of the organism if re-represented in the anterior insula on the same side of the brain.
These representations provide the substrate for a subjective evaluation of interoceptive state.

The concept of homeostatic emotions, regardless of the valence of the emotion, is consistent with the reported activation of similar brain regions (insula, dACC) by a variety of both pleasant and unpleasant stimuli (reviewed in Vogt, 2005 [625]).

2. ORGANIZATION OF HOMEOSTATIC REFLEXES: PROCESSING OF PELVIC VISCERAL INFORMATION WITHIN HIERARCHICAL ORGANIZED HOMEOSTATIC REFLEXES

a) Enteric reflexes within the Enteric Nervous System

The hierarchal organization of homeostatic reflexes within the central neuroaxis, and which are pertinent to both anorectum and urinary bladder is shown in **Figure 35B.** A fundamental difference in the regulation of the distal gut and the urinary bladder is the prominent role of the enteric nervous system (ENS) as the primary reflex circuit mediating both filling and emptying of the distal colon. The ENS, which is considered the third division of the ANS [626], is made up of two ganglionated plexus situated between the different muscle layers of the gut (myenteric plexus) and the mucosa (submucosal plexus). Even in the absence of any central input, these enteric (or intrinsic) reflexes can generate proximal to distal propulsion of colonic contents (peristaltic reflex), and relaxation of the internal anal sphincter [627]. While the enteric reflex circuitry within the anorectum is unique to fecal continence, the spinal and supraspinal reflexes mediated by extrinsic afferents overlap considerably for bladder and rectum.

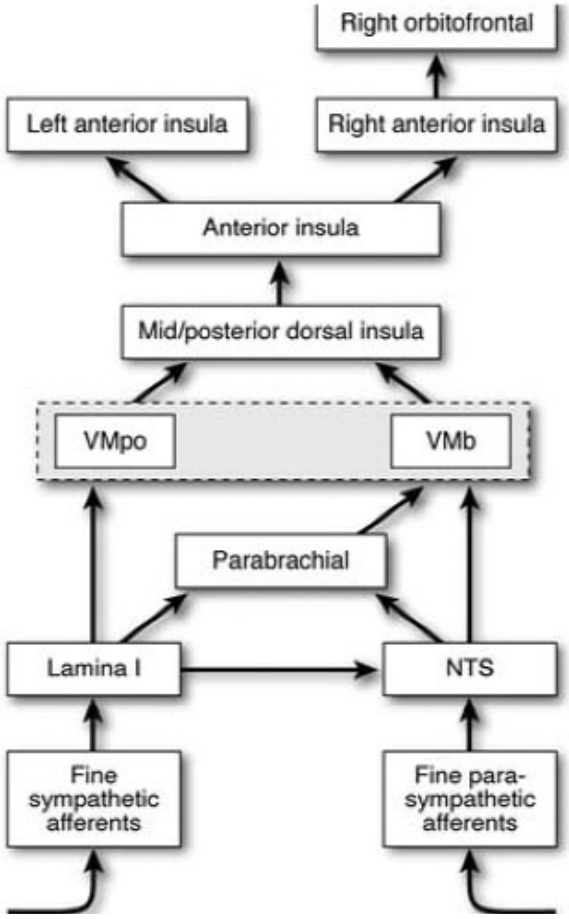


Figure 35B: Ascending projections of homeostatic afferents: Spino-thalamo-cortical system. Summary diagram illustrating the projections in primates of homeostatic afferent pathways from lamina I (spinal) and NTS (vagal) to thalamic nuclei, and the two cortical regions involved in the sensory (insula) and motivational (ACC) dimensions of homeostatic emotions.

b) Lamina I afferents provide input to spinal and supraspinal reflexes.

The small-diameter afferent fibers that innervate the pelvic viscera course peripherally with sympathetic and parasympathetic nerves (e.g. splanchnic, pelvic, vagus), and with somatic nerves (e.g. pudendal). Many innervate the mesenteric ganglia and play a role in the communication between the central nervous system and the ganglionated plexus of the ENS. Many of these extrinsic afferents respond to chemical and mechanical stimuli over a broad range of thresholds and response slopes, so that they might be considered to represent a broad continuum of response properties. However, they can also be regarded as separable into different classes, including low-threshold, high-threshold (nociceptive), "silent" (unresponsive unless inflamed), and thermosensitive (primarily warm) [628, 629]. These classes may correspond to different functional roles and different subjective feelings. Within the gut, there are also several distinct anatomical patterns of innervation, with some fibers ending within the longitudinal muscle sheets, others ending within the enteric ganglia (intraganglionic laminar endings, IGLEs), [630] and others in close proximity to enterochromaffin [631] or to mast cells [632].

c) Spinal and supraspinal reflexes

The lamina I neurons that receive the small-diameter fiber inputs have projections within the spinal cord and brainstem (**Figure 36A**). In the spinal cord, their only major projection is to the sympathetic cell column of the thoracolumbar spinal cord, where autonomic pre-ganglionic output neurons are located. In the brainstem, they project exclusively to the recognized homeostatic integration sites (e.g. caudal and rostral ventrolateral medulla, catecholamine cell groups A1-2 and A5-7, Barrington's nucleus ("pontine micturition center", PMC), PBN, periaqueductal gray [PAG]), which also receive parasympathetic afferent activity by way of the NTS and which are heavily interconnected with the hypothalamus and the amygdala complex (including the bed nucleus stria terminalis, BNST). The NTS that receives small-diameter fibers from parasympathetic nerves similarly projects to all of these sites. These spinal and bulbar

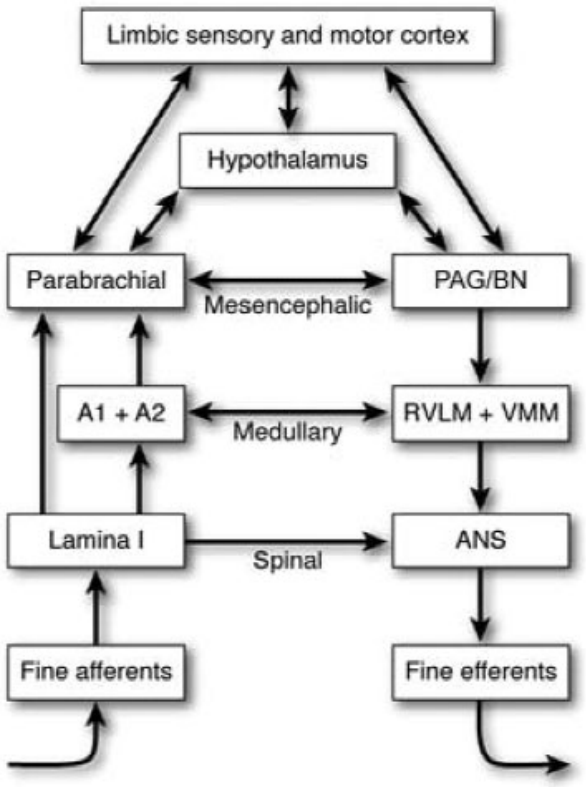


Figure 36: Hierarchical organization of homeostatic reflex systems involving the sympathetic nervous system. A. Homeostatic afferents that report the physiological condition of all tissues in the body, including the GI tract, terminate in lamina I of the dorsal horn. The ascending projections of these neurons provide the basis for reflex arcs at the spinal, medullary and mesencephalic levels. Limbic, paralimbic and prefrontal centers provide modulatory influences on the gain of these reflexes.

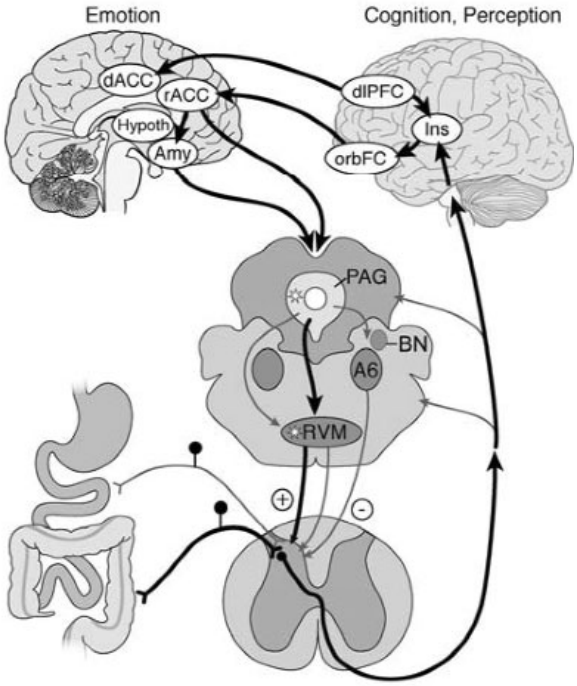


Figure 36B: Cortical modulation of homeostatic afferent input to the central nervous system. PFC regions (dorsolateral PFC [dlPFC], orbitofrontal cortex [orbFC]) modulate activity in limbic and paralimbic regions (amygdala [amy], ACC subregions, and hypothalamus [Hypoth]), which in turn regulate activity of descending inhibitory and facilitatory descending pathways through the PAG and pontomedullary nuclei. Activity in these corticolimbic pontine networks mediates the effect of cognitions and emotions on the perception of homeostatic feelings, including visceral pain and discomfort. From Mayer et al. (635)

projections from lamina I and from the NTS provide the substrate for the hierarchical, modality-selective somato-autonomic reflexes activated by spinal small-diameter afferents that are crucial for homeostatic control of all tissues of the body [633].

Traditionally, the pontine regulation of the pelvic viscera had been considered specific for the urinary bladder and was accordingly referred to as the pontine micturition center (PMC). However, more recent work in rodents has clearly established a role of this pontine nucleus (Barrington's nucleus, BN) as the anatomical substrate for the co-regulation of both bladder and rectosigmoid with central arousal circuits (via projections to the locus coeruleus) and with forebrain function [634] (**Figure 37**). Efferent projections from BN to the dorsal motor nucleus of the vagus (DMV) and to the sacral parasympathetic nucleus give this nucleus a unique role in the regulation of both upper and lower GI function through the two divisions of the parasympathetic nervous system [634]. Efferent projections from BN to the noradrenergic locus coeruleus are an important pathway by which afferent signals from both pelvic viscera can activate central arousal mechanisms, and hyperresponsiveness of these arousal circuits have been implicated in central pain amplification in functional visceral disorders such as IBS and PBS/IC [635]. The forebrain connections from the BN make this brain region ideally positioned to coordinate the activity of both pelvic viscera with behaviors that are appropriate for evacuation. Valentino et al. demonstrated evidence for the existence of 3 types of neuronal populations in BN, one of which is synaptically linked to both the bladder and the colon, and the other two populations which are specifically linked to either viscera [460, 452]. The authors concluded that these neuroanatomical substrates may underlie the central coordination of bladder and colon function and, in pathological states, may play a role in disorders characterized by a co-existence of bladder and colon symptoms.

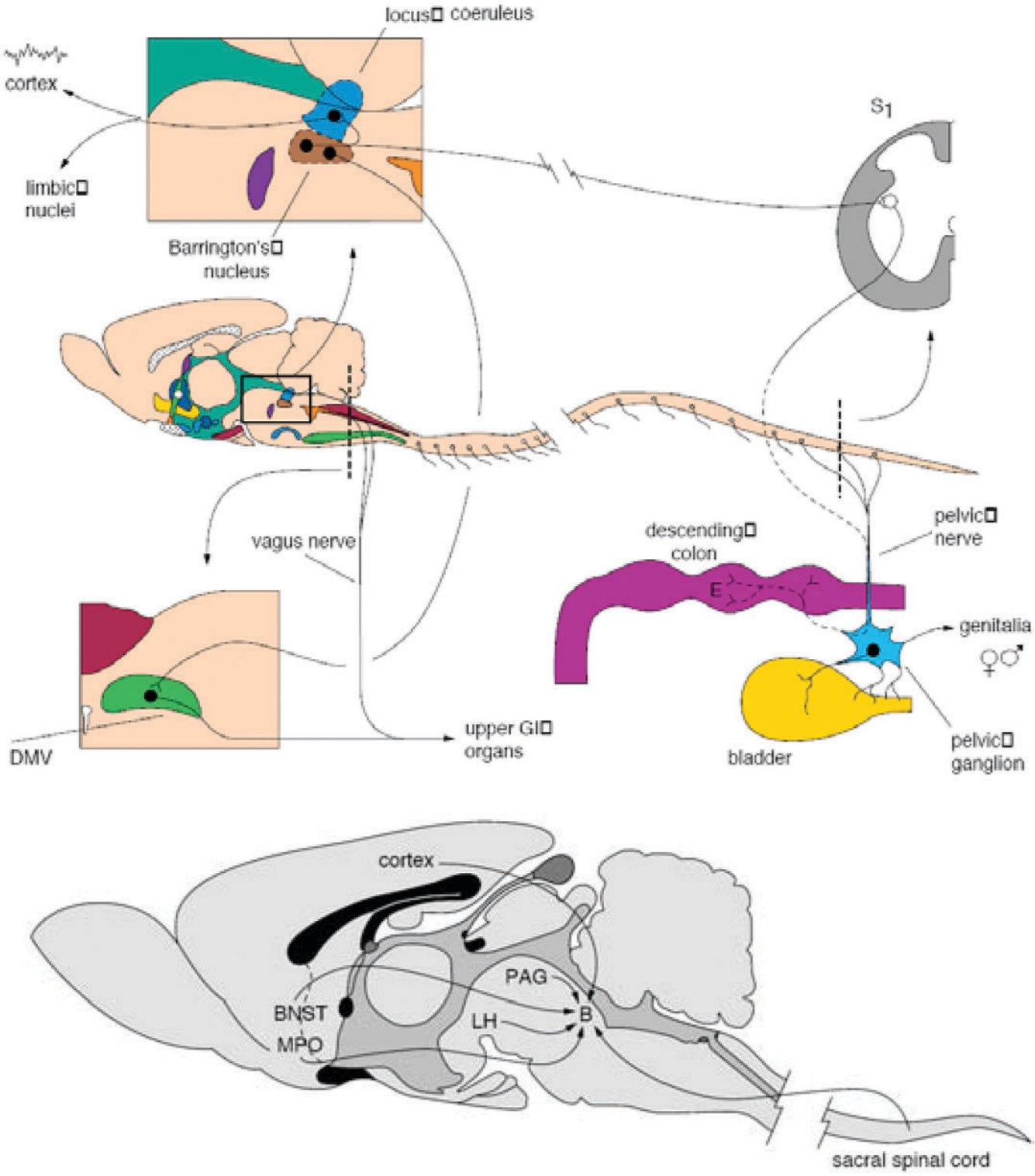


Figure 37: Role of Barrington's nucleus in the co-regulation of pelvic viscera. A, Barrington's nucleus (or "pontine micturition center", PMC) is in a unique strategic position to co-regulate upper and lower gastrointestinal function via vagal and sacral parasympathetic projections, and to influence higher brain functions via projections to cortical and emotional circuits. Close interactions with the noradrenergic locus coeruleus mediate the effect of pelvic visceral afferent input on emotional arousal circuits (ascending noradrenergic projections) as well as on descending pain modulation (descending noradrenergic projections).
B, Afferent projections to Barrington's nucleus as identified by regtrograde and anterograde tracing studies. Different cell groups within Barrington's nucleus receive either converging input from both pelvic viscera, or selective input from either bladder or rectum. BN receives descending projections from the periaqueductal grey (PAG), the lateral hypothalamus (LH), the bed nucleus striae terminalis (BNST), the medial preoptic nucleus (MPO) and cortical regions, including the ACC. From Valentino et al. [634]

d) Spino-thalamic input to interoceptive cortex

In sub-primates, ascending lamina I homeostatic afferent activity is integrated mainly in several brainstem sites (A1, PBN, PAG), which then provide an integrated signal to behavioral control regions in the forebrain. In primates, however, there is a novel, additional lamina I STT projection to a specific thalamo-cortical relay nucleus (VMpo) in posterolateral thalamus, which in turn projects to a discrete portion of dorsal posterior insular cortex (Fig. 35**)** [636]. This interoceptive cortex contains modality-selective representations of all afferent activity from lamina I (i.e., sympathetic afferent input) and from the NTS (i.e., parasympathetic input). In monkeys, this pathway is just visible, but in humans, it is greatly enlarged. Many functional imaging studies in humans confirm role of the insular cortex in pain, hunger, thirst, temperature, itch, muscle sensation, sensual touch, and cardiorespiratory activity, (reviewed in Craig, 2002 [636]), and it can be regarded as primary sensory cortex for the physiological condition of the body in primates. By contrast, in sub-primates the insular cortex appears to have a primordial role in modulating brainstem homeostatic integration (in PBN and other sites) and shows convergent, non-selective responses to homeostatic afferent inputs. The differences in the neuroanatomy of the insular cortex and its subregions have potentially important implications for the pathophysiology of IBS and BPS/IC. The modulation of visceral afferent input reaching the posterior insula is modulated by emotional arousal circuits and by cognitive input at the levels of the mid and anterior insula, respectively [624].

e) Spino thalamic input to limbic behavioral motor cortex

The ascending homeostatic afferent lamina I pathway in primates and humans also provides a direct thalamo-cortical pathway (by way of MDvc in medial thalamus) that activates the dACC (Fig. 35). In sub-primates, the dACC receives only integrated homeostatic input from the brainstem [636, 637]. On the basis of functional imaging and lesion studies in humans, the dACC can be directly associated with the affective aspect of visceral and somatic pain (unpleasantness), and with volition, behavioral motivation, as well as autonomic and motor responses [636,637,638,639,640]. Its interconnections with PFC regions, insular, and ventral striatal regions, along with its strong descending projections to the brainstem, particularly the PAG and the BN, strongly support the idea that it can be regarded generally as the limbic behavioral motor cortex, just as the insula can be regarded as the limbic sensory cortex [625-641].

3. CONSCIOUS PERCEPTION OF INPUT FROM THE BODY AS ASPECTS OF HOMEOSTASIS

Pain or discomfort from the pelvic viscera is often regarded as a distinct feeling, but from the perspective laid out above, pain can be viewed as another homeostatic feeling. It has characteristics exactly comparable to other feelings from the body. Pain normally originates with a change in the condition of the tissues of the body, a physiological imbalance that automatic (subconscious) homeostatic systems alone cannot rectify. It comprises both a sensation and an affective behavioral drive with accompanying autonomic adjustments. Depending on conditions, pain can be unpleasant (as usual) or pleasant (such as when it relieves an intense itch). Pain also generates characteristic reflexive motor patterns as do experimental gut and bladder stimuli. The behavioral motivation of pain is normally correlated with the intensity of the sensory input, but this can vary under different behavioral, autonomic and emotional conditions, so that pain can become intolerable or it can disappear, similar to any other homeostatic emotion (e.g. hunger). The modulation of the motivational aspect of a homeostatic emotion like pain can occur via inhibitory or excitatory prefrontal influences on brain circuits involving the dACC [640-642,643].

Viewing rectal or urinary urge and pain as homeostatic emotions provides a ready explanation of the interactions of these feelings from the body, including “gut feelings,” with other homeostatic conditions (including blood pressure, level of arousal, mood and affect) because homeostasis is an integrated, dynamic process. This conceptual perspective also provides a firm basis for explaining the interactions of pain and non-painful visceral discomfort with emotional status, emotional arousal or attention (i.e., the psychological dimension of pain), and it unifies the different conditions that can cause different types of pain/discomfort from different tissues under a common homeostatic function – the maintenance of the integrity of the body. All animals respond with emotional behavior to stimuli that in humans cause a feeling of pain [644]. An example relevant to preclinical studies in visceral pain mechanisms is the so called visceromotor reflex seen in response to colorectal or urinary bladder distension in rodents [645]. In primates, novel thalamo-cortical projections have emerged from this basic homeostatic system that provides direct pathways to encephalized cortical mechanisms for highly resolved sensations and motivations. In humans, these novel pathways are further elaborated, re-represented, and integrated with other forebrain emotional components in the anterior insula and in the orbitofrontal cortex (the fronto-insular region) (**Figures 35A, B**).

The concept of homeostatic emotions has considerable implications for the study of mechanisms underlying visceral pain and autonomic dysregulation in patients with symptom based (or “functional) disorders of the GI and urinary tract. In the majority of these patients, primary symptoms (urgency, sensation of fullness, pain) are directly related to the altered perception of homeostatic feelings associated with the intestine,

rectum or urinary bladder (visceral hypersensitivity). Since rodents, the animals most commonly used in experiments to model these disorders, do not have the forebrain structures to generate the same conscious emotional feelings of humans, findings obtained in such animals (using so-called pseudo-affective reflex responses) may reflect primarily the phylogenetically shared enteric, spinal and brainstem components of homeostatic pathways (e.g. reflexes), but may provide little or no insight into the uniquely human experience of abdominal pain and discomfort, nor to the modulatory cortical influences related to this experience.

The VMpo also has a collateral projection to a portion of sensorimotor cortex (area 3a) that is intercalated between the primary somatosensory area and the primary motor area. Similarly, vagal afferent activation occurs in the lateral portion of area 3a of the primary sensorimotor region by way of the NTS and VMb. These projections can be associated with cortical control of the reflex actions of skeletal muscle in response to homeostatic afferent inputs, a role subsumed under the term "viscero-somatic" integration.

The forebrain interoceptive representation of the body's condition in the dorsal posterior insula is successively re-represented in the middle insula and then in the right (non-dominant) anterior insula (636). Functional imaging data show that the right anterior insula is associated with subjective awareness of homeostatic emotions (e.g. visceral and somatic painful and non-painful sensations, temperature, sexual arousal, hunger, and thirst) as well as all emotions (e.g. anger, fear, disgust). This region is intimately interconnected with the dACC, which is co-active in such studies. Thus, viewing feelings from the body as homeostatic emotions enables neuroanatomical explanations of interactions with other homeostatic functions and with emotions at the level of the forebrain. Finally, recent evidence showing asymmetry in the sympathetic and parasympathetic afferent re-representations and control mechanisms in the left and right insula/orbitofrontal cortex and ACC seems to accord with the psychophysiological evidence for the forebrain asymmetry of positive and negative emotions [646]. In this context, it is of considerable interest that studies of central representation of both bladder [482] and rectal distensions [635] have repeatedly shown activation of the *right* ventrolateral PFC.

4. DESCENDING MODULATION OF HOMEOSTATIC REFLEXES AND FEELINGS

Lamina I neurons not only receive input via somatic and visceral afferents, but also receive descending (facilitatory and inhibitory) modulation directly from brainstem pre-autonomic sources, including serotonergic nuclei within the rostral ventral medulla and the noradrenergic pontine locus coeruleus complex (**Figure 36**). Indeed, lamina I and the spinal autonomic columns are the only regions in the spinal cord that receive descending modulation from the hypothalamus. Thus, the activity of lamina I neurons that ultimately produces the various feelings from the body is modulated by various tonic and phasically active, descending inhibitory and facilitatory pathways [647,648,649], whose primary purpose is control of homeostatic integration [650,651]. As pointed out by Mason [651,652,653], these serotonergic descending pathways from the PAG and ventromedial medulla are not only involved in descending pain modulation, but play a prominent role in various homeostatic functions including micturition and continence.

