

CHAPTER 7

Committee 3

Neural Control

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ABBREVIATIONS

5-HT	5-hydroxytryptamine	MCP	Medial collateral pathway
Ach	Acetylcholine	MPG	Major pelvic ganglia
ADP	Adenosine Diphosphate	MPO	Medial preoptic region of the hypothalamus
APF	Antiproliferative factor	Nav1.8/Nav1.9	Voltage gated sodium channel 1.8/1.9
ASIA A	American Spinal Injuries Association group A in the classification	NDO	Neurogenic detrusor overactivity
ATP	Adenosine Triphosphate	NE	Norepinephrine
BCR	Bulbo-cavernosal reflex	NGF	Nerve Growth Factor (NT-1)
BDNF	Brain Derived Neurotrophic Factor	NK1,2,3	Neurokinin receptors Tyoes 1,2,3
BOO	Bladder Outlet Obstruction	NKA	Neurokinin A
bFGF	Fibroblast growth factor	NMDA	N-methyl D aspartate
CB-1/CB-2	Cannabinoid receptors ?	NO	Nitric oxide
CC	Central canal of the spinal cord	NOS	Nitric Oxide Synthase
ChAT	Choline acetyl transferase	NPY	Neruoepetide Y
CGRP	Calcitonin gene-related peptide	NT-1	Neurotrophin 1 (Nerve Growth Factor)
CMG	Cystometrogram	NT-3-4	Neurotrophins-3-4
CNEMG	Concentric needle electromyography	ORL1	Opiate receptor-like receptor 1
CNS	Central Nervous System	OP4	Opiate receptor class 4
cSCI	Complete supra-sacral spinal cord lesion	P2X	A type of purinergic receptor
CTB	Cholera toxin B	P2Y	A type of purinergic receptor
DBH	Beta hydroxylase	p75[NTR]	A receptor for NGF
DCM	Dorsal commissure	PACAP	Pituitary adenylate cyclase activating polypeptide
DCN	Dorsal Nerve of Clitoris	PAG	Periaqueductal Grey matter of the midbrain
DCV	Dense Cored Vesicles	PAR	Pudendo-Anal Reflex
DENS	Direct electrical nerve stimulation	PAR 1-4	Protease-Activated Receptors 1-4
DH	Dorsal Horn	PEA	Palmitoylethanolamide
DO	Detrusor Overactivity	PET	Positron emission tomography
DPN	Dorsal Nerve of Penis	PG	Prostaglandin
DRG	Dorsal Root Ganglion	PGE ₂	Prostaglandin E2
EFS	Electrical field stimulation	PGN	Parasympathetic preganglionic neurons
EFV	End fill volume of the bladder	PKC	PhosphoKinase C
EMG	Electromyography	PMC	Pontine micturition center
ENaC	Epithelial sodium channel	S1-S4	Sacral segments 1-4
ENK	Enkephalin	SCG	Sympathetic Chain Ganglia
EUS	External Urethral Sphincter	SCI	Spinal Cord Injury
FIC	Feline interstitial cystitis	iSCI	Incomplete Spinal Cord Injury
fMRI	Functional magnetic resonance imaging	SHR	Spontaneously Hypertensive Rat
GABA	Gamma-Amino-Butyric Acid	SIF	Small, intensely fluorescent cells
GABA-B	Gamma-Amino-Butyric Acid B-receptor	SM	Smooth Muscle
Gal	Galanin	Som	Somatostatin
GNDF	Glial derived neurotrophic factor	SP	Substance P
HGN	Hypogastric Nerve	SPN	Sacral parasympathetic nucleus
HO-2	Haem-oxygenase-2	TH	Tyrosine Hydroxylase
IC