Recent evidence (in rats) suggests that the gain of the spino-bulbo-spinal reflex loops originating from neurokinin 1-receptor-containing lamina I neurons is not constant but can be modulated by peripheral primary afferent inputs from inflamed tissue or damaged nerves, [654] adapting the homeostatic response to the overall state of the organism. Thus, since the descending forebrain control of these pre-autonomic brainstem regions originates in brain networks associated with attention, emotion generation (i.e. the limbic system) and emotion regulation (PFC), these anatomic connections provide the basis for corticolimbic modulation of the afferent activity associated with feelings from the body, including pain and discomfort, at the spinal level as well as at the brainstem and forebrain levels (**Figure 36**).

5. BRAIN CIRCUITS ACTIVATED BY ACUTE VISCERAL STIMULI: EVIDENCE FROM FUNCTIONAL BRAIN IMAGING STUDIES IN HUMANS

Visceral sensations, including discomfort, hunger, fullness, early satiety, nausea, bloating or abdominal pain in humans are a subjective, conscious experience, that result from the modulation of homeostatic feelings by cognitive (attention, expectation), emotional (arousal, anxiety) and motivational factors, as well as memories of past experiences. Thus, the conscious experience is an image of the homeostatic state of the body represented in the insular cortex (**Figure 35**) and is further modified by these cortical and limbic inputs. In principal, altered perception of visceral stimuli could result from activity changes in visceral afferent signal processing areas alone (reflecting increased visceral afferent input to the brain from the gut), from alterations in distinct but overlapping pain modulation circuits ("central pain amplification"), or from variable combinations of these two overlapping circuitries [655,656,657] (**Figure 37**).

a) Activation of Regions involved in Homeostatic Emotions in Studies of the Human Brain-Gut Axis

Studies published during the past 5 years using widely

different experimental paradigms have confirmed the consistent activation of the central homeostatic afferent network consistent with the robust activation of homeostatic afferent pathways despite varying experimental paradigms and analysis techniques [658,659,660,661,662,663]. On the other hand, the variable activations of PFC and limbic regions, and the seemingly contradictory results on sex differences in reported studies is reflective of the fact that experimental paradigms and analysis techniques varied, and that the majority of these studies did not take into account cognitive and emotional aspects of pain modulation.

b) Modulation of Homeostatic Afferent Network Matrix by Cognitive and Emotional Stimuli

The brain has multiple ways to modulate the perception of afferent information and this modulation is influenced by the environmental context, the emotional state of the individual (e.g. fear, anxiety, or anger), cognitive factors (e.g. expectation, attention), or memories of previous sensory events (conditioned responses) (**Figure 36B**). As outlined in the earlier sections of this review, these top-down influences can modulate homeostatic afferent input to the central nervous system at multiple levels (**Figure 37A**). Considerable progress has been made both on a preclinical and, more recently, on a clinical level to identify brain regions, circuits and mechanisms which play a role in the facilitation and inhibition of the subjective pain experience [649-664], Both clinical and preclinical studies support a role for right orbitofrontal and right ventrolateral PFC in mediating inhibition of emotional and pain responses (reviewed in Lieberman et al. 2004 [643]) and for dorsolateral PFC in pain modulation [642-665].

• Cognitive Modulation

Attention. Aziz's group (666) examined the modulatory role of attention on the brain responses to non-painful visceral (esophageal) distension in 7 healthy volunteers (6 males). Brain responses to phasic visual and esophageal stimuli were presented simultaneously while subjects were asked to focus their attention on either the esophageal or the visual stimulus (selective attention) or both (divided attention). Selective attention on the esophageal stimulus was associated with activation of sensory (somatosensory cortex) and cognitive (dACC) networks, while selective attention on the visual stimulus activated the visual cortex. During the divided attention task, more brain regions in the sensory and cognitive domains were activated to process esophageal stimuli, in comparison to those processing visual stimuli. These findings emphasize the importance of attentional processes in the modulation of sensory information from the body and the relative biological importance placed on visceral sensation, compared to other sensory modalities.

Expectation. A variety of studies in the somatic pain literature have evaluated brain responses to the expectation of an aversive stimulus [662-667, 668, 669,670]. These studies suggest that the brain can either up- or down-regulate sensory and limbic brain regions based on previous experience, familiarity with the stimulus and expected intensity [668]. Several early studies have shown some evidence of activation of the homeostatic afferent network during conditioned anticipation of a visceral stimulus [662-667]. Berman et al. studied the brain fMRI BOLD response to anticipated (cue condition) and delivered mild and moderate rectal distension in 12 healthy women and 14 female IBS patients [671]. Distension increased activity in the homeostatic brain regions and decreased activity in the infragenual cingulate. As in previous studies comparing IBS patients and control subjects, the increases were more extensive in the IBS patients, with significant differences in midcingulate and dorsal brainstem. During cued anticipation of distension, activity *decreased* in the insula, dACC, amygdala and dorsal brainstem in healthy women, but not in IBS patients, consistent with a top-down modulation of homeostatic afferent networks by cortical regions. Three self-rated measures of negative affect during scanning were higher in IBS patients than healthy women ($P < 0.001$), and the anticipatory BOLD decreases in bilateral dorsal brainstem, centered in the pontine LCC, were inversely correlated with all three measures. The amplitude of anticipatory decrease in the LCC was associated with greater activation by subsequent distension in right orbitofrontal cortex and bilateral supragenual ACC – regions previously implicated in corticolimbic inhibition. When the cue was followed by a sham distension (e.g. no change in distension pressure from the resting pressure of 5 mmHG occurred), only IBS patients showed activation of the insula cortex, and this activation was limited to the more anterior portions of the insula. These findings are consistent with top-down modulation of the anterior insula by PFC influences, and with an enhanced top-down facilitation in IBS patients. In summary, these findings suggest that in healthy women, the brain decreases activity within the homeostatic brain matrix in expectation of a certain, inescapable pelvic pain stimulus. A failure to generate this down-regulation in IBS patients, and an inappropriate activation of the anterior insula during a sham distension, may be related to differences in cortically-mediated coping styles, emotional factors and linked arousal systems.

Yaguez et al. [672] studied brain responses during different phases of visceral aversive conditioning in 8 healthy volunteers (5 males) using fMRI. The authors used a classical conditioning paradigm in which different colored circles were used as conditioned stimuli and were paired with painful esophageal distension (learning phase), airpuff to the wrist or nothing. Brain responses during the learning phase

(delivery of aversive esophageal distension) were seen in the homeostatic brain matrix and in somatosensory cortex. During the anticipation and extinction phase of the paradigm, brain activity resembled that seen during actual esophageal distension, including activation in insula and dACC. These findings emphasize the importance of cognitive influences, such as expectation and memory recall in top-down modulation of brain regions involved in the processing of homeostatic information from the body.

Hypervigilance. Several lines of evidence indicate that IBS patients and other functional disorders have hypervigilance for symptom-relevant sensations [673]. Repeated exposure to experimental visceral stimuli can lead to decreased hypervigilance and, therefore, discomfort. In a longitudinal study of IBS patients exposed to 6 sessions of rectal inflations over a 1-year period, we examined regional cerebral blood flow to the inflations and anticipation of inflations using $H_2{}^{15}O$-PET at the first and last session [674]. Subjective ratings of the rectal inflations normalized over the 12 months of the study, while IBS symptom severity did not, indicating decreased vigilance independent of changes in perceived disease activity. In response to rectal distension, stable activation of regions of the homeostatic afferent network (including thalamus and anterior insula) was observed over the 12-month period, while activity in limbic, paralimbic and pontine regions decreased. During the anticipation condition, there were significant decreases in dACC, amygdala, and dorsal brainstem (perhaps involving the LCC) activation at 12 months. One way to interpret these findings is that brain regions processing the feeling and the motivational dimension of the homeostatic emotion were affected differentially by the habituation process: while insula activation remained constant, dACC activation progressively decreased. An analysis examining the covariation of these brain regions, as well as preliminary results from an effective connectivity modeling approach to the data [675] supported the hypothesis of changes in an arousal network including limbic, pontine and cortical areas underlying the decreased perception seen over the multiple stimulation studies.

In summary**,** brain imaging studies of cognitive and affective modulation of perceptual and brain responses to visceral stimuli strongly support the concept of a homeostatic afferent network in the brain. The fact that components of this network can be modulated differentially by top-down corticolimbic influences has important implications for a better understanding of symptom generation/modulation in functional GI disorders.

c) Modulation of Homeostatic Emotions by Descending Modulation

Since the beginning of the 20th century it has been known that the brain can tonically inhibit spinal cord excitability, thereby regulating the amount of peripheral sensory information reaching the central nervous system. More recent evidence has demonstrated the activity of both pain inhibitory and facilitatory mechanisms which can tonically and phasically regulate spinal cord excitability [647,648,649]. While top-down tonic pain inhibitory modulation appears to predominate in healthy individuals during basal conditions, an up-regulation of descending pain facilitatory systems has been demonstrated in the maintenance of hyperalgesia in animal models of peripheral nerve injury [654]. An alteration in the balance between inhibitory and facilitatory pain modulatory systems has been proposed as a possible mechanism underlying chronic pain syndromes such as fibromyalgia [676] and IBS [677,678].

Zambreanu and coworkers were the first to demonstrate the activation of brainstem regions in the context of central sensitization in healthy human volunteers [679]. Using 3T fMRI, they compared whole brain responses, including the brainstem, to punctuate mechanical stimulation in an area of secondary hyperalgesia (induced by heat/capsaicin sensitization model) or in a control area. They found greater activation during stimulation of the hyperalgesic region in several cortical regions, including posterior insula, ACC and posterior cingulate cortex, as well as thalamus and pons. The brainstem activation was localized to the NCF (possibly involving PBN) and the PAG, brain regions that receive input from corticolimbic networks (including the rostral ACC), send projections to the rostroventral medulla and are part of a cortico-limbic-pontine pain modulation circuit [680,681] (**see also Figure 36**). There is preliminary evidence to suggest that patients with IBS may also show abnormal activation of brain circuits involved in pain modulation [660-682,683]. Two studies were performed by Wilder-Smith and coworkers [660- 683] to study the central correlates of heterotopic pain inhibition. In the first study, they performed an fMRI study in 10 female patients with IBS (5 constipated-, 5 diarrhea-predominant bowel habit) and 10 female healthy control subjects to test the hypothesis that IBS patients show abnormal activation of diffuse noxious inhibitory control (DNIC) systems in response to a noxious stimulus [660]. DNIC activation can be quantified by the perceptual modulation of a painful stimulus (in this case noxious rectal balloon distension) by a secondary heterotypically applied nociceptive stimulus (in this case ice water immersion of the foot). They found that subjective pain ratings of rectal volume distension by the heterotypic cold pain stimulus was reduced in healthy controls but not in the IBS patient group, suggesting an inadequate activation of DNICs in the IBS patients. Following the heterotypic cold stimulus, a complex set of differences in response to rectal pain were found among the controls and the two IBS bowel habit-based sub-groups. These included decreased activation in insula, thalamus and PAG in

the control group (perhaps reflecting the DNIC process) that was absent in the IBS patients. In the second study, similar perceptual results were found and differences were also found between IBS and controls during expectation of rectal pain, without actual distension [683].

More recent brain imaging studies in patient populations provide more direct support for alterations in cortico-limbic-pontine pain modulation networks in IBS patients leading to visceral hypersensitivity. Mayer et al. [682] examined three groups of male subjects, ulcerative colitis patients with quiescent disease (n = 9), patients with IBS (n = 9), and healthy male controls (n = 9), during actual and expected but undelivered rectal distensions using $H_2{}^{15}O$-PET. This study found similar responses in all three groups in the homeostatic afferent network (anterior insula and dACC). However, IBS patients compared to both the ulcerative colitis and control groups showed consistently greater activation of limbic/paralimbic brain regions (amygdala, hypothalamus, ventral/rostral ACC, dorsomedial PFC), suggestive of increased activation of pain facilitatory pathways. In addition, the results showed activation in the ulcerative colitis and control subjects, but not IBS patients, in the lateral frontal regions and a brain region including the PAG. A connectivity analysis using structural equation modeling supported these regions acting as part of a pain inhibition network that involves lateral and medial frontal influences on the PAG.

In summary, a small group of hypothesis-driven studies aimed at evaluating specific mechanisms of visceral pain modulation (e.g. descending modulation) are beginning to demonstrate the activation of cortico-limbic-pontine networks, which may be the biological substrate of such endogenous modulation. The concept emerging from these studies is the ability of brain regions and networks involved in emotional generation (limbic circuits) as well as in emotion regulation (orbitofrontal, ventrolateral PFC) to modulate activity within the homeostatic brain matrix [655, 656]. Observations obtained with rectal and with bladder stimuli show many similarities in these activations [482]. Differences in the activation of these networks by cognitive or somatic stimuli between IBS patients and control populations suggest a possible role of such differences in the pathophysiology of enhanced visceral perception in IBS.

The concept of homeostatic emotions with a sensory feeling dimension (processed in the anterior insula) and a motivational dimension (processed in the dACC) provides a general framework to understand the interactions between peripheral afferent signals arising from the pelvic viscera, and centrally-mediated influences (including psychological factors, cognitions and emotions) on the conscious perception and associated autonomic responses to such stimuli. The deconstruction of symptoms into distinct contributions of specific brain networks mediating a variety of cognitive, emotional and motivational influences on the basic homeostatic afferent processing network may help to understand the pathophysiology of a variety of chronic disorders, including IBS and PBS/IC. A whole range of chronic disorders characterized by physical or emotional discomfort and pain (including functional visceral and somatic disorders, as well as disorders of mood and affect) can be conceptualized as disorders of homeostatic emotions. The ability to study a neurobiological substrate (e.g. brain activity) rather than relying on highly variable subjective symptoms, may make it possible to obtain insights about the role of genetic factors, receptor physiology, and drug interventions from much smaller samples of subjects, compared to epidemiological or traditional pharmacological studies.

REFERENCES

1. Andrew J, Nathan PW. Lesions of the anterior frontal lobes and disturbances of micturition and defaecation. Brain. 1964;87:233-62.
2. Fowler CJ, Griffiths D, De Groat WC. The neural control of micturition. Nature Reviews Neuroscience. 2008;9:453-66.
3. DasGupta R, Kavia RB, Fowler CJ. Cerebral mechanisms and voiding dysfunction. BJU International. 2007;99:731-4.
4. Kavia RB, Dasgupta R, Fowler CJ. Functional imaging and the central control of the bladder. J Comp Neurol. 2005;493:27-32.
5. Apodaca G. The uroepithelium: Not just a passive barrier. Traffic. 2004;5:117-28.
6. Lewis SA. Everything you wanted to know about the bladder epithelium but were afraid to ask. Am J Physiol Renal Physiol. [Invited Review]. 2000;278:F867-F74.
7. Hicks M. The mammalian urinary bladder: an accomodating organ. Biol Rev. 1975;50:215-46.
8. Liang FX, Riedel,I., Deng,F.M., Zhou,G., Wu, C., Wu,W.R., Kong,W.P., Moll,R., Sun,T.T. Organization of uroplakin subunits: transmembrane topology, pair formation and plaque composition. J Biochem. 2001;355:13-8.
9. Wu X.R, Lin J.H, Walz T., Haner M., Yu J., Aebi U., Sun T.T. Mammalian uroplakins. A group of highly conserved urothelial differentiation-related membrane proteins. J. BiolChem. 1994;269:13716-24.
10. Sun Y, Keay,S., DeDeyne,P., Chai,T.C. Augmented stretch activated adenosine triphosphate release from bladder uroepithelial cells in patients with interstitial cystitis. J Urol. 2001;166:1951-56.
11. Acharya P, Beckel JM, Ruiz WG, Wang E, Rojas R, Birder LA, et al. Distribution of the tight junction proteins ZPO-1, occludin, and claudin-4, -8, and -12 in bladder epithelium. Am J Physiol Renal Physiol. 2004 August 2004;287(2):F305-F18.
12. Parsons CL, Greenspan, C., Moore, S.W., Mulholland, S.G. Role of surface mucin in primary antibacterial defense of bladder. Urology. 1977;9:48-52.
13. Parson CL, Boychuk,D., Jones,S., Hurst,R., Callahan, H. Bladder surface glycosaminoglycans: an epithelial permeability barrier. Journal of Urology. 1990;143:139-42.
14. Parsons CL, Lilly JD, Stein P. Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J Urology. 1991;145(4):732-5.

15. Wang E, Truschel ST, Apodaca G. Analysis of hydrostatic pressure-induced changes in umbrella cell surface area. Methods. 2003;30:207-17.
16. Truschel ST, Wang E, Ruiz WG, Leung SM, Rojas R, Lavelle J, et al. Stretch-regulated exocytosis/endocytosis in bladder umbrella cells. Molecular Biology of the Cell. 2002;13:830-43.
17. Balestreire EM, Apodaca,G. Apical EGF receptor signaling: regulation of stretch-dependent exocytosis in bladder umbrella cells. Mol Biol Cell. 2007;18:1312-23.
18. Cheng J, Huang,H., Zhang,Z.T. Overexpression of epidermal growth factor receptor in urothelium elicits urothelial hyperplasia and promotes bladder tumor growth. Cancer Research. 2002;62:4157-63.
19. Apodaca G, Balestreire,E., Birder,L.A. The uroepithelial-associated sensory web. Kidney International. 2007;72:1057-64.
20. Birder LA, DeGroat WC. Mechanisms of disease: involvement of the urothelium in bladder dysfunction. Nature Clinical Practice. [Review]. 2007;4(1):46-54.
21. Martin BF. Cell replacement and differentiation in transitional epithelium: a histological and autographical study of the guinea-pig bladder and ureter. J Anat. 1972;112:433-55.
22. Lavelle J, Meyers,S., Ramage,R., Bastacky,S., Doty,D., Apodaca,G., Zeidel,M.L. Bladder permeability barrier: recovery from selective injury of surface epithelial cells. American Journal of Physiology. 2002;283:F242-53.
23. Bassuk JA, Cockrane,K., Mitchell,M.E. Induction of urothelial cell proliferation by fibroblast growth factor-7 in RAG1-deficient mice. Adv Exp Med Biol. 2003;539:623-33.
24. de Boer WI, Vermeij,M., Diez de Medina, S.G., Bindels,E., Radvanyi,F., van der Kwast, T., Chopin,D. Functions of fibroblast and transforming growth factors in primary organoid-like cultures of normal human urothelium. Lab Invest. 1996;75:147-56.
25. Anderson G, Palermo J, Schilling J, Roth R, Heuser J, Hultgren S. Intracellular bacterial biofilm-like pods in urinary tract infections. Science. 2003;301:105-7.
26. Apodaca G, Kiss S, Ruiz WG, Meyers S, Zeidel M, Birder LA. Disruption of bladder epithelium barrier function after spinal cord injury. Am J Physiol Renal Physiol. 2003 May 2003;284(5):F966-F76.
27. Lavelle JP, Meyers SA, Ruiz WG, Buffington CAT, Zeidel M, Apodaca G. Urothelial pathophysiological changes in feline interstitial cystitis: A human model. Am J Physiol Renal Physiol. 2000;278:F540-F53.
28. Conrads TP, Tocci,G.M., Hood,B.L., Zhang,C.O., Guo,L., Koch,K.R., Michejda,C.J., Veenstra,T.D., Keay,S.K. CKAP4/p63 is a receptor for the frizzled-8 protein-related antiproliferative factor from interstitial cystitis patients. J Biol Chem. 2006;281:37836-43.
29. Keay SK, Zhang CO, Shoenfelt J, Erickson DR, Whitmore K, Warren JW, et al. Sensitivity and specificity of antiproliferative factor, heparin-binding epidermal growth factor-like growth factor, and epidermal growth factor as urine markers for interstitial cystitis. Urology. 2001;57(6 Suppl 1):9-14.
30. Keay SK, Szekely Z, Conrads TP, Veenstra TD, Barchi JJJ, Zhang CO, et al. An antiproliferative factor from interstitial cystitis patients is a frizzled 8 protein-related sialoglycopeptide. Proc Natl Acad Sci USA. 2004;101(32):11803-8.
31. Schilling J, Hultgren S. Recent advances into the pathogenesis of recurrent urinary tract infections: the bladder as a reservoir for uropathogenic Escherichia coli. Int J Antimicrob Agents. 2002;19:457-560.
32. Thomas JC, DeMarco,R.T., Pope,J.C. Molecular biology of ureteral bud and trigonal development. Curr Urol Rep. 2005;6:146-51.
33. Liang FX, Bosland,M.C., Huang,H., Romih,R., Baptiste,S., Deng,F.M., Wu,X.R., Shapiro,E., Sun,T.T. Cellular basis of urothelial squamous metaplasia: roles of lineage heterogeneity and cell replacement. J Cell Biol. 2005;171:835-44.
34. Birder LA, Nealen,M.L., Kiss,S., de Groat,W.C., Caterina,M.J., Wang,E., Apodaca,G., Kanai,A.J. Beta-adrenoceptor agonists stimulate endothelial nitric oxide synthase in rat urinary bladder urothelial cells. J Neuroscience. 2002;22:8063-70.
35. Jen PY, Dixon,J.S., Gosling,J.A. Immunohistochemical localization of neuromarkers and neuropeptides in human fetal and neonatal urinary bladder. Br J Urol. 1995;75:230-5.
36. Kunze A, Neuhaus,J., Stolzenburg,J.U. Quantitative immunohistochemical study of the innervation of the guinea-pig lower urinary tract. BJU Int. 2006;98:424-9.
37. Apostolidis A, Brady,C.M., Yiangou,Y., Davis,J., Fowler,C.J., Anand,P. Capsaicin receptor TRPV1 in urothelium of neurogenic human bladders and effect of intravesical resiniferatoxin. Urology. 2005;65:400-5.
38. Brady CM, Apostolidis,A., Yiangou,Y., Baecker,P.A., Ford,A.P., Freeman,A., Jacques,T.S., Fowler,C.J., Anand,P. P2X3-immunoreactive nerve fibres in neurogenic detrusor overactivity and the effect of intravesical resiniferatoxin. Eur Urol. 2004;46:247-53.
39. Ost D, Roskams,T., Van der Aa,F., de Ridder,D. Topography of the vanilloid receptor in the human bladder: more than just the nerve fibers. Journal of Urology. 2002;168:293-7.
40. Sui GP, Wu,C., Fry,C.H. Electrical characteristics of suburothelial cells isolated from the human bladder. Journal of Urology. 2004;171:938-43.
41. Brading AF, McCloskey,K.D. Mechanisms of disease: specialized interstitial cells of the urothelium: an assessment of current knowledge. Nature Clinical Practice Urology. 2005;2:546-54.
42. Ikeda Y, Fry C, Hayashi F, Stolz D, Griffiths D, Kanai AJ. Role of gap junctions in spontaneous activity of the rat bladder. Am J Physiol Renal Physiol. 2007;293(4):F1018-F25.
43. Chopra B, Gever J, Barrick SR, Hanna-Mitchell AT, Beckel JM, Ford AP, et al. Expression and function of rat urothelial P2Y receptors. Am J Physiol Renal Physiol. 2008;294(4):F821-F9.
44. Chopra B, Barrick, S.R., Meyers,S., Beckel,J.M., Zeidel,M.L., Ford,A.P., de Groat,W.C., Birder,L.A. Expression and function of bradykinin B1 and B2 receptors in normal and inflamed rat urinary bladder urothelium. J Physiol. 2005;562:859-71.
45. Burnstock G. Purine-mediated signalling in pain and visceral perception. Trends in Pharmcol Sci. 2001;22:182-8.
46. Chess-Williams R. Muscarinic receptors of the urinary bladder: detrusor, urothelial and prejunctional. Auton Autocoid Pharmacol. 2002;22:133-45.
47. Birder LA, Barrick SR, Roppolo JR, Kanai AJ, DeGroat WC, Kiss S, et al. Feline interstitial cystitis results in mechanical hypersensitivity and altered ATP release from bladder urothelium. Am J Physiol Renal Physiol. 2003;285:F423-F9.
48. Ferguson DR, Kennedy,I., Burton,T.J. ATP is released from rat urinary bladder epithelial cells by hydrostatic pressure changes-a possible sensory mechanism? J Physiol. 1997;505:503-11.
49. Beckel JM, Kanai AJ, Lee SJ, DeGroat WC, Birder LA. Expression of functional nicotinic acetylcholine receptors in rat urinary bladder epithelial cells. Am J Physiol Renal Physiol. 2006;290(1):F103-F10.
50. Ossovskaya VS, Bunnett,N.W. Protease-activated receptors: contribution to physiology and disease. Physiol Rev. 2004;84:579-621.

51. Carattino MD, Sheng,S., Kleyman,T.R. Mutations in the pore region modify epithelial sodium channel gating by shear stress. J Biol Chem. 2005;280:4393-401.
52. LeBerge J, Malley SE, Zvarova K, Vizzard MA. Expression of corticotropin-releasing factor and CRF receptors in micturition pathways after cyclophosphamide-induced cystitis. Am J Physiol Regul Integr Comp Physiol.2006;291(3):R692-R703.
53. Hanna-Mitchell AT, Beckel JM, Barbadora S, Kanai AJ, DeGroat WC, Birder LA. Non-neuronal acetylcholine and urinary bladder urothelium. Life Sci. 2007;80(24-25):2298-302.
54. Andersson KE, Persson,K. Nitric oxide synthase and the lower urinary tract: posible implications for physiology and pathophysiology. Scand J Urol Nephrol. 1995;175:43-53.
55. Templeman L, Chapple CR, Chess-Williams R. Urothelium derived inhibitory factor and cross-talk among receptors in the trigone of the bladder of the pig. J Urology. 2002;167:742-5.
56. Wang EC, Lee JM, Ruiz WG, Balestreire EM, von Bodungen M, Barrick SR, et al. ATP and purinergic receptor-dependent membrane traffic in bladder umbrella cells. J Clin Invest. 2005;115(9):2412-22.
57. Sui GP, Wu,C., Fry, C.H. Characterization of the purinergic receptor subtype on guinea-pig suburothelial myofibroblasts. BJU Int. 2006;97:1327-31.
58. Fry CH, Sui,G.P., Kanai,A.J., Wu,C. The function of suburothelial myofibroblasts in the bladder. Neurourol Urodyn. 2007;26:914-9.
59. Du S, Araki I, Mikami Y, Zakoji H, Beppu M, Yoshiyama M, et al. Amiloride-sensitive ion channels in urinary bladder epithelium involved in mechanosensory transduction by modulating stretch-evoked adenosine triphosphate release. Urology.2007;69(3):590-5.
60. Yu W, Zacharia,L.C., Jackson,E.K., Apodaca,G. Adenosine receptor expression and function in bladder uroepithelium. Am J Physiol. 2006;291:C254-65.
61. Birder LA, Apodaca,G., de Groat,W.C., Kanai,A.J. Adrenergic and capsaicin-evoked nitric oxide release from urothelium and afferent nerves in urinary bladder. Am J Physiol. 1998;275:F226-9.
62. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature. 1997;389(6653):816-24.
63. Hicks GA. TRP channels as therapeutic targets: hot property, or time to cool down? Neurogastroenterol Motil. 2006;18:590-4.
64. Chancellor MB, de Groat,W.C. Intravesical capsaicin and resiniferatoxin therapy: spicing up the ways to treat the overactive bladder. J Urol. 1999;162:3-11.
65. Szallasi A, Blumber,P.M. Vanilloid (capsaicin) receptors and mechanisms. Pharmacol Rev. 1999;51:150-221.
66. Birder LA, Nakamura Y, Kiss S, Nealen ML, Barrick SR, Kanai AJ, et al. Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci. 2002;5(9):856-60.
67. Liedtke W. TRPV4 plays an evolutionary conserved role in the transduction of osmotic and mechanical stimuli in live animals. Pflugers Arch. 2005;451:176-80.
68. Gevaert T, Vriens,J., Segal,A., Evaraerts,W., Roskams,T., Talavera,K., Owsianik,G., Liedtke,W., Daelemans,D., Dewachter,I., Van Leuven,F., Voets,T., De Ridder,D., Nilius,B. Deletion of the transient receptor potential cation channel TRPV4 impairs murine bladder voiding. J Clin Invest. 2007;117:3453-62.
69. Birder LA, Kullmann,F.A., Lee,H., Barrick,S., de Groat,W.C., Kanai,A., Caterina,M. Activation of urothelial transient recepto potential vanilloid 4 by 4alpha-phorbol 12,13-didecanoate contributes to altered bladder reflexes in the rat. J Pharm Exp Ther. 2007;323:227-35.
70. Combrisson H, Allix,S., Robain,G. Influence of temperature on urethra to bladder micturition reflex in the awake ewe. Neurourol Urodyn. 2007;26:290-5.
71. Du S, Araki,I., Yoshiyama,M., Nomura,T., Takeda,M. Transient receptor potential channel A1 involved in sensory transduction of rat urinary bladder through C-fiber pathway. Urology. 2007;70:826-31.
72. Andrade EL, Ferreira,J., Andre,E., Calixto,J.B. Contractile mechanisms coupled to TRPA1 recepto activation in rat urinary bladder. Biochem Pharmacol. 2006;72:104-14.
73. Sun Y, Chai,T.C. Augmented extracellular ATP signaling in bladder urothelial cells from patients with interstitial cystitis. Am J Physiol. 2006;290:C27-34.
74. Zarghooni S, Wunsch,J., Bodenbenner,M., Bruggmann,D., Grando,S.A., Schwantes, U., Wess,J., Kummer,W., Lips,K.S. Expression of muscarinic and nicotinic acetylcholine receptors in the mouse urothelium. Life Sci. 2007;80:2308-13.
75. Kullman FA, Artim,D.E., Birder,L.A., de Groat,W.C. Activation of muscarinic receptos in rat bladder sensory pathways alters reflex bladder activity. J Neuroscience. 2008;28:1977-87.
76. Kullman FA, Artim D, Beckel JM, Barrick SR, DeGroat WC, Birder LA. Heterogeneity of muscarinic receptor-mediated Ca2+ responses in cultured urothelial cells from rat. Am J Physiol Renal Physiol. 2008;294(4):F971-F81.
77. Chancellor MB, Fowler,C.J., Apostolidis,A., de Groat,W.C., Smith,C.P., Somogyi,G.T., Aoki,R. Drug insight: biological effects of botulinum toxin A in the lower urinary tract. Nat Clin Prac Urol. 2008;5:319-28.
78. Bosse Y, Pare,P.D., Seow,C.Y. Airway wall remodeling in asthma: from the epithelial layer to the adventitia. Curr Allergy Asthma Rep. 2008;8:357-66.
79. Hendrix S. Neuroimmune communication in skin: Far from peripheral. J Invest Derm. 2008;128:260-1.
80. Lumplin EA, Caterina,M.J. Mechanisms of sensory transduction in the skin. Nature Rev. 2007;445:858-65.
81. Janig W, Morrison JF. Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception. Prog Brain Res. 1986;67:87-114.
82. de Groat WC. Spinal cord projections and neuropeptides in visceral afferent neurons. Prog Brain Res. 1986;67:165-87.
83. Torrens M, Morrison JFB. The physiology of the lower urinary tract. 1987.
84. Iggo A. Tension receptors in the stomach and the urinary bladder. J Physiol. 1955;128(3):593-607.
85. Xu L, Gebhart GF. Characterization of mouse lumbar splanchnic and pelvic nerve urinary bladder mechanosensory afferents. J Neurophysiol. 2007 Nov 14.
86. Zagorodnyuk VP, Costa M, Brookes SJ. Major classes of sensory neurons to the urinary bladder. Auton Neurosci. 2006 Jun 30;126-127:390-7.
87. de Groat WC, Booth AM, Yoshimura N, C.A.Maggi. Neurophysiology of micturition and its modification in animal models of human disease. The Autonomic Nervous System, Vol3, Chapter 8, Nervous Control of the Urogenital System. London: Harwood Academic Publishers, U.K.; 1993. p. 227-89.
88. Wiseman OJ, Brady CM, Hussain IF, Dasgupta P, Watt H, Fowler CJ, et al. The ultrastructure of bladder lamina propria nerves in healthy subjects and patients with detrusor hyperreflexia. JUrol. 2002;168(5):2040-5.

89. Gabella G, Davis C. Distribution of afferent axons in the bladder of rats. J Neurocytol. 1998;27(3):141-55.

90. Smet PJ, Edyvane KA, Jonavicius J, Marshall VR. Neuropeptides and neurotransmitter-synthesizing enzymes in intrinsic neurons of the human urinary bladder. Journal of Neurocytology. 1996;25(2):112-24.

91. Smet PJ, Moore KH, Jonavicius J. Distribution and colocalization of calcitonin gene-related peptide, tachykinins, and vasoactive intestinal peptide in normal and idiopathic unstable human urinary bladder. Lab-Invest. 1997;77(1):37-49.

92. Drake MJ, Hedlund P, Mills IW, McCoy R, McMurray G, Gardner BP, et al. Structural and functional denervation of human detrusor after spinal cord injury. Lab Invest. 2000 Oct;80(10):1491-9.

93. Gu J, Blank MA, Huang WM, Islam KN, McGregor GP, Christofides N, et al. Peptide-containing nerves in human urinary bladder. Urology. 1984 Oct;24(4):353-7.

94. Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, et al. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature. 2000;407(6807):1011-5.

95. Cook SP, McCleskey EW. ATP, pain and a full bladder. Nature. 2000;407(6807):951-2.

96. Apostolidis A, Popat R, Yiangou Y, Cockayne D, Ford AP, Davis JB, et al. Decreased sensory receptors P2X3 and TRPV1 in suburothelial nerve fibers following intradetrusor injections of botulinum toxin for human detrusor overactivity. J Urol. 2005 Sep;174(3):977-82; discussion 82-3.

97. Namasivayam S, Eardley I, Morrison JF. Purinergic sensory neurotransmission in the urinary bladder: an in vitro study in the rat. BJUInt. 1999;84(7):854-60.

98. Vlaskovska M, et al., Burnstock G. P2x3 knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci. 2001;21:5670-7.

99. Rong W, Spyer KM, Burnstock G. Activation and sensitisation of low and high threshold afferent fibres mediated by P2X receptors in the mouse urinary bladder. J Physiol. 2002;541(Pt 2):591-600.

100. Andersson KE, Persson K. Nitric oxide synthase and the lower urinary tract: possible implications for physiology and pathophysiology. ScandJ UrolNephrolSuppl. 1995;175:43-53.

101. Birder LA, Kanai AJ, de Groat WC. DMSO: effect on bladder afferent neurons and nitric oxide release. JUrol. 1997;158(5):1989-95.

102. Buffington CA, Kiss S, Kanai AJ, Dineley K, Roppolo JR, de Groat WC, et al. Alterations in urothelium and bladder afferents in feline interstitial cystitis. Society for Neuroscience Abstracts. 2000;26 349.3.

103. de Groat WC, Kruse MN, Vizzard MA, Cheng CL, Araki I, Yoshimura N. Modification of urinary bladder function after spinal cord injury. AdvNeurol. 1997;72:347-64.

104. Yoshimura N, Seki S, de Groat WC. Nitric oxide modulates Ca(2+) channels in dorsal root ganglion neurons innervating rat urinary bladder. J Neurophysiol. 2001;86(1):304-11.

105. Ozawa H, Chancellor MB, Jung SY, Yokoyama T, Fraser MO, Yu Y, et al. Effect of intravesical nitric oxide therapy on cyclophosphamide-induced cystitis. JUrol. 1999;162(6):2211-6.

106. Pandita RK, Mizusawa H, Andersson KE. Intravesical oxyhemoglobin initiates bladder overactivity in conscious, normal rats. J Urol. 2000;164(2):545-50.

107. Sutherland RS, Kogan BA, Piechota HJ, Bredt DS. Vesicourethral function in mice with genetic disruption of neuronal nitric oxide synthase. J Urol. 1997;157(3):1109-16.

108. Lemack GE, Zimmern PE, Vazquez D, Connell JD, Lin VK. Altered response to partial bladder outlet obstruction in mice lacking inducible nitric oxide synthase. J Urol. 2000;163(6):1981-7.

109. Morrison JFB, Nimmo AJ, Whitaker EM. The effect of neonatal capsaicin treatment on the distribution of neurokinin binding sites in rat bladder. J Physiol. 1990;423:79-.

110. Wang MJ, Xiong SH, Li ZW. Neurokinin B potentiates ATP-activated currents in rat DRG neurons. Brain Res. 2001;923(1-2):157-62.

111. Wen J, Morrison JF. The effects of high urinary potassium concentration on pelvic nerve mechanoreceptors and 'silent' afferents from the rat bladder. AdvExpMedBiol. 1995;385:237-9.

112. Kibble A, Morrison JFB. In vivo pelvic afferent responses to a Neurokinin A agonist in the anaesthetised rat. J Physiol. 1996;491:148-9.

113. Kibble A, Morrison JFB. Decreases in mechanosensitivity of urinary bladder afferents in the anaesthetised rat following administration of the selective NK-2 antagonist SR48968. J Physiol. 1997;497:18-28.

114. Shea VK, Cai R, Crepps B, Mason JL, Perl ER. Sensory fibers of the pelvic nerve innervating the Rat's urinary bladder. J Neurophysiol. 2000;84(4):1924-33.

115. Sculptoreanu A, de Groat WC. Protein kinase C is involved in neurokinin receptor modulation of N- and L-type Ca2+ channels in DRG neurons of the adult rat. J Neurophysiol. 2003;90(1):21-31.

116. Everaerts W, Gevaert T, Nilius B, De Ridder D. On the origin of bladder sensing: Tr(i)ps in urology. Neurourol Urodyn. 2007 Sep 11.

117. Gevaert T, Vandepitte J, Hutchings G, Vriens J, Nilius B, De Ridder D. TRPV1 is involved in stretch-evoked contractile changes in the rat autonomous bladder model: a study with piperine, a new TRPV1 agonist. Neurourol Urodyn. 2007;26(3):440-50; discussion 51-3.

118. Gevaert T, Vandepitte J, Ost D, Nilius B, De Ridder D. Autonomous contractile activity in the isolated rat bladder is modulated by a TRPV1 dependent mechanism. Neurourol Urodyn. 2007;26(3):424-32; discussion 51-3.

119. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, et al. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science. 2000;288(5464):306-13.

120. Caterina MJ, Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. AnnuRevNeurosci. 2001;24:487-517.

121. Winston J, Toma H, Shenoy M, Pasricha PJ. Nerve growth factor regulates VR-1 mRNA levels in cultures of adult dorsal root ganglion neurons. Pain. 2001;89(2-3):181-6.

122. Gauldie SD, McQueen DS, Pertwee R, Chessell IP. Anandamide activates peripheral nociceptors in normal and arthritic rat knee joints. BrJ Pharmacol. 2001;132(3):617-21.

123. Tognetto M, Amadesi S, Harrison S, Creminon C, Trevisani M, Carreras M, et al. Anandamide excites central terminals of dorsal root ganglion neurons via vanilloid receptor-1 activation. J Neurosci. 2001;21(4):1104-9.

124. Howlett AC, Mukhopadhyay S. Cellular signal transduction by anandamide and 2-arachidonoylglycerol. ChemPhysLipids. 2000;108(1-2):53-70.

125. Jaggar SI, Sellaturay S, Rice AS. The endogenous cannabinoid anandamide, but not the CB2 ligand palmitoylethanolamide, prevents the viscero-visceral hyper-reflexia associated with inflammation of the rat urinary bladder. NeurosciLett. 1998;253(2):123-6.

126. Jaggar SI, Hasnie FS, Sellaturay S, Rice AS. The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. Pain. 1998;76(1-2):189-99.

127. Farquhar-Smith WP, Rice AS. Administration of endocannabinoids prevents a referred hyperalgesia associated with inflammation of the urinary bladder. Anesthesiology. 2001;94(3):507-13.

128. Birder LA, Nakamura Y, Kiss S, Nealen ML, Barrick S, Kanai AJ, et al. Altered urinary bladder function in mice lacking the vanilloid receptor TRPV1. Nat Neurosci. 2002 Sep;5(9):856-60.

129. Daly D, Rong W, Chess-Williams R, Chapple C, Grundy D. Bladder afferent sensitivity in wild-type and TRPV1 knockout mice. J Physiol. 2007 Sep 1;583(Pt 2):663-74.

130. Mukerji G, Waters J, Chessell IP, Bountra C, Agarwal SK, Anand P. Pain during ice water test distinguishes clinical bladder hypersensitivity from overactivity disorders. BMC Urol. 2006;6:31.

131. Tsukimi Y, Mizuyachi K, Yamasaki T, Niki T, Hayashi F. Cold response of the bladder in guinea pig: involvement of transient receptor potential channel, TRPM8. Urology. 2005 Feb;65(2):406-10.

132. Du S, Araki I, Yoshiyama M, Nomura T, Takeda M. Transient receptor potential channel A1 involved in sensory transduction of rat urinary bladder through C-fiber pathway. Urology. 2007 Oct;70(4):826-31.

133. Giuliani S, Lecci A, Tramontana M, Maggi CA. Nociceptin protects capsaicin-sensitive afferent fibers in the rat urinary bladder from desensitization. Naunyn Schmiedebergs ArchPharmacol. 1999;360(2):202-8.

134. Lazzeri M, Calo G, Spinelli M, Guerrini R, Salvadori S, Beneforti P, et al. Urodynamic effects of intravesical nociceptin/orphanin FQ in neurogenic detrusor overactivity: a randomized, placebo-controlled, double-blind study. Urology. 2003;61(5):946-50.

135. Su X, Sengupta JN, Gebhart GF. Effects of opioids on mechanosensitive pelvic nerve afferent fibers innervating the urinary bladder of the rat. J Neurophysiol. 1997;77(3):1566-80.

136. Tanner R, Chambers P, Khadra MH, Gillespie JI. The production of nerve growth factor by human bladder smooth muscle cells in vivo and in vitro. BJUInt. 2000;85(9):1115-9.

137. Okragly AJ, Niles AL, Saban R, Schmidt D, Hoffman RL, Warner TF, et al. Elevated tryptase, nerve growth factor, neurotrophin-3 and glial cell line-derived neurotrophic factor levels in the urine of interstitial cystitis and bladder cancer patients. J Urol. 1999;161(2):438-41.

138. Oddiah D, Anand P, McMahon SB, Rattray M. Rapid increase of NGF, BDNF and NT-3 mRNAs in inflamed bladder. Neuroreport. 1998;9(7):1455-8.

139. Vizzard MA. Changes in urinary bladder neurotrophic factor mRNA and NGF protein following urinary bladder dysfunction. ExpNeurol. 2000;161(1):273-84.

140. Spitsbergen JM, Clemow DB, McCarty R, Steers WD, Tuttle JB. Neurally mediated hyperactive voiding in spontaneously hypertensive rats. Brain Res. 1998;790(1-2):151-9.

141. Steinbacher BC, Jr., Nadelhaft I. Increased levels of nerve growth factor in the urinary bladder and hypertrophy of dorsal root ganglion neurons in the diabetic rat. Brain Res. 1998;782(1-2):255-60.

142. Dmitrieva N, McMahon SB. Sensitization of visceral afferents by nerve growth factor in the adult rat. Pain. 1996;66(1):87-97.

143. Dmitrieva N, Shelton D, Rice AS, McMahon SB. The role of nerve growth factor in a model of visceral inflammation. Neuroscience. 1997 May;78(2):449-59.

144. Jaggar SI, Scott HC, Rice AS. Inflammation of the rat urinary bladder is associated with a referred thermal hyperalgesia which is nerve growth factor dependent. BrJ Anaesth. 1999;83(3):442-8.

145. Yoshimura N, Bennett NE, Hayashi Y, Ogawa T, Nishizawa O, Chancellor MB, et al. Bladder overactivity and hyperexcitability of bladder afferent neurons after intrathecal delivery of nerve growth factor in rats. J Neurosci. 2006 Oct 18;26(42):10847-55.

146. Sculptoreanu A, de Groat WC, Buffington CA, Birder LA. Protein kinase C contributes to abnormal capsaicin responses in DRG neurons from cats with feline interstitial cystitis. Neurosci Lett. 2005 Jun 10-17;381(1-2):42-6.

147. Sculptoreanu A, de Groat WC, Buffington CA, Birder LA. Abnormal excitability in capsaicin-responsive DRG neurons from cats with feline interstitial cystitis. Exp Neurol. 2005 Jun;193(2):437-43.

148. Lommatzsch M, Braun A, Mannsfeldt A, Botchkarev VA, Botchkareva NV, Paus R, et al. Abundant production of brain-derived neurotrophic factor by adult visceral epithelia. Implications for paracrine and target-derived Neurotrophic functions. AmJ Pathol. 1999;155(4):1183-93.

149. Qiao LY, Grider JR. Up-regulation of calcitonin gene-related peptide and receptor tyrosine kinase TrkB in rat bladder afferent neurons following TNBS colitis. Exp Neurol. 2007 Apr;204(2):667-79.

150. Zvarova K, Murray E, Vizzard MA. Changes in galanin immunoreactivity in rat lumbosacral spinal cord and dorsal root ganglia after spinal cord injury. J Comp Neurol. 2004 Aug 2;475(4):590-603.

151. Yoshimura N, Seki S, Novakovic SD, Tzoumaka E, Erickson VL, Erickson KA, et al. The involvement of the tetrodotoxin-resistant sodium channel Na(v)1.8 (PN3/SNS) in a rat model of visceral pain. J Neurosci. 2001;21(21):8690-6.

152. Bennett DL, Dmietrieva N, Priestley JV, Clary D, McMahon SB. trkA, CGRP and IB4 expression in retrogradely labelled cutaneous and visceral primary sensory neurones in the rat. NeurosciLett. 1996;206(1):33-6.

153. Yoshimura N, de Groat WC. Plasticity of Na+ channels in afferent neurones innervating rat urinary bladder following spinal cord injury. JPhysiol. 1997;503 (Pt 2):269-76.

154. Black JA, Cummins TR, Yoshimura N, de Groat WC, Waxman SG. Tetrodotoxin-resistant sodium channels Na(v)1.8/SNS and Na(v)1.9/NaN in afferent neurons innervating urinary bladder in control and spinal cord injured rats. Brain Res. 2003;963(1-2):132-8.

155. Seki S, Sasaki K, Fraser MO, Igawa Y, Nishizawa O, Chancellor MB, et al. Immunoneutralization of nerve growth factor in lumbosacral spinal cord reduces bladder hyperreflexia in spinal cord injured rats. J Urol. 2002;168(5):2269-74.

156. Yamane H, de Groat WC, Sculptoreanu A. Effects of ralfinamide, a Na(+) channel blocker, on firing properties of nociceptive dorsal root ganglion neurons of adult rats. Exp Neurol. 2007 Nov;208(1):63-72.

157. Castleden CM, Duffin HM, Clarkson EJ. In vivo and in vitro studies on the effect of sodium antagonists on the bladder in man and rat. Age Ageing. 1983;12(3):249-55.

158. Lapointe SP, Wang B, Kennedy WA, Shortliffe LM. The effects of intravesical lidocaine on bladder dynamics of children with myelomeningocele. J Urol. 2001;165(6 Pt 2):2380-2.

159. Yokoyama O, Komatsu K, Kodama K, Yotsuyanagi S, Niikura S, Namiki M. Diagnostic value of intravesical lidocaine for overactive bladder. J Urol. 2000;164(2):340-3.

160. Reuther K, Aagaard J, Jensen KS. Lignocaine test and detrusor instability. BrJ Urol. 1983;55(5):493-4.

161. Sethia KK, Smith JC. The effect of pH and lignocaine on detrusor instability. BrJ Urol. 1987;60(6):516-8.

162. Chalfin SA, Bradley WE. The etiology of detrusor hyperreflexia in patients with infravesical obstruction. J Urol. 1982;127(5):938-42.

163. Thor KB, Morgan,C., Nadelhaft,I., Houston,M., de Groat,W.C. Organization of afferent and efferent pathways in the pudendal nerve of the femal cat. J Comp Neurol. 1989;288:263-79.

164. de Groat WC. Spinal cord projections and neuropeptides in visceral afferent neurons. Prog Br Research. 1986;67:165.

165. Vanderhorst VG, Mouton,L.J., Blok,B.F., Holstege,G. Distinct cell groups in the lumbosacral cord of the cat project to different areas in the periaqueductal gray. J Comp Neurol. 1996;376:361-85.

166. Vanderhorst VG, Ulfake,B. The organization of the brainstem and spinal cord of the mouse: relationships between monoaminergic, cholinergic and spinal projection systems. J Chem Neuroanat. 2006;31:2-36.

167. Vizzard MA, Erickson,V.L., Card,J.P., Roppolo,J.R., de Groat,W.C. Transneuronal labeling of neurons in the adult rat brainstem and spinal cord after injection of pseudorabies virus into the urethra. J Comp Neurol. 1995;335:629-40.

168. McMahon SB, Morrison,J.F. Spinal neurones with long projections activated from the abdominal viscera of the cat. J Physiol. 1982;322:1-20.

169. McMahon SB, Morrison,J.F. Two groups of spinal interneurones that respond to stimulation of the abdominal viscera of the cat. J Physiol. 1982;322:21-34.

170. Nathan PW, Smith,M.C. The centripetal pathway from the bladder and urethra within the spinal cord. J Neurochemistry. 1951;14:262.

171. Morgan C, Nadelhaft,I., de Groat,W.C. The distribution of visceral primary afferents from the pelvic nerve to Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J Comp Neurol. 1981;201:415.

172. Jiang CH, Lindstrom,S. Prolonged enhancement of the micturition reflex in the repetitive stimulation of bladder afferents. J Physiol. 1999;517:599.

173. Gustafson KJ, Creasey,G.H., Grill,W.M. A urethral afferent mediated excitatory bladder reflex exists in humans. Neurosci Lett. 2004;360:9.

174. Shefchyk SJ, Buss,R.R. Urethral pudendal afferent-evoked bladder and sphincter reflexes in decerebrate and acute spinal cats. Neurosci Lett. 1998;244:137.

175. Pezzone MA, Liang,R., Fraser,M.O. A model of neural cross-talk and irritation in the pelvis: implications for the overlap of chronic pelvic pain disorders. Gastroenterology. 2005;128:1953-64.

176. Tai C, Wang,J., Wang,X., de Groat, W.C., Roppolo, J.R. Bladder inhbition or voiding induced by pudendal nerve stimulation in chronic spinal cord injured cats. Neurourol Urodyn. 2007;26:570-7.

177. Woock JP, Yoo,P.B., Grill,W.M. Activation and inhibition of the micturition reflex by penile afferents in the cat. Am J Physiol. 2008;294:R1880-90.

178. Floyd K, McMahon,S.B., Morrison,J.F. Inhibitory interactions between colonic and vesical afferents in the micturition reflex of the cat. J Physiol. 1982;322:45.

179. deGroat WC, Fraser,M.O., Yoshiyama,M. Neural control of the urethra. Scand J Urol Nephrol. 2001;35.

180. Malykhina AP. Neural mechanisms of pelvic organ cross-sensitization. Neuroscience. 2007;149:660-72.

181. Clemens JQ, Meenan RT, O'Keeffe Rosetti MC, Kimes TA. Case-control study of medical comorbidities in women with interstitial cystitis. J Urology. 2008 June 2008;179:2222-5.

182. Zinck ND, Rafuse,V.F., Downie,J.W. Sprouting of CGRP primary afferents in lumbosacral spinal cord precedes emergence of bladder activity after spinal injury. Exp Neurol. 2007;204:777-90.

183. Shehab SA, Spike,R.C., Todd,A.J. Do central terminals of intact myelinated primary afferents sprout into the superficial dorsal horn of rat spinal cord after injury to a neighboring peripheral nerve? J Comp Neurol. 2004;28:474-37.

184. Zvara P, Braas,K.M., May,V., Vizzard,M.A. A role for pituitary adenylate cyclase activating poly peptide (PACAP) in detrusor hyperreflexia after spinal cord injury. Ann NY Acad Sci. 2006;1070:622-8.

185. Kakizaki H, Yoshiyama,M., de Groat,W.C. Role of NMDA and AMPA glutamatergic transmission in spinal c-fos expression after urinary tract irritation. Am J Physiol. 1996;270:R990-6.

186. Kakizaki H, Yoshiyama,M., Roppolo,J.R., Booth,A.M., de Groat,W.C. Role of spinal glutamatergic transmission in the ascending limb of the micturition reflex pathway in the rat. J Pharm Exp Ther. 1998;285:22-7.

187. Lecci A, Maggi,C.A. Tachykinins as modulators of the micturition reflex in the central and peripheral nervous system. Regul Peptides. 2001;15:1-18.

188. Seki S, Erickson,K.A., Seki,M., Nishizawa,O., Igawa,Y., Ogawa,T., de Groat,W.C. Chancellor,M.B., Yoshimura,N. Elimination of rat spinal neurons expressing neurokinin 1 receptors reduces bladder overactivity and spinal c-fos expression induced by bladder irritation. Am J Physiol. 2005;288:F466-73.

189. Cheng CL, Ma,C.P., de Groat,W.C. Effect of capsaicin on micturition and associated reflexes in chronic spinal rats. Brain Res. 1995;678:40.

190. Shaham S. Glia-neuron interactions in the nervous system of Caenorhabditis elegans. Curr Opin Neurobiol. 2006 Oct;16(5):522-8.

191. Bass NH, Hess HH, Pope A, Thalheimer C. Quantitative cytoarchitectonic distribution of neurons, glia, and DNa in rat cerebral cortex. J Comp Neurol. 1971 Dec;143(4):481-90.

192. Swanson N, Swanson L. Cajal RS: Histologie du syste`me nerveux de l'homme et des verte´bres. (1909) English translation. [Translation of title: Histology of the nervous system of man and vertebrates. Oxford University Press; 1995.

193. Peters A, Palay,S,Webster,H. The Fine Structure of the Nervous System. Third ed.: Oxford University Press; 1991.

194. Theodosis DT, Poulain DA, Oliet SH. Activity-dependent structural and functional plasticity of astrocyte-neuron interactions. Physiol Rev. 2008 Jul;88(3):983-1008.

195. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci. 1999 May;22(5):208-15.

196. DeLeo J, Sorkin,LS,Watkins,LR, editor. Immune and Glial Regulation of Pain: IASP Press; 2007.

197. D'Mello R, Dickenson AH. Spinal cord mechanisms of pain. Br J Anaesth. 2008 Jul;101(1):8-16.

198. Ren K, Dubner R. Neuron-glia crosstalk gets serious: role in pain hypersensitivity. Curr Opin Anaesthesiol. 2008 Oct;21(5):570-9.

199. Scholz J, Woolf CJ. The neuropathic pain triad: neurons, immune cells and glia. Nat Neurosci. 2007 Nov;10(11):1361-8.

200. Tanga FY, Raghavendra V, DeLeo JA. Quantitative real-

time RT-PCR assessment of spinal microglial and astrocytic activation markers in a rat model of neuropathic pain. Neurochem Int. 2004 Jul-Aug;45(2-3):397-407.

201. Sun YN, Luo JY, Rao ZR, Lan L, Duan L. GFAP and Fos immunoreactivity in lumbo-sacral spinal cord and medulla oblongata after chronic colonic inflammation in rats. World J Gastroenterol. 2005 Aug 21;11(31):4827-32.
202. Hanna-Mitchell AT, Chib MK, Roppolo JR, Kanai AJ, de Groat WC, Buffington CA, et al. A Role for Spinal Cord Astrocytes in the Pain Syndrome Interstitial Cystitis and Colitis 12th World Congress on Pain; 2008 August 17-22, 2008; Glasgow,UK. 2008.
203. Ashton-Miller JA, DeLancey JO. Functional anatomy of the female pelvic floor. Ann N Y Acad Sci. 2007 Apr;1101:266-96.
204. Dorschner W, Biesold M, Schmidt F, Stolzenburg JU. The dispute about the external sphincter and the urogenital diaphragm. J Urol. 1999 Dec;162(6):1942-5.
205. Mirilas P, Skandalakis JE. Urogenital diaphragm: an erroneous concept casting its shadow over the sphincter urethrae and deep perineal space. J Am Coll Surg. 2004 Feb;198(2):279-90.
206. Sebe P, Fritsch H, Oswald J, Schwentner C, Lunacek A, Bartsch G, et al. Fetal development of the female external urinary sphincter complex: an anatomical and histological study. J Urol. 2005 May;173(5):1738-42; discussion 42.
207. Sebe P, Schwentner C, Oswald J, Radmayr C, Bartsch G, Fritsch H. Fetal development of striated and smooth muscle sphincters of the male urethra from a common primordium and modifications due to the development of the prostate: an anatomic and histologic study. Prostate. 2005 Mar 1;62(4):388-93.
208. Barber MD, Bremer RE, Thor KB, Dolber PC, Kuehl TJ, Coates KW. Innervation of the female levator ani muscles. Am J Obstet Gynecol. 2002 Jul;187(1):64-71.
209. Pierce LM, Coates KW, Kramer LA, Bradford JC, Thor KB, Kuehl TJ. Effects of bilateral levator ani nerve injury on pelvic support in the female squirrel monkey. Am J Obstet Gynecol. 2008 May;198(5):585 e1-8.
210. Pierce LM, Rankin MR, Foster RT, Dolber PC, Coates KW, Kuehl TJ, et al. Distribution and immunohistochemical characterization of primary afferent neurons innervating the levator ani muscle of the female squirrel monkey. Am J Obstet Gynecol. 2006 Oct;195(4):987-96.
211. Pierce LM, Reyes M, Thor KB, Dolber PC, Bremer RE, Kuehl TJ, et al. Innervation of the levator ani muscles in the female squirrel monkey. Am J Obstet Gynecol. 2003 May;188(5):1141-7.
212. Thuroff JW, Bazeed MA, Schmidt RA, Luu DH, Tanagho EA. Regional topography of spinal cord neurons innervating pelvic floor muscles and bladder neck in the dog: a study by combined horseradish peroxidase histochemistry and autoradiography. Urol Int. 1982;37(2):110-20.
213. Bremer RE, Barber MD, Coates KW, Dolber PC, Thor KB. Innervation of the levator ani and coccygeus muscles of the female rat. Anat Rec A Discov Mol Cell Evol Biol. 2003 Nov;275(1):1031-41.
214. Grigorescu BA, Lazarou G, Olson TR, Downie SA, Powers K, Greston WM, et al. Innervation of the levator ani muscles: description of the nerve branches to the pubococcygeus, iliococcygeus, and puborectalis muscles. Int Urogynecol J Pelvic Floor Dysfunct. 2008 Jan;19(1):107-16.
215. Wallner C, van Wissen J, Maas CP, Dabhoiwala NF, Deruiter MC, Lamers WH. The Contribution of the Levator Ani Nerve and the Pudendal Nerve to the Innervation of the Levator Ani Muscles; a Study in Human Fetuses. Eur Urol. 2007 Nov 20.
216. McKenna KE, Nadelhaft I. The organization of the pudendal nerve in the male and female rat. J Comp Neurol. 1986 Jun 22;248(4):532-49.
217. Pierce LM, Reyes M, Thor KB, Dolber PC, Bremer RE, Kuehl TJ, et al. Immunohistochemical evidence for the interaction between levator ani and pudendal motor neurons in the coordination of pelvic floor and visceral activity in the squirrel monkey. Am J Obstet Gynecol. 2005 May;192(5):1506-15.
218. Roppolo JR, Nadelhaft I, de Groat WC. The organization of pudendal motoneurons and primary afferent projections in the spinal cord of the rhesus monkey revealed by horseradish peroxidase. J Comp Neurol. 1985 Apr 22;234(4):475-88.
219. Thor KB, Morgan C, Nadelhaft I, Houston M, De Groat WC. Organization of afferent and efferent pathways in the pudendal nerve of the female cat. J Comp Neurol. 1989 Oct 8;288(2):263-79.
220. Ueyama T, Mizuno N, Nomura S, Konishi A, Itoh K, Arakawa H. Central distribution of afferent and efferent components of the pudendal nerve in cat. J Comp Neurol. 1984 Jan 1;222(1):38-46.
221. Ueyama T, Arakawa H, Mizuno N. Central distribution of efferent and afferent components of the pudendal nerve in rat. Anat Embryol (Berl). 1987;177(1):37-49.
222. Wieslander CK, Roshanravan SM, Wai CY, Schaffer JI, Corton MM. Uterosacral ligament suspension sutures: Anatomic relationships in unembalmed female cadavers. Am J Obstet Gynecol. 2007 Dec;197(6):672 e1-6.
223. Wallner C, Maas CP, Dabhoiwala NF, Lamers WH, DeRuiter MC. Innervation of the pelvic floor muscles: a reappraisal for the levator ani nerve. Obstet Gynecol. 2006 Sep;108(3 Pt 1):529-34.
224. Gosling JA, Dixon JS, Critchley HO, Thompson SA. A comparative study of the human external sphincter and periurethral levator ani muscles. Br J Urol. 1981 Feb;53(1):35-41.
225. Palmieri G, Panu R, Asole A, Sanna L, Farina V. Coccygeus and levator ani muscles in the rabbit: morphology and proprioceptive innervation. Biol Struct Morphog. 1988;1(4):142-6.
226. Borghi F, Di Molfetta L, Garavoglia M, Levi AC. Questions about the uncertain presence of muscle spindles in the human external anal sphincter. Panminerva Med. 1991 Jul-Sep;33(3):170-2.
227. Martin WD, Fletcher TF, Bradley WE. Innervation of feline perineal musculature. Anat Rec. 1974 Sep;180(1):15-29.
228. Rockswold GL, Bradley WE, Chou SN. Innervation of the external urethral and external anal sphincters in higher primates. J Comp Neurol. 1980 Sep 15;193(2):521-8.
229. Schroder HD, Reske-Nielsen E. Fiber types in the striated urethral and anal sphincters. Acta Neuropathol. 1983;60(3-4):278-82.
230. Todd JK. Afferent Impulses in the Pudendal Nerves of the Cat. J Exp Physiol Cogn Med Sci. 1964 Jul;49:258-67.
231. Bradley WE, Teague CT. Electrophysiology of pelvic and pudendal nerves in the cat. Exp Neurol. 1972 May;35(2):378-93.
232. Mackel R. Segmental and descending control of the external urethral and anal sphincters in the cat. J Physiol. 1979 Sep;294:105-22.
233. McMahon SB, Morrison JF, Spillane K. An electrophysiological study of somatic and visceral convergence in the reflex control of the external sphincters. J Physiol. 1982 Jul;328:379-87.
234. Thor KB, Hisamitsu T, Roppolo JR, Tuttle P, Nagel J, deGroat WC. Selective inhibitory effects of ethylketocyclazocine on

reflex pathways to the external urethral sphincter of the cat. J Pharmacol Exp Ther. 1989 Mar;248(3):1018-25.

235. Brown AG FR. The morphology of group Ia afferent fibre collaterals in the spinal cord of the cat. J Physiol. 1978;274:111-27.
236. Brown AG FR. The morphology of group Ib afferent fibre collaterals in the spinal cord of the cat. J Physiol. 1979;296:215-26.
237. Coates KW, Galan HL, Shull BL, Kuehl TJ. The squirrel monkey: an animal model of pelvic relaxation. Am J Obstet Gynecol. 1995 Feb;172(2 Pt 1):588-93.
238. Kramer LA, Gendron JM, Pierce LM, Runge VM, Shull BL, Kuehl TJ. Magnetic resonance imaging of the levator ani in the squirrel monkey: a comparison of muscle volume between a cohort with pelvic organ prolapse and matched normals. Am J Obstet Gynecol. 2006 May;194(5):1467-71.
239. Pacheco P, Camacho MA, Garcia LI, Hernandez ME, Carrillo P, Manzo J. Electrophysiological evidence for the nomenclature of the pudendal nerve and sacral plexus in the male rat. Brain Res. 1997 Jul 25;763(2):202-8.
240. Pacheco P, Martinez-Gomez M, Whipple B, Beyer C, Komisaruk BR. Somato-motor components of the pelvic and pudendal nerves of the female rat. Brain Res. 1989 Jun 19;490(1):85-94.
241. Kim RJ, Kerns JM, Liu S, Nagel T, Zaszczurynski P, Lin DL, et al. Striated muscle and nerve fascicle distribution in the female rat urethral sphincter. Anat Rec (Hoboken). 2007 Feb;290(2):145-54.
242. Praud C, Sebe P, Mondet F, Sebille A. The striated urethral sphincter in female rats. Anat Embryol (Berl). 2003 Sep;207(2):169-75.
243. Wunderlich M, Swash M. The overlapping innervation of the two sides of the external anal sphincter by the pudendal nerves. J Neurol Sci. 1983 Apr;59(1):97-109.
244. Gonzalez-Soriano J, Martin-Palacios S, Rodriguez-Veiga E, Triguero D, Costa G, Garcia-Pascual A. Nitric oxide synthase in the external urethral sphincter of the sheep: immunohistochemical and functional study. J Urol. 2003 May;169(5):1901-6.
245. Ho KM, Borja MC, Persson K, Brading AF, Andersson KE. Expression of nitric oxide synthase immunoreactivity in the human female intramural striated urethral sphincter. J Urol. 2003 Jun;169(6):2407-11.
246. Ho KM, McMurray G, Brading AF, Noble JG, Ny L, Andersson KE. Nitric oxide synthase in the heterogeneous population of intramural striated muscle fibres of the human membranous urethral sphincter. J Urol. 1998 Mar;159(3):1091-6.
247. Pullen AH, Humphreys P. Protracted elevation of neuronal nitric oxide synthase immunoreactivity in axotomised adult pudendal motor neurons. J Anat. 1999 May;194 (Pt 4):547-65.
248. Pullen AH, Humphreys P, Baxter RG. Comparative analysis of nitric oxide synthase immunoreactivity in the sacral spinal cord of the cat, macaque and human. J Anat. 1997 Aug;191 (Pt 2):161-75.
249. Stamler JS, Meissner G. Physiology of Nitric Oxide in Skeletal Muscle. Physiological Reviews. 2001;81(1):209-37.
250. Reitz A BS, Knapp PA, Müntener M, Wefer B, Schurch B. The effect of nitric oxide on the resting tone and the contractile behaviour of the external urethral sphincter: a functional urodynamic study in healthy humans. Eur Urol. 2004;45(3):367-73.
251. Elbadawi A, Schenk EA. A new theory of the innervation of bladder musculature. 2. Innervation of the vesicourethral junction and external urethral sphincter. J Urol. 1974 May;111(5):613-5.
252. Creed KE, Van Der Werf BA, Kaye KW. Innervation of the striated muscle of the membranous urethra of the male dog. J Urol. 1998 May;159(5):1712-6.
253. Onufrowicz B. On the arrangement and function of the cell groups of the sacral region of the spinal cord in man. Arch Neurol Psychopathol. 1900;3:387-411.
254. Nakagawa S. Onuf's nucleus of the sacral cord in a South American monkey (Saimiri): its location and bilateral cortical input from area 4. Brain Res. 1980 Jun 9;191(2):337-44.
255. Gerrits PO, Sie JA, Holstege G. Motoneuronal location of the external urethral and anal sphincters: a single and double labeling study in the male and female golden hamster. Neurosci Lett. 1997 May 2;226(3):191-4.
256. Kuipers R, Izhar Z, Gerrits PO, Miner W, Holstege G. Location of bladder and urethral sphincter motoneurons in the male guinea pig (Cavia porcellus). Neurosci Lett. 2004 May 13;362(1):57-60.
257. Blok BF, Roukema G, Geerdes B, Holstege G. Location of external anal sphincter motoneurons in the sacral cord of the female domestic pig. Neurosci Lett. 1996 Oct 4;216(3):203-6.
258. Ulibarri C, Popper P, Micevych PE. Motoneurons dorsolateral to the central canal innervate perineal muscles in the Mongolian gerbil. J Comp Neurol. 1995 May 29;356(2):225-37.
259. Sasaki M. Morphological analysis of external urethral and external anal sphincter motoneurones of cat. J Comp Neurol. 1994 Nov 8;349(2):269-87.
260. Nadelhaft I, Degroat WC, Morgan C. Location and morphology of parasympathetic preganglionic neurons in the sacral spinal cord of the cat revealed by retrograde axonal transport of horseradish peroxidase. J Comp Neurol. 1980 Sep 1;193(1):265-81.
261. Jankowska E, Padel Y, Zarzecki P. Crossed disynaptic inhibition of sacral motoneurones. J Physiol. 1978 Dec;285:425-44.
262. Shefchyk SJ. Spinal mechanisms contributing to urethral striated sphincter control during continence and micturition: "how good things might go bad". Prog Brain Res. 2006;152:85-95.
263. Kawatani M, Nagel J, de Groat WC. Identification of neuropeptides in pelvic and pudendal nerve afferent pathways to the sacral spinal cord of the cat. J Comp Neurol. 1986 Jul 1;249(1):117-32.
264. Rampal G, Mignard P. Behaviour of the urethral striated sphincter and of the bladder in the chronic spinal cat. Implications at the Central Nervous System Level. Pflugers Arch. 1975;353(1):33-42.
265. Rampal G, Mignard P. Organization of the nervous control of urethral sphincter. A study in the anaesthetized cat with intact central nervous system. Pflugers Arch. 1975;353(1):21-31.
266. Holstege G, Tan J. Supraspinal control of motoneurons innervating the striated muscles of the pelvic floor including urethral and anal sphincters in the cat. Brain. 1987 Oct;110 (Pt 5):1323-44.
267. Miller AD, Nonaka S, Siniaia MS, Jakus J. Multifunctional ventral respiratory group: bulbospinal expiratory neurons play a role in pudendal discharge during vomiting. J Auton Nerv Syst. 1995 Sep 5;54(3):253-60.
268. Bors E BK. Bulbocavernosus reflex. J Urol. 1959;82(1):128-30.
269. Chen Z, Anderson DL, Faison WL, Baer PG. Biphasic urethral sphincter responses to acetic acid infusion into the lower urinary tract in anesthetized cats. J Urol. 2001 Oct;166(4):1539-48.

270. Thor KB, Katofiasc MA. Effects of duloxetine, a combined serotonin and norepinephrine reuptake inhibitor, on central neural control of lower urinary tract function in the chloralose-anesthetized female cat. J Pharmacol Exp Ther. 1995 Aug;274(2):1014-24.

271. Thor KB, Muhlhauser MA. Vesicoanal, urethroanal, and urethrovesical reflexes initiated by lower urinary tract irritation in the rat. Am J Physiol. 1999 Oct;277(4 Pt 2):R1002-12.

272. Danuser H, Bemis K, Thor KB. Pharmacological analysis of the noradrenergic control of central sympathetic and somatic reflexes controlling the lower urinary tract in the anesthetized cat. J Pharmacol Exp Ther. 1995 Aug;274(2):820-5.

273. Hurtado EA, Smith PP, Smith CP, Boone TB, Somogyi GT. Urethral afferent signaling leads to activation of the external urethral sphincter and abdominal wall muscles. Neurourology and Urodynamics. 2008;27(2):105.

274. Danuser H, Thor KB. Spinal 5-HT2 receptor-mediated facilitation of pudendal nerve reflexes in the anaesthetized cat. Br J Pharmacol. 1996 May;118(1):150-4.

275. Thor KB, Langdale C, Ukai M, Karicheti V. Characterization of a Spinal Urine Storage Reflex Inhibitory Center (SUSRIC) and its regulation by 5-HT1A receptors. . Society for Neuroscience Annual Meeting abstracts. 2008.

276. Wefer B, Reitz A, Knapp PA, Bannowsky A, Juenemann KP, Schurch B. Conditioning stimulus can influence an external urethral sphincter contraction evoked by a magnetic stimulation. Neurourol Urodyn. 2005;24(4):311-7; discussion 8.

277. Morgan C, Nadelhaft I, de Groat WC. The distribution of visceral primary afferents from the pelvic nerve to Lissauer's tract and the spinal gray matter and its relationship to the sacral parasympathetic nucleus. J Comp Neurol. 1981 Sep 20;201(3):415-40.

278. Blok BF, van Maarseveen JT, Holstege G. Electrical stimulation of the sacral dorsal gray commissure evokes relaxation of the external urethral sphincter in the cat. Neurosci Lett. 1998 Jun 12;249(1):68-70.

279. Sie JA, Blok BF, de Weerd H, Holstege G. Ultrastructural evidence for direct projections from the pontine micturition center to glycine-immunoreactive neurons in the sacral dorsal gray commissure in the cat. J Comp Neurol. 2001 Jan 22;429(4):631-7.

280. Goodwin RJ SM, Fowler CJ. The neurophysiology of urinary retention in young women and its treatment by neuromodulation. World J Urol. 1998;16(5):305-7.

281. Gu B, Olejar KJ, Reiter JP, Thor KB, Dolber PC. Inhibition of bladder activity by 5-hydroxytryptamine1 serotonin receptor agonists in cats with chronic spinal cord injury. J Pharmacol Exp Ther. 2004 Sep;310(3):1266-72.

282. Gu B, Thor KB, Reiter JP, Dolber PC. Effect of 5-hydroxytryptamine serotonin receptor agonists on noxiously stimulated micturition in cats with chronic spinal cord injury. J Urol. 2007 Jun;177(6):2381-5.

283. Thor KB, Katofiasc MA, Danuser H, Springer J, Schaus JM. The role of 5-HT(1A) receptors in control of lower urinary tract function in cats. Brain Res. 2002 Aug 16;946(2):290-7.

284. Boers J, Ford TW, Holstege G, Kirkwood PA. Functional heterogeneity among neurons in the nucleus retroambiguus with lumbosacral projections in female cats. J Neurophysiol. 2005 Oct;94(4):2617-29.

285. Vanderhorst VG, Holstege G. Caudal medullary pathways to lumbosacral motoneuronal cell groups in the cat: evidence for direct projections possibly representing the final common pathway for lordosis. J Comp Neurol. 1995 Aug 28;359(3):457-75.

286. Vanderhorst VG, Holstege G. Nucleus retroambiguus projections to lumbosacral motoneuronal cell groups in the male cat. J Comp Neurol. 1997 May 26;382(1):77-88.

287. Kenton K, Brubaker L. Relationship between levator ani contraction and motor unit activation in the urethral sphincter. Am J Obstet Gynecol. 2002 Aug;187(2):403-6.

288. Enck P, Vodusek DB. Electromyography of pelvic floor muscles. J Electromyogr Kinesiol. 2006 Dec;16(6):568-77.

289. Kojima M, Matsuura T, Kimura H, Nojyo Y, Sano Y. Fluorescence histochemical study on the noradrenergic control to the anterior column of the spinal lumbosacral segments of the rat and dog, with special reference to motoneurons innervating the perineal striated muscles (Onuf's nucleus). Histochemistry. 1984;81(3):237-41.

290. Kojima M, Takeuchi Y, Goto M, Sano Y. Immunohistochemical study on the distribution of serotonin fibers in the spinal cord of the dog. Cell Tissue Res. 1982;226(3):477-91.

291. Kojima M, Takeuchi Y, Goto M, Sano Y. Immunohistochemical study on the localization of serotonin fibers and terminals in the spinal cord of the monkey (Macaca fuscata). Cell Tissue Res. 1983;229(1):23-36.

292. Rajaofetra N, Passagia JG, Marlier L, Poulat P, Pellas F, Sandillon F, et al. Serotoninergic, noradrenergic, and peptidergic innervation of Onuf's nucleus of normal and transected spinal cords of baboons (Papio papio). J Comp Neurol. 1992 Apr 1;318(1):1-17.

293. Holstege G, Griffiths D, de Wall H, Dalm E. Anatomical and physiological observations on supraspinal control of bladder and urethral sphincter muscles in the cat. J Comp Neurol. 1986 Aug 22;250(4):449-61.

294. Bernabe J, Julia-Guilloteau V, Denys P, Chartier-Kastler E, Alexandre L, Peeters M, et al. Peripheral neural lesion-induced stress urinary incontinence in anaesthetized female cats. BJU Int. 2008 Jun 11.

295. Kamo I, Torimoto K, Chancellor MB, de Groat WC, Yoshimura N. Urethral closure mechanisms under sneeze-induced stress condition in rats: a new animal model for evaluation of stress urinary incontinence. Am J Physiol Regul Integr Comp Physiol. 2003 Aug;285(2):R356-65.

296. Yoshiyama M, de Groat WC. Effect of bilateral hypogastric nerve transection on voiding dysfunction in rats with spinal cord injury. Exp Neurol. 2002 May;175(1):191-7.

297. Yoshiyama M, Roppolo JR, De Groat WC. Interactions between NMDA and AMPA/kainate receptors in the control of micturition in the rat. Eur J Pharmacol. 1995 Dec 4;287(1):73-8.

298. Yoshiyama M, Roppolo JR, de Groat WC. Effects of LY215490, a competitive alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist, on the micturition reflex in the rat. J Pharmacol Exp Ther. 1997 Feb;280(2):894-904.

299. Yoshiyama M, Roppolo JR, Thor KB, de Groat WC. Effects of LY274614, a competitive NMDA receptor antagonist, on the micturition reflex in the urethane-anaesthetized rat. Br J Pharmacol. 1993 Sep;110(1):77-86.

300. Shefchyk SJ, Espey MJ, Carr P, Nance D, Sawchuk M, Buss R. Evidence for a strychnine-sensitive mechanism and glycine receptors involved in the control of urethral sphincter activity during micturition in the cat. Exp Brain Res. 1998 Apr;119(3):297-306.

301. Miyazato M, Sasatomi K, Hiragata S, Sugaya K, Chancellor MB, de Groat WC, et al. GABA receptor activation in the lumbosacral spinal cord decreases detrusor overactivity in spinal cord injured rats. J Urol. 2008 Mar;179(3):1178-83.

302. Teague CT, Merrill DC. Effect of baclofen and dantrolene on bladder stimulator-induced detrusor-sphincter dyssynergia in dogs. Urology. 1978 May;11(5):531-5.

303. Leyson JF, Martin BF, Sporer A. Baclofen in the treatment of detrusor-sphincter dyssynergia in spinal cord injury patients. J Urol. 1980 Jul;124(1):82-4.

304. Nanninga JB, Frost F, Penn R. Effect of intrathecal baclofen on bladder and sphincter function. J Urol. 1989 Jul;142(1):101-5.

305. Steers WD, Meythaler JM, Haworth C, Herrell D, Park TS. Effects of acute bolus and chronic continuous intrathecal baclofen on genitourinary dysfunction due to spinal cord pathology. J Urol. 1992 Dec;148(6):1849-55.

306. Thor KB. Targeting serotonin and norepinephrine receptors in stress urinary incontinence. Int J Gynaecol Obstet. 2004 Jul;86 Suppl 1:S38-52.

307. Dmochowski RR, Miklos JR, Norton PA, Zinner NR, Yalcin I, Bump RC. Duloxetine versus placebo for the treatment of North American women with stress urinary incontinence. J Urol. 2003 Oct;170(4 Pt 1):1259-63.

308. Millard RJ, Moore K, Rencken R, Yalcin I, Bump RC. Duloxetine vs placebo in the treatment of stress urinary incontinence: a four-continent randomized clinical trial. BJU Int. 2004 Feb;93(3):311-8.

309. Miyazato M, Kaiho Y, Kamo I, Chancellor MB, Sugaya K, de Groat WC, et al. Effect of duloxetine, a norepinephrine and serotonin reuptake inhibitor, on sneeze-induced urethral continence reflex in rats. Am J Physiol Renal Physiol. 2008 Jul;295(1):F264-71.

310. Norton PA, Zinner NR, Yalcin I, Bump RC. Duloxetine versus placebo in the treatment of stress urinary incontinence. Am J Obstet Gynecol. 2002 Jul;187(1):40-8.

311. van Kerrebroeck P, Abrams P, Lange R, Slack M, Wyndaele JJ, Yalcin I, et al. Duloxetine versus placebo in the treatment of European and Canadian women with stress urinary incontinence. BJOG. 2004 Mar;111(3):249-57.

312. Boy S, Reitz A, Wirth B, Knapp PA, Braun PM, Haferkamp A, et al. Facilitatory neuromodulative effect of duloxetine on pudendal motor neurons controlling the urethral pressure: a functional urodynamic study in healthy women. Eur Urol. 2006 Jul;50(1):119-25.

313. Klarskov N, Scholfield D, Soma K, Darekar A, Mills I, G. L. Evaluation Of The Sensitivity Of Urethral Pressure Reflectometry And Urethral Pressure Profilometry To Detect Pharmacological Augmentation Of Urethral Pressure, Using [S,S]-Reboxetine. J Urol. 2008;179(4, Suppl.):521-2.

314. Zinner N, Scholfield D, Soma K, Darekar A, Grant L, Mills I. A Phase 2, 8-Week, Multi-Center, Randomized Double-Blind, Placebo Controlled, Parallel Group Study Evaluating The Efficacy, Tolerability And Safety Of [S,S] - Reboxetine (Pnu-165442g) For Stress Urinary Incontinence In Women. J Urol. 2008;179(4, Suppl.):569-70.

315. Ranson RN, Dodds AL, Smith MJ, Santer RM, Watson AH. Age-associated changes in the monoaminergic innervation of rat lumbosacral spinal cord. Brain Res. 2003 May 16;972(1-2):149-58.

316. Danuser H, Thor KB. Inhibition of central sympathetic and somatic outflow to the lower urinary tract of the cat by the alpha 1 adrenergic receptor antagonist prazosin. J Urol. 1995 Apr;153(4):1308-12.

317. Gajewski J, Downie JW, Awad SA. Experimental evidence for a central nervous system site of action in the effect of alpha-adrenergic blockers on the external urinary sphincter. J Urol. 1984 Aug;132(2):403-9.

318. Burgard EC, Portbury S, Thor KB. Electrophysiology of motor neurons innervating the urethral sphincter of female rats. Society for Neuroscience Annual Meeting abstracts. 2008.

319. Downie JW, Espey MJ, Gajewski JB. Alpha 2-adrenoceptors not imidazole receptors mediate depression of a sacral spinal reflex in the cat. Eur J Pharmacol. 1991 Mar 26;195(2):301-4.

320. Ramage AG, Wyllie MG. A comparison of the effects of doxazosin and terazosin on the spontaneous sympathetic drive to the bladder and related organs in anaesthetized cats. Eur J Pharmacol. 1995 Dec 29;294(2-3):645-50.

321. Krier J TK, de Groat WC. Effects of clonidine on the lumbar sympathetic pathways to the large intestine and urinary bladder of the cat. Eur J Pharmacol 1979;59 (1-2):47-53.

322. Conlon K, Miner W, Christy C, McCleary S, Brinkman H, Rees H, et al. Identification of 5-HT2C-mediated mechanisms involved in urethral sphincter reflexes. Abstract Society for Neuroscience. 2005;Program No. 48.14.

323. Mbaki Y, Ramage AG. Investigation of the role of 5-HT(2) receptor subtypes in the control of the bladder and the urethra in the anaesthetized female rat. Br J Pharmacol. 2008 Jul 7.

324. Ogier R, Tribollet E, Suarez P, Raggenbass M. Identified motoneurons involved in sexual and eliminative functions in the rat are powerfully excited by vasopressin and tachykinins. J Neurosci. 2006 Oct 18;26(42):10717-26.

325. Holmes GM, Rogers RC, Bresnahan JC, Beattie MS. Differential effects of intrathecal thyrotropin-releasing hormone (TRH) on perineal reflexes in male rats. Physiol Behav. 1997 Jan;61(1):57-63.

326. Kimura Y, Hamada K, Taniguchi N, Ukai Y, Yoshikuni Y, Kimura K. CNS-mediated influence of TRH and its analog, NS-3, on the function of the rabbit lower urinary tract. J Auton Nerv Syst. 1996 Aug 27;60(1-2):1-11.

327. Holmes GM. 5-Hydroxytryptamine2C receptors on pudendal motoneurons innervating the external anal sphincter. Brain Res. 2005 Sep 28;1057(1-2):65-71.

328. Xu C, Giuliano F, Sun XQ, Brisorgueil MJ, Leclerc P, Verge D, et al. Serotonin 5-HT2A and 5-HT5A receptors are expressed by different motoneuron populations in rat Onuf's nucleus. J Comp Neurol. 2007 Jun 1;502(4):620-34.

329. Kruse MN, Belton AL, de Groat WC. Changes in bladder and external urethral sphincter function after spinal cord injury in the rat. Am J Physiol. 1993 Jun;264(6 Pt 2):R1157-63.

330. Leung PY, Johnson CS, Wrathall JR. Comparison of the effects of complete and incomplete spinal cord injury on lower urinary tract function as evaluated in unanesthetized rats. Exp Neurol. 2007 Nov;208(1):80-91.

331. Chang HY, Cheng CL, Chen JJ, de Groat WC. Serotonergic drugs and spinal cord transections indicate that different spinal circuits are involved in external urethral sphincter activity in rats. Am J Physiol Renal Physiol. 2007 Mar;292(3):F1044-53.

332. Lin SY, Chen GD, Liao JM, Pan SF, Chen MJ, Chen JC, et al. Estrogen modulates the spinal N-methyl-D-aspartic acid-mediated pelvic nerve-to-urethra reflex plasticity in rats. Endocrinology. 2006 Jun;147(6):2956-63.

333. Lin TB. Dynamic pelvic-pudendal reflex plasticity mediated by glutamate in anesthetized rats. Neuropharmacology. 2003 Feb;44(2):163-70.

334. Lin TB. Tetanization-induced pelvic-to-pudendal reflex plasticity in anesthetized rats. Am J Physiol Renal Physiol. 2004 Aug;287(2):F245-51.

335. Chen GD, Peng HY, Tung KC, Cheng CL, Chen YJ, Liao JM, et al. Descending facilitation of spinal NMDA-dependent reflex potentiation from pontine tegmentum in rats. Am J Physiol Renal Physiol. 2007 Oct;293(4):F1115-22.

336. Gu B, Fraser MO, Thor KB, Dolber PC. Induction of bladder sphincter dyssynergia by kappa-2 opioid receptor agonists in the female rat. J Urol. 2004 Jan;171(1):472-7.

337. Truitt WA, Coolen LM. Identification of a potential ejaculation generator in the spinal cord. Science. 2002 Aug 30;297(5586):1566-9.

338. Xu C, Yaici ED, Conrath M, Blanchard P, Leclerc P, Benoit G, et al. Galanin and neurokinin-1 receptor immunoreactive [corrected] spinal neurons controlling the prostate and the bulbospongiosus muscle identified by transsynaptic labeling in the rat. Neuroscience. 2005;134(4):1325-41.

339. McKenna K, Chung S, McVary K. A model for the study of sexual function in anesthetized male and female rats. Am J Physiol. 1991;261(5 Pt 2):R1276-85.

340. Dolber PC, Gu B, Zhang X, Fraser MO, Thor KB, Reiter JP. Activation of the external urethral sphincter central pattern generator by a 5-HT(1A) receptor agonist in rats with chronic spinal cord injury. Am J Physiol Regul Integr Comp Physiol. 2007 Apr;292(4):R1699-706.

341. Looney C, Thor KB, Ricca D, Marson L. Differential effects of simultaneous or sequential administration of paroxetine and WAY-100,635 on ejaculatory behavior. Pharmacol Biochem Behav. 2005 Nov;82(3):427-33.

342. Mendelson SD, Gorzalka BB. 5-HT1A receptors: differential involvement in female and male sexual behavior in the rat. Physiol Behav. 1986;37(2):345-51.

343. Leyton M, Stewart J. The stimulation of central kappa opioid receptors decreases male sexual behavior and locomotor activity. Brain Res. 1992 Oct 23;594(1):56-74.

344. Yoshimura N, Seki S, Erickson KA, Erickson VL, Chancellor MB, de Groat WC. Histological and electrical properties of rat dorsal root ganglion neurons innervating the lower urinary tract. J Neurosci. 2003 May 15;23(10):4355-61.

345. Glazer EJ, Basbaum AI. Leucine enkephalin: localization in and axoplasmic transport by sacral parasympathetic preganglionic neurons. Science. 1980 Jun 27;208(4451):1479-81.

346. Blanco CE, Popper P, Micevych P. alpha-CGRP mRNA levels in motoneurons innervating specific rat muscles. Brain Res Mol Brain Res. 1997 Mar;44(2):253-61.

347. Schroder HD. Somatostatin in the caudal spinal cord: an immunohistochemical study of the spinal centers involved in the innervation of pelvic organs. J Comp Neurol. 1984 Mar 1;223(3):400-14.

348. Holstege JC, Van Dijken H, Buijs RM, Goedknegt H, Gosens T, Bongers CM. Distribution of dopamine immunoreactivity in the rat, cat and monkey spinal cord. J Comp Neurol. 1996 Dec 23;376(4):631-52.

349. Mantyh PW, Allen CJ, Rogers S, DeMaster E, Ghilardi JR, Mosconi T, et al. Some sensory neurons express neuropeptide Y receptors: potential paracrine inhibition of primary afferent nociceptors following peripheral nerve injury. J Neurosci. 1994 Jun;14(6):3958-68.

350. Thor KB, Nickolaus S, Helke CJ. Autoradiographic localization of 5-hydroxytryptamine1A, 5-hydroxytryptamine1B and 5-hydroxytryptamine1C/2 binding sites in the rat spinal cord. Neuroscience. 1993 Jul;55(1):235-52.

351. Kawatani M, Suzuki T, de Groat WC. Corticotropin releasing factor-like immunoreactivity in afferent projections to the sacral spinal cord of the cat. J Auton Nerv Syst. 1996 Dec 14;61(3):218-26.

352. van Dijken H, Dijk J, Voom P, Holstege JC. Localization of dopamine D2 receptor in rat spinal cord identified with immunocytochemistry and in situ hybridization. Eur J Neurosci. 1996 Mar;8(3):621-8.

353. Doly S, Fischer J, Brisorgueil MJ, Verge D, Conrath M. 5-HT5A receptor localization in the rat spinal cord suggests a role in nociception and control of pelvic floor musculature. J Comp Neurol. 2004 Aug 30;476(4):316-29.

354. Charlton CG, Helke CJ. Autoradiographic localization and characterization of spinal cord substance P binding sites: high densities in sensory, autonomic, phrenic, and Onuf's motor nuclei. J Neurosci. 1985 Jun;5(6):1653-61.

355. Lewinter RD, Skinner K, Julius D, Basbaum AI. Immunoreactive TRPV-2 (VRL-1), a capsaicin receptor homolog, in the spinal cord of the rat. J Comp Neurol. 2004 Mar 15;470(4):400-8.

356. Zhang M, Moller M, Broman J, Sukiasyan N, Wienecke J, Hultborn H. Expression of calcium channel CaV1.3 in cat spinal cord: light and electron microscopic immunohistochemical study. J Comp Neurol. 2008 Mar 1;507(1):1109-27.

357. Koliatsos VE, Price DL, Clatterbuck RE. Motor neurons in Onuf's nucleus and its rat homologues express the p75 nerve growth factor receptor: sexual dimorphism and regulation by axotomy. J Comp Neurol. 1994 Jul 22;345(4):510-27.

358. MacLennan AJ, Devlin BK, Neitzel KL, McLaurin DL, Anderson KJ, Lee N. Regulation of ciliary neurotrophic factor receptor alpha in sciatic motor neurons following axotomy. Neuroscience. 1999;91(4):1401-13.

359. Nacimiento W, Topper R, Fischer A, Mobius E, Oestreicher AB, Gispen WH, et al. B-50 (GAP-43) in Onuf's nucleus of the adult cat. Brain Res. 1993 Jun 4;613(1):80-7.

360. Arvidsson U, Risling M, Frisen J, Piehl F, Fried K, Hokfelt T, et al. trkC-like immunoreactivity in the primate descending serotoninergic system. Eur J Neurosci. 1994 Feb 1;6(2):230-6.

361. Morgan C, Nadelhaft I, de Groat WC. Location of bladder preganglionic neurons within the sacral parasympathetic nucleus of the cat. Neurosci Lett. 1979 Oct;14(2-3):189-94.

362. Petras JM, Cummings JF. Sympathetic and parasympathetic innervation of the urinary bladder and urethra. Brain Res. 1978 Sep 22;153(2):363-9.

363. Nadelhaft I, Roppolo J, Morgan C, de Groat WC. Parasympathetic preganglionic neurons and visceral primary afferents in monkey sacral spinal cord revealed following application of horseradish peroxidase to pelvic nerve. J Comp Neurol. 1983 May 1;216(1):36-52.

364. Nadelhaft I, Booth AM. The location and morphology of preganglionic neurons and the distribution of visceral afferents from the rat pelvic nerve: a horseradish peroxidase study. J Comp Neurol. 1984 Jun 20;226(2):238-45.

365. Liao JM, Cheng CL, Lee SD, Chen GD, Chen KJ, Yang CH, et al. Impaired micturition reflex caused by acute selective dorsal or ventral root(s) rhizotomy in anesthetized rats. Neurourol Urodyn. 2006;25(3):283-9.

366. Dering MA, Santer RM, Watson AH. Age-related changes in the morphology of preganglionic neurons projecting to the rat hypogastric ganglion. J Neurocytol. 1996 Oct;25(10):555-63.

367. Dering MA, Santer RM, Watson AH. Age-related changes in the morphology of preganglionic neurons projecting to the paracervical ganglion of nulliparous and multiparous rats. Brain Res. 1998 Jan 12;780(2):245-52.

368. Santer RM, Dering MA, Ranson RN, Waboso HN, Watson AH. Differential susceptibility to ageing of rat preganglionic neurones projecting to the major pelvic ganglion and of their afferent inputs. Auton Neurosci. 2002 Feb 28;96(1):73-81.

369. Vizzard MA, Erdman SL, Forstermann U, de Groat WC. Differential distribution of nitric oxide synthase in neural pathways to the urogenital organs (urethra, penis, urinary bladder) of the rat. Brain Res. 1994;646:279-91.

370. Ishizuka O, Alm P, Larsson B, Mattiasson A, Andersson KE. Facilitatory effect of pituitary adenylate cyclase activating polypeptide on micturition in normal, conscious rats. Neuroscience. 1995 Jun;66(4):1009-14.

371. Vera PL, Nadelhaft I. Anatomical evidence for two spinal

'afferent-interneuron-efferent' reflex pathways involved in micturition in the rat: a 'pelvic nerve' reflex pathway and a 'sacrolumbar intersegmental' reflex pathway. Brain Res. 2000 Nov 10;883(1):107-18.

372. Birder LA, de Groat WC. Induction of c-fos expression in spinal neurons by nociceptive and nonnociceptive stimulation of LUT. Am J Physiol. 1993 Aug;265(2 Pt 2):R326-33.
373. Brindley GS. An implant to empty the bladder or close the urethra. J Neurol Neurosurg Psychiatry. 1977 Apr;40(4):358-69.
374. Brindley GS. The first 500 patients with sacral anterior root stimulator implants: general description. Paraplegia. 1994 Dec;32(12):795-805.
375. Lin VW, Wolfe V, Frost FS, Perkash I. Micturition by functional magnetic stimulation. J Spinal Cord Med. 1997 Apr;20(2):218-26.
376. Bycroft JA, Craggs MD, Sheriff M, Knight S, Shah PJ. Does magnetic stimulation of sacral nerve roots cause contraction or suppression of the bladder? Neurourol Urodyn. 2004;23(3):241-5.
377. Anneren G, Meurling S, Olsen L. Megacystis-microcolon-intestinal hypoperistalsis syndrome (MMIHS), an autosomal recessive disorder: clinical reports and review of the literature. Am J Med Genet. 1991 Nov 1;41(2):251-4.
378. Richardson CE, Morgan JM, Jasani B, Green JT, Rhodes J, Williams GT, et al. Megacystis-microcolon-intestinal hypoperistalsis syndrome and the absence of the alpha3 nicotinic acetylcholine receptor subunit. Gastroenterology. 2001;121:350-7.
379. Xu W, Orr-Urtreger A, Nigro F, Gelber S, Sutcliffe CB, Armstrong D, et al. Multiorgan autonomic dysfunction in mice lacking the beta2 and the beta4 subunits of neuronal nicotinic acetylcholine receptors. Journal of Neuroscience. 1999;19:9298-305.
380. De Biasi M, Nigro F, Xu W. Nicotinic acetylcholine receptors in the autonomic control of bladder function. Eur J Pharmacol. 2000 Mar 30;393(1-3):137-40.
381. Li MZ, Masuko S. Target specific organization and neuron types of the dog pelvic ganglia: a retrograde-tracing and immunohistochemical study. Arch Histol Cytol. 2001 Aug;64(3):267-80.
382. Wanigasekara Y, Kepper ME, Keast JR. Immunohistochemical characterisation of pelvic autonomic ganglia in male mice. Cell Tissue Res. 2003 Feb;311(2):175-85.
383. Gabella G. Intramural neurons in the urinary bladder of the guinea-pig. Cell Tissue Res. 1990 Aug;261(2):231-7.
384. Brading AF, Greenland JE, Mills IW, McMurray G, Symes S. Blood supply to the bladder during filling. Scand J Urol Nephrol Suppl. 1999;201:25-31.
385. Leissner J, Allhoff EP, Wolff W, Feja C, Hockel M, Black P, et al. The pelvic plexus and antireflux surgery: topographical findings and clinical consequences. J Urol. 2001 May;165(5):1652-5.
386. Maas CP, Kenter GG, Trimbos JB, Deruiter MC. Anatomical basis for nerve-sparing radical hysterectomy: immunohistochemical study of the pelvic autonomic nerves. Acta Obstet Gynecol Scand. 2005 Sep;84(9):868-74.
387. Butler-Manuel SA, Buttery LD, A'Hern RP, Polak JM, Barton DP. Pelvic nerve plexus trauma at radical hysterectomy and simple hysterectomy: the nerve content of the uterine supporting ligaments. Cancer. 2000 Aug 15;89(4):834-41.
388. Warburton AL, Santer RM. Sympathetic and sensory innervation of the urinary tract in young adult and aged rats: a semi-quantitative histochemical and immunohistochemical study. Histochem J. 1994 Feb;26(2):127-33.
389. Warburton AL, Santer RM. Localisation of NADPH-diaphorase and acetylcholinesterase activities and of tyrosine hydroxylase and neuropeptide-Y immunoreactivity in neurons of the hypogastric ganglion of young adult and aged rats. J Auton Nerv Syst. 1993 Nov;45(2):155-63.
390. Ho KM, Ny L, McMurray G, Andersson KE, Brading AF, Noble JG. Co-localization of carbon monoxide and nitric oxide synthesizing enzymes in the human urethral sphincter. J Urol. 1999 Jun;161(6):1968-72.
391. Gabella G, Berggren T, Uvelius B. Hypertrophy and reversal of hypertrophy in rat pelvic ganglion neurons. J Neurocytol. 1992;21:649-62.
392. Dixon JS, Gilpin SA, Gilpin CJ, Gosling JA. Intramural ganglia of the human urinary bladder. Br J Urol. 1983 Apr;55(2):195-8.
393. Keast JR, Kawatani M, De Groat WC. Sympathetic modulation of cholinergic transmission in cat vesical ganglia is mediated by alpha 1- and alpha 2-adrenoceptors. Am J Physiol. 1990 Jan;258(1 Pt 2):R44-50.
394. Scheepe JR, van den Hoek J, Junemann KP, Alken P. Endothelin-A-receptor antagonist LU 302146 inhibits electrostimulation-induced bladder contractions in vivo. Neurourol Urodyn. 2006;25(5):468-72.
395. Sullivan ME, Mumtaz FH, Khan MA, Dashwood MR, Thompson CS, Mikhailidis DP, et al. Endothelins in the urinary tract. BJU Int. 2000 Jul;86(1):97-106.
396. Garcia-Pascual A, Larsson B, Andersson KE. Contractile effects of endothelin-1 and localization of endothelin binding sites in rabbit lower urinary tract smooth muscle. Acta Physiol Scand. 1990 Dec;140(4):545-55.
397. Saenz de Tejada I, Mueller JD, de Las Morenas A, Machado M, Moreland RB, Krane RJ, et al. Endothelin in the urinary bladder. I. Synthesis of endothelin-1 by epithelia, muscle and fibroblasts suggests autocrine and paracrine cellular regulation. J Urol. 1992 Oct;148(4):1290-8.
398. Latifpour J, Fukumoto Y, Weiss RM. Regional differences in the density and subtype specificity of endothelin receptors in rabbit urinary tract. Naunyn Schmiedebergs Arch Pharmacol. 1995 Nov;352(5):459-68.
399. Ek A, Alm P, Andersson KE, Persson CG. Adrenergic and Cholinergic Nerves of the Human Urethra and Urinary Bladder. A histochemical study. Acta Physiol Scand. 1977 Mar;99(3):345-52.
400. Somogyi GT, Tanowitz M, de Groat WC. Prejunctional facilitatory alpha 1-adrenoceptors in the rat urinary bladder. Br J Pharmacol. 1995 Apr;114(8):1710-6.
401. Szell EA, Yamamoto T, de Groat WC, Somogyi GT. Smooth muscle and parasympathetic nerve terminals in the rat urinary bladder have different subtypes of alpha(1) adrenoceptors. Br J Pharmacol. 2000 Aug;130(7):1685-91.
402. Drake MJ, Gardner BP, Brading AF. Innervation of the detrusor muscle bundle in neurogenic detrusor overactivity. BJU International. 2003;91:702-10.
403. Vignes JR, Deloire MS, Petry KG, Nagy F. Characterization and restoration of altered inhibitory and excitatory control of micturition reflex in experimental autoimmune encephalomyelitis in rats. J Physiol. 2007 Jan 15;578(Pt 2):439-50.
404. Miyazato M, Sugaya K, Nishijima S, Ashitomi K, Hatano T, Ogawa Y. Inhibitory effect of intrathecal glycine on the micturition reflex in normal and spinal cord injury rats. Exp Neurol. 2003 Sep;183(1):232-40.
405. Mertens P, Ghaemmaghami C, Bert L, Perret-Liaudet A, Sindou M, Renaud B. Amino acids in spinal dorsal horn of patients during surgery for neuropathic pain or spasticity. Neuroreport. 2000 Jun 5;11(8):1795-8.
406. Xiao CG, Du MX, Li B, Liu Z, Chen M, Chen ZH, et al. An

artificial somatic-autonomic reflex pathway procedure for bladder control in children with spina bifida. J Urol. 2005 Jun;173(6):2112-6.

407. Xiao CG, Du MX, Dai C, Li B, Nitti VW, de Groat WC. An artificial somatic-central nervous system-autonomic reflex pathway for controllable micturition after spinal cord injury: preliminary results in 15 patients. J Urol. 2003 Oct;170(4 Pt 1):1237-41.
408. Xiao CG, de Groat WC, Godec CJ, Dai C, Xiao Q. "Skin-CNS-bladder" reflex pathway for micturition after spinal cord injury and its underlying mechanisms. J Urol. 1999 Sep;162(3 Pt 1):936-42.
409. de Groat WC, Yoshimura N. Pharmacology of the lower urinary tract. Annu Rev Pharmacol Toxicol. 2001;41:691-721.
410. Keast JR, Stephensen TM. Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal transport. J Comp Neurol. 2000 Sep 4;424(4):577-87.
411. Gougis S, Prud'homme MJ, Rampin O. Presence of the N-methyl-D-aspartic acid R1 glutamatergic receptor subunit in the lumbosacral spinal cord of male rats. Neurosci Lett. 2002 May 3;323(3):224-8.
412. Nishizawa O, Igawa Y, Satoh T, Yamashiro S, Sugaya K. Effects of glutamate receptor antagonists on lower urinary tract function in conscious unanesthetized rats. Adv Exp Med Biol. 1999;462:275-81.
413. Matsumoto G, Hisamitsu T, de Groat WC. Role of glutamate and NMDA receptors in the descending limb of the spinobulbospinal micturition reflex pathway of the rat. Neurosci Lett. 1995 Jan 2;183(1-2):58-61.
414. Shefchyk SJ. Spinal cord neural organization controlling the urinary bladder and striated sphincter. Prog Brain Res. 2002;137:71-82.
415. Miyazato M, Sugaya K, Nishijima S, Ashitomi K, Ohyama C, Ogawa Y. Rectal distention inhibits bladder activity via glycinergic and GABAergic mechanisms in rats. J Urol. 2004 Mar;171(3):1353-6.
416. de Groat WC. Influence of central serotonergic mechanisms on lower urinary tract function. Urology. 2002 May;59(5 Suppl 1):30-6.
417. Mizukawa K. The segmental detailed topographical distribution of monoaminergic terminals and their pathways in the spinal cord of the cat. Anat Anz. 1980;147(2):125-44.
418. Skagerberg G, Bjorklund A. Topographic principles in the spinal projections of serotonergic and non-serotonergic brainstem neurons in the rat. Neuroscience. 1985 Jun;15(2):445-80.
419. Chen SY, Wang SD, Cheng CL, Kuo JS, De Groat WC, Chai CY. Glutamate activation of neurons in CV-reactive areas of cat brain stem affects urinary bladder motility. Am J Physiol. 1993 Oct;265(4 Pt 2):F520-9.
420. McMahon SB, Spillane K. Brain stem influences on the parasympathetic supply to the urinary bladder of the cat. Brain Res. 1982 Feb 25;234(2):237-49.
421. Sugaya K, Ogawa Y, Hatano T, Koyama Y, Miyazato T, Oda M. Evidence for involvement of the subcoeruleus nucleus and nucleus raphe magnus in urine storage and penile erection in decerebrate rats. J Urol. 1998 Jun;159(6):2172-6.
422. Lecci A, Giuliani S, Santicioli P, Maggi CA. Involvement of 5-hydroxytryptamine1A receptors in the modulation of micturition reflexes in the anesthetized rat. J Pharmacol Exp Ther. 1992 Jul;262(1):181-9.
423. Ryall RW, DeGroat WC. The microelectrophoretic administration of noradrenaline, 5-hydroxytryptamine, acetylcholine and glycine to sacral parasympathetic preganglionic neurones. Brain Res. 1972 Feb 25;37(2):345-7.
424. Thor KB, Hisamitsu T, de Groat WC. Unmasking of a neonatal somatovesical reflex in adult cats by the serotonin autoreceptor agonist 5-methoxy-N,N-dimethyltryptamine. Brain Res Dev Brain Res. 1990 Jun 1;54(1):35-42.
425. Tai C, Miscik CL, Ungerer TD, Roppolo JR, de Groat WC. Suppression of bladder reflex activity in chronic spinal cord injured cats by activation of serotonin 5-HT1A receptors. Exp Neurol. 2006 Jun;199(2):427-37.
426. Lumb BM. Brainstem control of visceral afferent pathways in the spinal cord. Prog Brain Res. 1986;67:279-93.
427. de Groat WC, Yoshiyama M, Ramage AG, Yamamoto T, Somogyi GT. Modulation of voiding and storage reflexes by activation of alpha1-adrenoceptors. Eur Urol. 1999;36 Suppl 1:68-73.
428. Yoshiyama M, Yamamoto T, de Groat WC. Role of spinal alpha(1)-adrenergic mechanisms in the control of lower urinary tract in the rat. Brain Res. 2000 Nov 3;882(1-2):36-44.
429. Wada T, Otsu T, Hasegawa Y, Mizuchi A, Ono H. Characterization of alpha 1-adrenoceptor subtypes in rat spinal cord. Eur J Pharmacol. 1996 Sep 26;312(2):263-6.
430. Wu W, Elde R, Wessendorf MW. Organization of the serotonergic innervation of spinal neurons in rats—III. Differential serotonergic innervation of somatic and parasympathetic preganglionic motoneurons as determined by patterns of co-existing peptides. Neuroscience. 1993 Jul;55(1):223-33.
431. Pascual JI, Insausti R, Gonzalo LM. Urinary bladder innervation in male rat: termination of primary afferents in the spinal cord as determined by transganglionic transport of WGA-HRP. J Urol. 1993 Aug;150(2 Pt 1):500-4.
432. Ljungdahl A, Hokfelt T, Nilsson G. Distribution of substance P-like immunoreactivity in the central nervous system of the rat—I. Cell bodies and nerve terminals. Neuroscience. 1978;3(10):861-943.
433. Sasek CA, Seybold VS, Elde RP. The immunohistochemical localization of nine peptides in the sacral parasympathetic nucleus and the dorsal gray commissure in rat spinal cord. Neuroscience. 1984 Jul;12(3):855-73.
434. Micevych PE, Coquelin A, Arnold AP. Immunohistochemical distribution of substance P, serotonin, and methionine enkephalin in sexually dimorphic nuclei of the rat lumbar spinal cord. J Comp Neurol. 1986 Jun 8;248(2):235-44.
435. Johansson O, Hokfelt T, Pernow B, Jeffcoate SL, White N, Steinbusch HW, et al. Immunohistochemical support for three putative transmitters in one neuron: coexistence of 5-hydroxytryptamine, substance P- and thyrotropin releasing hormone-like immunoreactivity in medullary neurons projecting to the spinal cord. Neuroscience. 1981;6(10):1857-81.
436. Maxwell L, Maxwell DJ, Neilson M, Kerr R. A confocal microscopic survey of serotoninergic axons in the lumbar spinal cord of the rat: co-localization with glutamate decarboxylase and neuropeptides. Neuroscience. 1996 Nov;75(2):471-80.
437. Lecci A, Maggi CA. Tachykinins as modulators of the micturition reflex in the central and peripheral nervous system. Regul Pept. 2001 Sep 15;101(1-3):1-18.
438. Mersdorf A, Schmidt RA, Kaula N, Tanagho EA. Intrathecal administration of substance P in the rat: the effect on bladder and urethral sphincteric activity. Urology. 1992 Jul;40(1):87-96.
439. Gilbey MP, McKenna KE, Schramm LP. Effects of substance P on sympathetic preganglionic neurones. Neurosci Lett. 1983 Oct 31;41(1-2):157-9.

440. Goettl VM, Tejwani GA, Neff NH, Hadjiconstantinou M. Decreased neuropeptide content in the spinal cord of aged rats: the effect of GM1 ganglioside. Neuroreport. 1999 Feb 25;10(3):513-6.

441. Johnson H, Ulfhake B, Dagerlind A, Bennett GW, Fone KC, Hokfelt T. The serotoninergic bulbospinal system and brainstem-spinal cord content of serotonin-, TRH-, and substance P-like immunoreactivity in the aged rat with special reference to the spinal cord motor nucleus. Synapse. 1993 Sep;15(1):63-89.

442. Ranson RN, Priestley DJ, Santer RM, Watson AH. Changes in the substance P-containing innervation of the lumbosacral spinal cord in male Wistar rats as a consequence of ageing. Brain Res. 2005 Mar 2;1036(1-2):139-44.

443. Vodusek DB, Light JK, Libby JM. Detrusor inhibition induced by stimulation of pudendal nerve afferents. Neurourol Urodyn. 1986;5:2381-9.

444. Sundin T, Carlsson CA, Kock NG. Detrusor inhibition induced from mechanical stimulation of the anal region and from electrical stimulation of pudendal nerve afferents. An experimental study in cats. Invest Urol. 1974 Mar;11(5):374-8.

445. Nomoto Y, Yoshida A, Ikeda S, Kamikawa Y, Harada K, Ohwatashi A, et al. Effect of menthol on detrusor smooth-muscle contraction and the micturition reflex in rats. Urology. 2008 Sep;72(3):701-5.

446. Foreman RD. Integration of viscerosomatic sensory input at the spinal level. Prog Brain Res. 2000;122:209-21.

447. Kaddumi EG, Hubscher CH. Convergence of multiple pelvic organ inputs in the rat rostral medulla. J Physiol. 2006 Apr 15;572(Pt 2):393-405.

448. Bruggemann J, Shi T, Apkarian AV. Squirrel monkey lateral thalamus. II. Viscerosomatic convergent representation of urinary bladder, colon, and esophagus. J Neurosci. 1994 Nov;14(11 Pt 2):6796-814.

449. Dalmose AL, Rijkhoff NJ, Andersen IS, Stefania D, Jorgensen TM, Djurhuus JC. Bladder and urethral responses to pelvic nerve stimulation in the pig. Scand J Urol Nephrol Suppl. 2002(210):34-45.

450. Persson K, Pandita RK, Spitsbergen JM, Steers WD, Tuttle JB, Andersson KE. Spinal and peripheral mechanisms contributing to hyperactive voiding in spontaneously hypertensive rats. Am J Physiol. 1998 Oct;275(4 Pt 2):R1366-73.

451. Kruse MN, Mallory BS, Noto H, Roppolo JR, de Groat WC. Modulation of the spinobulbospinal micturition reflex pathway in cats. Am J Physiol. 1992 Mar;262(3 Pt 2):R478-84.

452. Rouzade-Dominguez ML, Miselis R, Valentino RJ. Central representation of bladder and colon revealed by dual transsynaptic tracing in the rat: substrates for pelvic visceral coordination. Eur J Neurosci. 2003 Dec;18(12):3311-24.

453. Keast JR, De Groat WC. Segmental distribution and peptide content of primary afferent neurons innervating the urogenital organs and colon of male rats. J Comp Neurol. 1992 May 22;319(4):615-23.

454. Winnard KP, Dmitrieva N, Berkley KJ. Cross-organ interactions between reproductive, gastrointestinal, and urinary tracts: modulation by estrous stage and involvement of the hypogastric nerve. Am J Physiol Regul Integr Comp Physiol. 2006 Dec;291(6):R1592-601.

455. Dmitrieva N, Berkley KJ. Influence of estradiol on micturition thresholds in the rat: involvement of the hypogastric nerve. Am J Physiol Regul Integr Comp Physiol. 2005 Dec;289(6):R1724-8.

456. Malykhina AP, Qin C, Greenwood-van Meerveld B, Foreman RD, Lupu F, Akbarali HI. Hyperexcitability of convergent colon and bladder dorsal root ganglion neurons after colonic inflammation: mechanism for pelvic organ cross-talk. Neurogastroenterol Motil. 2006 Oct;18(10):936-48.

457. Qin C, Malykhina AP, Akbarali HI, Foreman RD. Cross-organ sensitization of lumbosacral spinal neurons receiving urinary bladder input in rats with inflamed colon. Gastroenterology. 2005 Dec;129(6):1967-78.

458. Wall PD, Hubscher CH, Berkley KJ. Intraspinal modulation of neuronal responses to uterine and cervix stimulation in rat L1 and L6 dorsal horn. Brain Res. 1993 Sep 17;622(1-2):71-8.

459. Hubscher CH, Kaddumi EG, Johnson RD. Brain stem convergence of pelvic viscerosomatic inputs via spinal and vagal afferents. Neuroreport. 2004 Jun 7;15(8):1299-302.

460. Rouzade-Dominguez ML, Pernar L, Beck S, Valentino RJ. Convergent responses of Barrington's nucleus neurons to pelvic visceral stimuli in the rat: a juxtacellular labelling study. Eur J Neurosci. 2003 Dec;18(12):3325-34.

461. Sugaya K, de Groat WC. Bladder volume-dependent excitatory and inhibitory influence of lumbosacral dorsal and ventral roots on bladder activity in rats. Biomed Res. 2007 Aug;28(4):169-75.

462. Lagou M, Gillespie J, Kirkwood T, Harvey I, Drake MJ. Muscarinic stimulation of the mouse isolated whole bladder: physiological responses in young and ageing mice. Auton Autacoid Pharmacol. 2006 Jul;26(3):253-60.

463. Gevaert T, Ost D, De Ridder D. Comparison study of autonomous activity in bladders from normal and paraplegic rats. Neurourol Urodyn. 2006;25(4):368-78; discussion 79-80.

464. Finney SM, Stewart LH, Gillespie JI. Cholinergic activation of phasic activity in the isolated bladder: possible evidence for M3- and M2-dependent components of a motor/sensory system. BJU Int. 2007 Sep;100(3):668-78.

465. Sugaya K, de Groat WC. Inhibitory control of the urinary bladder in the neonatal rat in vitro spinal cord-bladder preparation. Brain Res Dev Brain Res. 2002 Sep 20;138(1):87-95.

466. Sugaya K, de Groat WC. Effects of MK-801 and CNQX, glutamate receptor antagonists, on bladder activity in neonatal rats. Brain Res. 1994 Mar 21;640(1-2):1-10.

467. Sugaya K, De Groat WC. Micturition reflexes in the in vitro neonatal rat brain stem-spinal cord-bladder preparation. Am J Physiol. 1994 Mar;266(3 Pt 2):R658-67.

468. Lagou M, Gillespie JI, Andersson KE, Kirkwood T, Drake MJ. Bladder volume alters cholinergic responses of the isolated whole mouse bladder. J Urol. 2006 Feb;175(2):771-6.

469. Drake M, Gillespie J, Hedlund P, Harvey I, Lagou M, Andersson KE. Muscarinic stimulation of the rat isolated whole bladder: pathophysiological models of detrusor overactivity. Auton Autacoid Pharmacol. 2006 Jul;26(3):261-6.

470. Grol S, van Koeveringe GA, de Vente J, van Kerrebroeck PE, Gillespie JI. Regional differences in sensory innervation and suburothelial interstitial cells in the bladder neck and urethra. BJU Int. 2008 Sep;102(7):870-7.

471. de Jongh R, van Koeveringe GA, van Kerrebroeck PE, Markerink-van Ittersum M, de Vente J, Gillespie JI. Damage to the bladder neck alters autonomous activity and its sensitivity to cholinergic agonists. BJU Int. 2007 Oct;100(4):919-29.

472. Gillespie JI, Harvey IJ, Drake MJ. Agonist and nerve induced phasic activity in the isolated whole bladder of the guinea pig. Experimental Physiology. 2003;88:343-57.

473. Kanai A, Golin F, Roppolo J, Birder L, Tai C, Meyers S, et al. Age-induced changes in urinary bladder activity detected using voltage- and calcium-sensitive dyes. FASEB J. 2003:A649.

474. Kanai A, Roppolo J, Ikeda Y, Zabbarova I, Tai C, Birder L, et al. Origin of spontaneous activity in neonatal and adult rat bladders and its enhancement by stretch and muscarinic agonists. Am J Physiol Renal Physiol. 2007 Mar;292(3):F1065-72.

475. Drake MJ, Harvey IJ, Gillespie JI. Autonomous activity in the isolated guinea pig bladder. Experimental Physiology. 2003;88(1):19-30.

476. Coolsaet BL, Van Duyl WA, Van Os-Bossagh P, De Bakker HV. New concepts in relation to urge and detrusor activity. Neurourol Urodyn. 1993;12(5):463-71.

477. Drake MJ, Mills IW, Gillespie JI. Model of peripheral autonomous modules and a myovesical plexus in normal and overactive bladder function. Lancet. 2001 Aug 4;358(9279):401-3.

478. Smith TK, Robertson WJ. Synchronous movements of the longitudinal and circular muscle during peristalsis in the isolated guinea-pig distal colon. J Physiol. 1998 Jan 15;506 (Pt 2):563-77.

479. Drake MJ. The integrative physiology of the bladder. Ann R Coll Surg Engl. 2007 Sep;89(6):580-5.

480. Zvara P, Vizzard MA. Exogenous overexpression of nerve growth factor in the urinary bladder produces bladder overactivity and altered micturition circuitry in the lumbosacral spinal cord. BMC Physiol. 2007;7:9.

481. de Groat WC. Integrative control of the lower urinary tract: preclinical perspective. Br J Pharmacol. 2006 Feb;147 Suppl 2:S25-40.

482. Fowler CJ, Griffiths D, de Groat WC. The neural control of micturition. Nat Rev Neurosci. 2008 Jun;9(6):453-66.

483. Holstege G. Micturition and the soul. J Comp Neurol. 2005 Dec 5;493(1):15-20.

484. Blok BF, De Weerd H, Holstege G. Ultrastructural evidence for a paucity of projections from the lumbosacral cord to the pontine micturition center or M-region in the cat: a new concept for the organization of the micturition reflex with the periaqueductal gray as central relay. J Comp Neurol. 1995 Aug 21;359(2):300-9.

485. Athwal BS, Berkley KJ, Hussain I, Brennan A, Craggs M, Sakakibara R, et al. Brain responses to changes in bladder volume and urge to void in healthy men. Brain. 2001 Feb;124(Pt 2):369-77.

486. Kavia RB, Dasgupta R, Fowler CJ. Functional imaging and the central control of the bladder. J Comp Neurol. 2005 Dec 5;493(1):27-32.

487. Griffiths D, Derbyshire S, Stenger A, Resnick N. Brain control of normal and overactive bladder. J Urol. 2005 Nov;174(5):1862-7.

488. Taniguchi N, Miyata M, Yachiku S, Kaneko S, Yamaguchi S, Numata A. A study of micturition inducing sites in the periaqueductal gray of the mesencephalon. J Urol. 2002 Oct;168(4 Pt 1):1626-31.

489. Sugaya K, Roppolo JR, Yoshimura N, Card JP, de Groat WC. The central neural pathways involved in micturition in the neonatal rat as revealed by the injection of pseudorabies virus into the urinary bladder. Neurosci Lett. 1997 Feb 28;223(3):197-200.

490. Vizzard MA, Erickson VL, Card JP, Roppolo JR, de Groat WC. Transneuronal labeling of neurons in the adult rat brainstem and spinal cord after injection of pseudorabies virus into the urethra. J Comp Neurol. 1995 May 15;355(4):629-40.

491. Holstege G, Kuypers HG, Boer RC. Anatomical evidence for direct brain stem projections to the somatic motoneuronal cell groups and autonomic preganglionic cell groups in cat spinal cord. Brain Res. 1979 Aug 3;171(2):329-33.

492. Mallory BS, Roppolo JR, de Groat WC. Pharmacological modulation of the pontine micturition center. Brain Res. 1991 Apr 19;546(2):310-20.

493. Noto H, Roppolo JR, Steers WD, de Groat WC. Excitatory and inhibitory influences on bladder activity elicited by electrical stimulation in the pontine micturition center in the rat. Brain Res. 1989 Jul 17;492(1-2):99-115.

494. Komiyama A, Kubota A, Hidai H. Urinary retention associated with a unilateral lesion in the dorsolateral tegmentum of the rostral pons. J Neurol Neurosurg Psychiatry. 1998 Dec;65(6):953-4.

495. Kuipers R, Mouton LJ, Holstege G. Afferent projections to the pontine micturition center in the cat. J Comp Neurol. 2006 Jan 1;494(1):36-53.

496. Holstege G. Descending motor pathways and the spinal motor system: limbic and non-limbic components. Prog Brain Res. 1991;87:307-421.

497. Rizvi TA, Ennis M, Aston-Jones G, Jiang M, Liu WL, Behbehani MM, et al. Preoptic projections to Barrington's nucleus and the pericoerulear region: architecture and terminal organization. J Comp Neurol. 1994 Sep 1;347(1):1-24.

498. Gjone R, Setekleiv J. Excitatory and Inhibitory Bladder Responses to Stimulation of the Cerebral Cortex in the Cat. Acta Physiol Scand. 1963 Dec;59:337-48.

499. Redfern WS, Yardley CP. Contribution of the periaqueductal grey matter to the development of hypertension in the spontaneously hypertensive rat. J Auton Nerv Syst. 1990 Dec;31(3):231-9.

500. Bandler R, Keay KA. Columnar organization in the midbrain periaqueductal gray and the integration of emotional expression. Prog Brain Res. 1996;107:285-300.

501. Valentino RJ, Page ME, Luppi PH, Zhu Y, Van Bockstaele E, Aston-Jones G. Evidence for widespread afferents to Barrington's nucleus, a brainstem region rich in corticotropin-releasing hormone neurons. Neuroscience. 1994 Sep;62(1):125-43.

502. Nadelhaft I, Vera PL, Card JP, Miselis RR. Central nervous system neurons labelled following the injection of pseudorabies virus into the rat urinary bladder. Neurosci Lett. 1992 Aug 31;143(1-2):271-4.

503. Blok BF, Holstege G. Ultrastructural evidence for a direct pathway from the pontine micturition center to the parasympathetic preganglionic motoneurons of the bladder of the cat. Neurosci Lett. 1997 Feb 7;222(3):195-8.

504. Sasaki M. Bladder contractility-related neurons in Barrington's nucleus: axonal projections to the spinal cord in the cat. J Comp Neurol. 2002 Aug 5;449(4):355-63.

505. Sasaki M. Feed-forward and feedback regulation of bladder contractility by Barrington's nucleus in cats. J Physiol. 2004 May 15;557(Pt 1):287-305.

506. Sugaya K, Ogawa Y, Hatano T, Nishijima S, Matsuyama K, Mori S. Ascending and descending brainstem neuronal activity during cystometry in decerebrate cats. Neurourol Urodyn. 2003;22(4):343-50.

507. Matsumoto G, Hisamitsu T, de Groat WC. Non-NMDA glutamatergic excitatory transmission in the descending limb of the spinobulbospinal micturition reflex pathway of the rat. Brain Res. 1995 Sep 25;693(1-2):246-50.

508. Blok BF. Central pathways controlling micturition and urinary continence. Urology. 2002 May;59(5 Suppl 1):13-7.

509. Bradley WE, Conway CJ. Bladder representation in the pontine-mesencephalic reticular formation. Exp Neurol. 1966 Nov;16(3):237-49.

510. Koshino K. Spontaneous potential activities related to the intravesical pressure in the pontine area of the cat. Jpn J Physiol. 1970 Jun 15;20(3):272-80.

511. Blok BF. Brain control of the lower urinary tract. Scand J Urol Nephrol Suppl. 2002(210):11-5.

512. Simms AE, Paton JF, Pickering AE. Hierarchical recruitment of the sympathetic and parasympathetic limbs of the baroreflex in normotensive and spontaneously hypertensive rats. J Physiol. 2007 Mar 1;579(Pt 2):473-86.

513. Paton JF, Deuchars J, Ahmad Z, Wong LF, Murphy D, Kasparov S. Adenoviral vector demonstrates that angiotensin II-induced depression of the cardiac baroreflex is mediated by endothelial nitric oxide synthase in the nucleus tractus solitarii of the rat. J Physiol. 2001 Mar 1;531(Pt 2):445-58.

514. Paton JF, Lonergan T, Deuchars J, James PE, Kasparov S. Detection of angiotensin II mediated nitric oxide release within the nucleus of the solitary tract using electron-paramagnetic resonance (EPR) spectroscopy. Auton Neurosci. 2006 Jun 30;126-127:193-201.

515. Simms AE, Paton JF, Pickering AE. Disinhibition of the cardiac limb of the arterial baroreflex in rat: a role for metabotropic glutamate receptors in the nucleus tractus solitarii. J Physiol. 2006 Sep 15;575(Pt 3):727-38.

516. Sasaki M. Properties of Barrington's neurones in cats: units that fire inversely with micturition contraction. Brain Res. 2005 Feb 1;1033(1):41-50.

517. Schreihofer AM, Guyenet PG. The baroreflex and beyond: control of sympathetic vasomotor tone by GABAergic neurons in the ventrolateral medulla. Clin Exp Pharmacol Physiol. 2002 May-Jun;29(5-6):514-21.

518. Stornetta RL, Rosin DL, Wang H, Sevigny CP, Weston MC, Guyenet PG. A group of glutamatergic interneurons expressing high levels of both neurokinin-1 receptors and somatostatin identifies the region of the pre-Botzinger complex. J Comp Neurol. 2003 Jan 20;455(4):499-512.

519. Holstege G, Kuypers HG. The anatomy of brain stem pathways to the spinal cord in cat. A labeled amino acid tracing study. Prog Brain Res. 1982;57:145-75.

520. Blok BF, Holstege G. Two pontine micturition centers in the cat are not interconnected directly: implications for the central organization of micturition. J Comp Neurol. 1999 Jan 11;403(2):209-18.

521. Sakakibara R, Nakazawa K, Shiba K, Nakajima Y, Uchiyama T, Yoshiyama M, et al. Firing patterns of micturition-related neurons in the pontine storage centre in cats. Auton Neurosci. 2002 Jul 31;99(1):24-30.

522. Vargas LC, Marques TA, Schenberg LC. 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. 2000 Feb 11;280(1):45-8.

523. Liu Z, Sakakibara R, Nakazawa K, Uchiyama T, Yamamoto T, Ito T, et al. Micturition-related neuronal firing in the periaqueductal gray area in cats. Neuroscience. 2004;126(4):1075-82.

524. Matsuura S, Allen GV, Downie JW. Volume-evoked micturition reflex is mediated by the ventrolateral periaqueductal gray in anesthetized rats. Am J Physiol. 1998 Dec;275(6 Pt 2):R2049-55.

525. Sakakibara R, Hattori T, Kita K, Yamanishi T, Yasuda K. Urodynamic and cardiovascular measurements in patients with micturition syncope. Clin Auton Res. 1997 Oct;7(5):219-21.

526. Smith JC, Abdala AP, Koizumi H, Rybak IA, Paton JF. Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms. J Neurophysiol. 2007 Dec;98(6):3370-87.

527. Yoshiyama M, de Groat WC. Supraspinal and spinal alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and N-methyl-D-aspartate glutamatergic control of the micturition reflex in the urethane-anesthetized rat. Neuroscience. 2005;132(4):1017-26.

528. Burgard EC, Fraser MO, Thor KB. Serotonergic modulation of bladder afferent pathways. Urology. 2003 Oct;62(4 Suppl 1):10-5.

529. Ito T, Sakakibara R, Nakazawa K, Uchiyama T, Yamamoto T, Liu Z, et al. Effects of electrical stimulation of the raphe area on the micturition reflex in cats. Neuroscience. 2006 Nov 3;142(4):1273-80.

530. Conley RK, Williams TJ, Ford AP, Ramage AG. The role of alpha(1)-adrenoceptors and 5-HT(1A) receptors in the control of the micturition reflex in male anaesthetized rats. Br J Pharmacol. 2001 May;133(1):61-72.

531. Yoshimura N, Kuno S, Chancellor MB, De Groat WC, Seki S. Dopaminergic mechanisms underlying bladder hyperactivity in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway. Br J Pharmacol. 2003 Aug;139(8):1425-32.

532. Yaguchi H, Soma H, Miyazaki Y, Tashiro J, Yabe I, Kikuchi S, et al. A case of acute urinary retention caused by periaqueductal grey lesion. J Neurol Neurosurg Psychiatry. 2004;75(8):1202-3.

533. Duffau H, Capelle L. Incontinence after brain glioma surgery: new insights into the cortical control of micturition and continence. Case report. J Neurosurg. 2005;102(1):148-51.

534. Yamamoto T, Sakakibara R, Uchiyama T, Liu Z, Ito T, Yamanishi T, et al. Lower urinary tract function in patients with pituitary adenoma compressing hypothalamus. J Neurol Neurosurg Psychiatry. 2005;76(3):390-4.

535. Blok BF, Holstege G. The central nervous system control of micturition in cats and humans. Behav Brain Res. 1998 May;92(2):119-25.

536. Holstege G. Micturition and the soul. J Comp Neurol. 2005;493:15-20.

537. Maurice-Williams RS. Micturition symptoms in frontal tumours. Journal of Neurology Neurosurgery and Psychiatry. 1974;37:431-6.

538. Sakakibara R, Hattori T, Yasuda K, Yamanishi T. Micturitional disturbance after acute hemispheric stroke: analysis of the lesion site by CT and MRI. Journal of Neurological Sciences. 1996;137:47-56.

539. Lang EW, Chesnut RM, Hennerici M. Urinary retention and space-occupying lesions of the frontal cortex. Eur Neurol. 1996;36(1):43-7.

540. Yamamoto S, Soma T, Hatayama T, Mori H, Yoshimura N. Neurogenic bladder induced by brain abscess. British Journal of Urology. 1995;76:272.

541. Blok BF, Sturms LM, Holstege G. Brain activation during micturition in women. Brain. 1998 Nov;121(Pt 11):2033-42.

542. Blok BF, Willemsen AT, Holstege G. A PET study on brain control of micturition in humans. Brain. 1997 Jan;120(Pt 1):111-21.

543. Nour S, Svarer C, Kristensen JK, Paulson OB, Law I. Cerebral activation during micturition in normal men. Brain. 2000 Apr;123(Pt 4):781-9.

544. Matsuura S, Kakizaki H, Mitsui T, Shiga T, Tamaki N, Koyanagi T. Human brain region response to distention or cold stimulation of the bladder: a positron emission tomography study. J Urol. 2002 Nov;168(5):2035-9.

545. Kuhtz-Buschbeck JP, Van der Horst C, Wolff S, Filippow N, Nabavi A, Jansen O, et al. Activation of the supplementary motor area (SMA) during voluntary pelvic floor muscle contractions-An fMRI study. NeuroImage. 2007;35:449-57.

546. Di Gangi Herms AMR, Veit R, Reisenauer C, Herms A, Grodd W, Enck P, et al. Functional imaging of stress urinary incontinence. NeuroImage. 2006;29:267-75.

547. Griffiths D, Derbyshire S, Stenger A, Resnick N. Brain control of normal and overactive bladder. J Urol. 2005;174:1862-7.

548. Blok BFM, Groen J, Bosch JLHR, Veltman DJ, Lammersma AA. Different brain effects during chronic and acute sacral modulation in urge incontinent patients with implanted neurostimulators. BJU Int. 2006;08:1238-43.

549. Yin Y, Shuke N, Okizaki A, Sato J, Aburano1 T, Li Y, et al. Cerebral activation during withholding urine with full bladder in healthy men using 99mTc-HMPAO SPECT. J Nucl Med. 2006;47(7):1093-8.

550. Griffiths D, Tadic SD, Schaefer W, Resnick NM. Cerebral control of the bladder in normal and urge-incontinent women. NeuroImage. 2007;37:1-7.

551. Andrew J, Nathan PW. The cerebral control of micturition. Proc RSM. 1965;58:553-5.

552. Seseke S, Baudewig J, Kallenberg K, R-H. R, Seseke F, Dechent P. Voluntary pelvic floor muscle control - an fMRI study. Neuroimage. 2006;31:1399-407.

553. Laplane D, Degos JD, Baulac M, Gray F. Bilateral infarction of the anterior cingulate gyri and of the fornices. Report of a case. J Neurol Sci. 1981;51(2):289-300.

554. Kuhtz-Buschbeck JP, van der Horst C, Pott C, Wolff S, Nabavi A, Jansen O, et al. Cortical representation of the urge to void: a functional magnetic resonance imaging study. J Urol. 2005;174:1477-81.

555. Griffiths D, Tadic SD. Bladder control, urgency, and urge incontinence: Evidence from functional brain imaging. Neurourol Urodyn. 2007;Dec 18, Epub ahead of print.

556. Andrew J, Nathan P, Spanos N. Disturbances of micturition and defaecation due to aneurysms of anterior communication or anterior cerebral arteries. Journal of Neurosurgery. 1966;24:1-10.

557. Barrington F. The effect of lesions of the hind- and mid-brani on micturition in the cat. Quart J Exp Physiol Cogn Med. 1925;15:81-102.

558. Holman E. Difficult urination associated with intracranial tumours of the posterior fossa. A physiologic and clinical study. Arch Neurol Psychiat. 1926;15:371-80.

559. Betts CD, Kapoor R, Fowler CJ. Pontine pathology and micturition dysfunction. Br J Urol. 1992;70:100-2.

560. Manente G, Melchionda D, Uncini A. Urinary retention in bilateral pontine tumour: evidence for a pontine micturition centre in humans. Journal of Neurology Neurosurgery and Psychiatry. 1996;61:528-36.

561. Sakakibara R, Hattori T, Fukutake T, Mori M, Yamanishi T, Yasuda K. Micturitional disturbance in herpetic brainstem encephalitis; contribution of the pontine micturition centre. J Neurol Neurosurg Psychiatry. 1998;64(2):269-72.

562. Ueki K. Disturbances of micturition observed in some patients with brain tumour. Neurologica Medica Chirurgica. 1960;2:25-33.

563. Renier WO, Gabreels FJM. Evaluation of diagnosis and non-surgical therapy in 24 children with a pontine tumour. Neuropaediatrie. 1980;11:262-73.

564. Sakakibara R, Hattori T, Yasuda K, Yamanishi T. Micturitional disturbance and the pontine tegmental lesion: urodynamic and MRI analyses of vascular cases. Journal of Neurological Sciences. 1996;141:105-10.

565. Critchley HD. Neural mechanisms of autonomic, affective, and cognitive integration. J Comp Neurol. 2005;493:154-66.

566. Nazif O, Teichman JM, Gebhart GF. Neural upregulation in interstitial cystitis. Urology. 2007 Apr;69(4 Suppl):24-33.

567. Wein AJ, Hanno PM. Targets for therapy of the painful bladder. Urology. 2002 May;59(5 Suppl 1):68-73.

568. Gonzalez RR, Fong T, Belmar N, Saban M, Felsen D, Te A. Modulating bladder neuro-inflammation: RDP58, a novel anti-inflammatory peptide, decreases inflammation and nerve growth factor production in experimental cystitis. J Urol. 2005 Feb;173(2):630-4.

569. Steers WD, Tuttle JB. Mechanisms of Disease: the role of nerve growth factor in the pathophysiology of bladder disorders. Nat Clin Pract Urol. 2006 Feb;3(2):101-10.

570. Okragly AJ, Niles AL, Saban R, Schmidt D, Hoffman RL, Warner TF, et al. Elevated tryptase, nerve growth factor, neurotrophin-3 and glial cell line-derived neurotrophic factor levels in the urine of interstitial cystitis and bladder cancer patients. J Urol. 1999 Feb;161(2):438-41; discussion 41-2.

571. Liu HT, Kuo HC. Intravesical botulinum toxin A injections plus hydrodistension can reduce nerve growth factor production and control bladder pain in interstitial cystitis. Urology. 2007 Sep;70(3):463-8.

572. Hu VY, Zvara P, Dattilio A, Redman TL, Allen SJ, Dawbarn D, et al. Decrease in bladder overactivity with REN1820 in rats with cyclophosphamide induced cystitis. J Urol. 2005 Mar;173(3):1016-21.

573. Tyagi P, Banerjee R, Basu S, Yoshimura N, Chancellor M, Huang L. Intravesical antisense therapy for cystitis using TAT-peptide nucleic acid conjugates. Mol Pharm. 2006 Jul-Aug;3(4):398-406.

574. Yoshimura N, de Groat WC. Neural control of the lower urinary tract. Int J Urol. 1997 Mar;4(2):111-25.

575. Brading AF, Turner WH. The unstable bladder: towards a common mechanism. Br J Urol. 1994 Jan;73(1):3-8.

576. Harrison SC, Ferguson DR, Doyle PT. Effect of bladder outflow obstruction on the innervation of the rabbit urinary bladder. Br J Urol. 1990 Oct;66(4):372-9.

577. Sibley GN. An experimental model of detrusor instability in the obstructed pig. Br J Urol. 1985 Jun;57(3):292-8.

578. Speakman MJ, Brading AF, Gilpin CJ, Dixon JS, Gilpin SA, Gosling JA. Bladder outflow obstruction—a cause of denervation supersensitivity. J Urol. 1987 Dec;138(6):1461-6.

579. German K, Bedwani J, Davies J, Brading AF, Stephenson TP. Physiological and morphometric studies into the pathophysiology of detrusor hyperreflexia in neuropathic patients. J Urol. 1995 May;153(5):1678-83.

580. Mills IW, Greenland JE, McMurray G, McCoy R, Ho KM, Noble JG, et al. Studies of the pathophysiology of idiopathic detrusor instability: the physiological properties of the detrusor smooth muscle and its pattern of innervation. J Urol. 2000 Feb;163(2):646-51.

581. Kinder RB, Mundy AR. Atropine blockade of nerve-mediated stimulation of the human detrusor. Br J Urol. 1985 Aug;57(4):418-21.

582. Turner WH, Brading AF. Smooth muscle of the bladder in the normal and the diseased state: pathophysiology, diagnosis and treatment. Pharmacol Ther. 1997 Aug;75(2):77-110.

583. Elbadawi A, Yalla SV, Resnick NM. Structural basis of geriatric voiding dysfunction. III. Detrusor overactivity. J Urol. 1993 Nov;150(5 Pt 2):1668-80.

584. Charlton RG, Morley AR, Chambers P, Gillespie JI. Focal changes in nerve, muscle and connective tissue in normal and unstable human bladder. BJU Int. 1999 Dec;84(9):953-60.

585. Williams JH, Turner WH, Sainsbury GM, Brading AF. Experimental model of bladder outflow tract obstruction in the guinea-pig. Br J Urol. 1993 May;71(5):543-54.

586. Brading AF. A myogenic basis for the overactive bladder. Urology. 1997 Dec;50(6A Suppl):57-67; discussion 8-73.

587. McMurray G, Casey JH, Naylor AM. Animal models in urological disease and sexual dysfunction. Br J Pharmacol. 2006 Feb;147 Suppl 2:S62-79.

588. Mitsui T, Kakizaki H, Matsuura S, Tanaka H, Yoshioka M, Koyanagi T. Chemical bladder irritation provokes c-fos expression in the midbrain periaqueductal gray matter of the rat. Brain Res. 2003 Mar 28;967(1-2):81-8.

589. Avelino A, Cruz F, Coimbra A. Intravesical resiniferatoxin desensitizes rat bladder sensory fibres without causing intense noxious excitation. A c-fos study. Eur J Pharmacol. 1999 Jul 28;378(1):17-22.

590. Steers WD, Kolbeck S, Creedon D, Tuttle JB. Nerve growth factor in the urinary bladder of the adult regulates neuronal form and function. J Clin Invest. 1991 Nov;88(5):1709-15.

591. Steers WD, Creedon DJ, Tuttle JB. Immunity to nerve growth factor prevents afferent plasticity following urinary bladder hypertrophy. J Urol. 1996 Jan;155(1):379-85.

592. Drake MJ, Hedlund P, Harvey IJ, Pandita RK, Andersson KE, Gillespie JI. Partial outlet obstruction enhances modular autonomous activity in the isolated rat bladder. J Urol. 2003 Jul;170(1):276-9.

593. Clemow DB, Steers WD, McCarty R, Tuttle JB. Altered regulation of bladder nerve growth factor and neurally mediated hyperactive voiding. Am J Physiol. 1998 Oct;275(4 Pt 2):R1279-86.

594. Clemow DB, McCarty R, Steers WD, Tuttle JB. Efferent and afferent neuronal hypertrophy associated with micturition pathways in spontaneously hypertensive rats. Neurourol Urodyn. 1997;16(4):293-303.

595. Lee K-S, Dean-McKinney T, Tuttle JB, Steers WD. Intrathecal antisense oligonucleotide against the tetrodotoxin-resistant sodium channel (Nav 1.8) reduces bladder hyperactivity in the spontaneously hypertensive rat. J Urol. 2002;167:38.

596. de Groat WC, Yoshimura N. Mechanisms underlying the recovery of lower urinary tract function following spinal cord injury. Prog Brain Res. 2006;152:59-84.

597. Yoshimura N, de Groat WC. Plasticity of Na+ channels in afferent neurones innervating rat urinary bladder following spinal cord injury. J Physiol. 1997 Sep 1;503 (Pt 2):269-76.

598. Yokoyama O, Yoshiyama M, Namiki M, de Groat WC. Role of the forebrain in bladder overactivity following cerebral infarction in the rat. Exp Neurol. 2000 Jun;163(2):469-76.

599. Yokoyama O, Mizuno H, Komatsu K, Akino H, Tanase K, Namiki M. Role of glutamate receptors in the development and maintenance of bladder overactivity after cerebral infarction in the rat. J Urol. 2004 Apr;171(4):1709-14.

600. Sakakibara R, Hattori T, Yasuda K, Yamanishi T. Micturitional disturbance after acute hemispheric stroke: analysis of the lesion site by CT and MRI. J Neurol Sci. 1996 Apr;137(1):47-56.

601. Ukimura O, Ushijima S, Honjo H, Iwata T, Suzuki K, Hirahara N, et al. Neuroselective current perception threshold evaluation of bladder mucosal sensory function. Eur Urol. 2004 Jan;45(1):70-6.

602. Kenton K, Lowenstein L, Simmons J, Brubaker L. Aging and overactive bladder may be associated with loss of urethral sensation in women. Neurourol Urodyn. 2007;26(7):981-4.

603. Geirsson G, Fall M, Lindstrom S. The ice-water test—a simple and valuable supplement to routine cystometry. Br J Urol. 1993 Jun;71(6):681-5.

604. Evans RJ. Intravesical therapy for overactive bladder. Curr Urol Rep. 2005 Nov;6(6):429-33.

605. Drake MJ, Harvey IJ, Gillespie JI, Van Duyl WA. Localized contractions in the normal human bladder and in urinary urgency. BJU Int. 2005 May;95(7):1002-5.

606. Torrisi G, Sampugnaro EG, Pappalardo EM, D'Urso E, Vecchio M, Mazza A. Postpartum urinary stress incontinence: analysis of the associated risk factors and neurophysiological tests. Minerva Ginecol. 2007 Oct;59(5):491-8.

607. Damaser MS, Broxton-King C, Ferguson C, Kim FJ, Kerns JM. Functional and neuroanatomical effects of vaginal distention and pudendal nerve crush in the female rat. J Urol. 2003 Sep;170(3):1027-31.

608. Lin YH, Liu G, Daneshgari F. A mouse model of simulated birth trauma induced stress urinary incontinence. Neurourol Urodyn. 2008;27(4):353-8.

609. Katofiasc MA, Nissen J, Audia JE, Thor KB. Comparison of the effects of serotonin selective, norepinephrine selective, and dual serotonin and norepinephrine reuptake inhibitors on lower urinary tract function in cats. Life Sci. 2002 Aug 2;71(11):1227-36.

610. Klarskov N, Scholfield D, Soma K, Darekar A, Mills I, Lose G. Evaluation of the sensitivity of urethral pressure reflectometry (UPR) and urethral pressure profilometry (UPP) to detect pharmacological augmentation of urethral pressure, using [s,s]-reboxetine. J Urol. 2008;179 Suppl(4):521-2.

611. Zinner NR, Scholfield D, Soma K, Darekar A, Grant L, Mills I. A Phase 2, 8-week, multicenter, randomized, double-blind, placebo controlled, parallel group study evaluating the efficacy, tolerability and safety of [s,s]-reboxetine (PNU-165442G) for stress urinary incontinence in women. J Urol. 2008;179 Suppl(4):569-70.

612. Greenland JE, Hvistendahl JJ, Andersen H, Jorgensen TM, McMurray G, Cortina-Borja M, et al. The effect of bladder outlet obstruction on tissue oxygen tension and blood flow in the pig bladder. BJU Int. 2000 Jun;85(9):1109-14.

613. Pessina F, McMurray G, Wiggin A, Brading AF. The effect of anoxia and glucose-free solutions on the contractile response of guinea-pig detrusor strips to intrinsic nerve stimulation and the application of excitatory agonists. J Urol. 1997 Jun;157(6):2375-80.

614. Barendrecht MM, Chichester P, Michel MC, Levin RM. Effect of short-term outlet obstruction on rat bladder nerve density and contractility. Auton Autacoid Pharmacol. 2007 Jan;27(1):47-53.

615. Rothrock NE, Lutgendorf SK, Hoffman A, Kreder KJ. Depressive symptoms and quality of life in patients with interstitial cystitis. J Urol. 2002 Apr;167(4):1763-7.

616. Clemens JQ, Meenan RT, O'Keeffe Rosetti MC, Kimes TA, Calhoun EA. Case-control study of medical comorbidities in women with interstitial cystitis. J Urol. 2008 Jun;179(6):2222-5.

617. Melville JL, Walker E, Katon W, Lentz G, Miller J, Fenner D. Prevalence of comorbid psychiatric illness and its impact on symptom perception, quality of life, and functional status in women with urinary incontinence. Am J Obstet Gynecol. 2002 Jul;187(1):80-7.

618. van der Vaart CH, Roovers JP, de Leeuw JR, Heintz AP. Association between urogenital symptoms and depression in community-dwelling women aged 20 to 70 years. Urology. 2007 Apr;69(4):691-6.

619. Clauw DJ, Schmidt M, Radulovic D, Singer A, Katz P, Bresette J. The relationship between fibromyalgia and interstitial cystitis. J Psychiatr Res. 1997 Jan-Feb;31(1):125-31.

620. Alagiri M, Chottiner S, Ratner V, Slade D, Hanno PM. Interstitial cystitis: unexplained associations with other chronic disease and pain syndromes. Urology. 1997 May;49(5A Suppl):52-7.

621. Brand K, Littlejohn G, Kristjanson L, Wisniewski S, Hassard T. The fibromyalgia bladder index. Clin Rheumatol. 2007 Dec;26(12):2097-103.

622. Fenton BW. Limbic associated pelvic pain: a hypothesis to explain the diagnostic relationships and features of patients with chronic pelvic pain. Med Hypotheses. 2007;69(2):282-6.

623. Holstege G, Bandler R, Saper CB, Holstege G, Bandler R, Saper CB. The Emotional Motor System. Amsterdam: Elsevier; 1996.

624. Craig AD. Interoception: The sense of the physiological condition of the body. Current Opinion in Neurobiology. 2003;13(4):500-5.

625. Vogt BA. Pain and emotion interactions in subregions of the cingulate gyrus. Nature Reviews Neuroscience. 2005;6(7):533-44.

626. Furness J. The Enteric Nervous System. Oxford: Blackwell; 2006.

627. Bharucha AE. Pelvic floor: anatomy and function. Neurogastroenterol Motil. 2006 Jul;18(7):507-19.

628. Cervero F, Belmonte C, Cervero F. Visceral nociceptors. Neurobiology of Nociceptors: Oxford University Press; 1996. p. 220-42.

629. Adelson DW, Wei JY, Kruger L. Warm-sensitive afferent splanchnic C-fiber units in vitro. Journal of Neurophysiology. 1997;77(6):2989-3002.

630. Grundy D, Al-Chaer ED, Aziz Q, Collins SM, Ke M, Tach, Y, et al. Fundamentals of neurogastroenterology: Basic science. Gastroenterology. 2006;130(5):1391-411.

631. Gershon MD. Nerves, reflexes, and the enteric nervous system: Pathogenesis of the irritable bowel syndrome. Journal of Clinical Gastroenterology. 2005;39(5 Suppl):S184-S93.

632. Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology. 2004;126(3):693-702.

633. Sato A, Schmidt RF. Somatosympathetic reflexes: Afferent fibers, central pathways, discharge characteristics. Physiological Reviews. 1973;53:916-47.

634. Valentino RJ, Miselis RR, Pavcovich LA. Pontine regulation of pelvic viscera: Pharmacological target for pelvic visceral dysfunction. Trends in Pharmacological Sciences. 1999;20:253-60.

635. Mayer EA, Naliboff BD, Craig AD. Neuroimaging of the brain-gut axis: From basic understanding to treatment of functional GI disorders. Gastroenterology. 2006;131(6):1925-42.

636. Craig AD. How do you feel? Interoception: The sense of the physiological condition of the body. Nature Reviews Neuroscience. 2002;3(8):655-66.

637. Johansen JP, Fields HL, Manning BH. The affective component of pain in rodents: Direct evidence for a contribution of the anterior cingulate cortex. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(14):8077-82.

638. Critchley HD, Corfield DR, Chandler MP, Mathias CJ, Dolan RJ. Cerebral correlates of autonomic cardiovascular arousal: A functional neuroimaging investigation in humans. Journal of Physiology. 2000;523(Pt 1):259-70.

639. Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar BK, et al. Human cingulate cortex and autonomic control: Converging neuroimaging and clinical evidence. Brain. 2003;126(Pt 10):2139-52.

640. Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC. Pain affect encoded in human anterior cingulate but not somatosensory cortex. Science. 1997;277:968-71.

641. Critchley HD. The human cortex responds to an interoceptive challenge. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(17):6333-4.

642. Lorenz J, Minoshima S, Casey KL. Keeping pain out of mind: the role of the dorsolateral prefrontal cortex in pain modulation. Brain. 2003;126(Pt 5):1079-91.

643. Lieberman MD, Jarcho JM, Berman S, Naliboff BD, Suyenobu BY, Mandelkern M, et al. The neural correlates of placebo effects: A disruption account. Neuroimage. 2004;22(1):447-55.

644. Darwin C. The Expression of the Emotions in Man and Animals. Chicago, IL The University of Chicago Press; 1965.

645. Ness TJ, Gebhart GF. Colorectal distension as a noxious visceral stimulus: Physiological and pharmacological characterization of pseudoaffective reflexes in the rat. Brain Research. 1988;450(1-2):153-69.

646. Craig AD. Forebrain emotional asymmetry: A neuroanatomical basis? Trends in Cognitive Sciences. 2005;9(12):566-71.

647. Gebhart GF. Descending modulation of pain. Neuroscience and Biobehavioral Reviews. 2004;27(8):729-37.

648. Porreca F, Ossipov MH, Gebhart GF. Chronic pain and medullary descending facilitation. Trends in Neurosciences. 2002;25(6):319-25.

649. Tracey I, Dunckley P. Importance of anti- and pro-nociceptive mechanisms in human disease. Gut. 2004;53(11):1553-5.

650. Foo H, Mason P. Brainstem modulation of pain during sleep and waking. Sleep Med Rev. 2003 Apr;7(2):145-54.

651. Mason P. Deconstructing endogenous pain modulations. Journal of Neurophysiology. 2005;94(3):1659-63.

652. Baez MA, Brink TS, Mason P. Roles for pain modulatory cells during micturition and continence. J Neurosci. 2005 Jan 12;25(2):384-94.

653. Mason P. Ventromedial medulla: pain modulation and beyond. J Comp Neurol. 2005 Dec 5;493(1):2-8.

654. Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. Nature Neuroscience. 2002;5(12):1319-26.

655. Petrovic P, Ingvar M. Imaging cognitive modulation of pain processing. Pain. 2002;95(1-2):1-5.

656. Villemure C, Bushnell MC. Cognitive modulation of pain: How do attention and emotion influence pain processing? Pain. 2002;95(3):195-9.

657. Price DD. Psychological and neural mechanisms of the affective dimension of pain. Science. 2000;288:1769-72.

658. Toogood JA, Barr AM, Stevens TK, Gati JS, Menon RS, Martin RE. Discrete functional contributions of cerebral cortical foci in voluntary swallowing: A functional magnetic resonance imaging (fMRI) "Go, No-Go" study. Experimental Brain Research. 2005;161(1):81-90.

659. Vandenbergh J, Dupont P, Fischler B, Bormans G, Persoons P, Janssens J, et al. Regional brain activation during proximal stomach distention in humans: A positron emission tomography study. Gastroenterology. 2005;128(3):564-73.

660. Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. Gut. 2004;53(11):1595-601.

661. Kern MK, Arndorfer RC, Hyde JS, Shaker R. Cerebral cortical representation of external anal sphincter contraction: Effect of effort. American Journal of Physiology - Gastrointestinal and Liver Physiology. 2004;286(2):G304-G11.

662. Naliboff BD, Berman S, Chang L, Derbyshire SWG,

Suyenobu B, Vogt BA, et al. Sex-related differences in IBS patients: Central processing of visceral stimuli. Gastroenterology. 2003;124(7):1738-47.

663. Hobson AR, Furlong PL, Worthen SF, Hillebrand A, Barnes GR, Singh KD, et al. Real-time imaging of human cortical activity evoked by painful esophageal stimulation. Gastroenterology. 2005;128(3):610-9.
664. Tracey I. Nociceptive processing in the human brain. Current Opinion in Neurobiology. 2005;15(4):478-87.
665. Apkarian AV, Sosa Y, Sonty S, Levy RM, Harden RN, Parrish TB, et al. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. Journal of Neuroscience. 2004;24(46):10410-5.
666. Gregory LJ, Yaguez L, Williams SC, Altmann C, Coen SJ, Ng V, et al. Cognitive modulation of the cerebral processing of human oesophageal sensation using functional magnetic resonance imaging. Gut. 2003;52(12):1671-7.
667. Naliboff BD, Derbyshire SWG, Munakata J, Berman S, Mandelkern M, Chang L, et al. Cerebral activation in irritable bowel syndrome patients and control subjects during rectosigmoid stimulation. Psychosomatic Medicine. 2001;63(3):365-75.
668. Porro CA, Cettolo V, Francescato MP, Baraldi P. Functional activity mapping of the mesial hemispheric wall during anticipation of pain. Neuroimage. 2003;19(4):1738-47.
669. Petrovic P, Kalso E, Petersson KM, Ingvar M. Placebo and opioid analgesia - imaging a shared neuronal network. Science. 2002;295(5560):1737-40.
670. Wager TD, Rilling JK, Smith EE, Sokolik A, Casey KL, Davidson RJ, et al. Placebo-induced changes in fMRI in the anticipation and experience of pain. Science. 2004;303(5661):1162-7.
671. Berman SM, Naliboff BD, Suyenobu B, Chang L, Bueller J, Ruby K, et al. Abnormal CNS response to anticipation of visceral distension in female patients with irritable bowel syndrome (IBS) an FMRI study. Gastroenterology. 2006;130(4 Suppl 2):A-78.
672. Yaguez L, Coen S, Gregory LJ, Amaro E, Jr., Altman C, Brammer MJ, et al. Brain response to visceral aversive conditioning: A functional magnetic resonance imaging study. Gastroenterology. 2005;128(7):1819-29.
673. Mayer EA, Craske MG, Naliboff BD. Depression, anxiety and the gastrointestinal system. Journal of Clinical Psychiatry. 2001;62(Suppl 8):28-36.
674. Naliboff BD, Berman S, Suyenobu B, Labus JS, Chang L, Stains J, et al. Longitudinal change in perceptual and brain activation response to visceral stimuli in irritable bowel syndrome patients. Gastroenterology. 2006;131(2):352-65.
675. Labus JS, Mayer EA, Berman SM, Suyenobu B, Chang L, Naliboff BD. Identification of brain networks associated with visceral perception and hypervigilance in patients with irritable bowel syndrome (IBS). Gastroenterology. 2006;130(4 Suppl 2):A-78.
676. Clauw DJ, Crofford LJ. Chronic widespread pain and fibromyalgia: What we know, and what we need to know. Best Practice & Research Clinical Rheumatology. 2003;17(4):685-701.
677. Munakata J, Naliboff B, Harraf F, Kodner A, Lembo T, Chang L, et al. Repetitive sigmoid stimulation induces rectal hyperalgesia in patients with irritable bowel syndrome. Gastroenterology. 1997;112(1):55-63.
678. Chang L, Naliboff BD, Labus JS, Schmulson M, Lee OY, Olivas TI, et al. Effect of sex on perception of rectosigmoid stimuli in irritable bowel syndrome. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2006;291:R277-R84.
679. Zambreanu L, Wise RG, Brooks JC, Iannetti GD, Tracey I. A role for the brainstem in central sensitisation in humans. Evidence from functional magnetic resonance imaging. Pain. 2005;114(3):397-407.
680. Petrovic P, Petersson KM, Hansson P, Ingvar M. Brainstem involvement in the initial response to pain. Neuroimage. 2004;22(2):995-1005.
681. Petrovic P, Dietrich T, Fransson P, Andersson J, Carlsson K, Ingvar M. Placebo in emotional processing — Induced expectations of anxiety relief activate a generalized modulatory network. Neuron. 2005;46(6):957-69.
682. Mayer EA, Berman S, Suyenobu B, Labus J, Mandelkern MA, Naliboff BD, et al. Differences in brain responses to visceral pain between patients with irritable bowel syndrome and ulcerative colitis. Pain. 2005;115(3):398-409.
683. Song GH, Venkatraman V, Ho KY, Chee MW, Yeoh KG, Wilder-Smith CH. Cortical effects of anticipation and endogenous modulation of visceral pain assessed by functional brain MRI in irritable bowel syndrome patients and healthy controls. Pain. 2006.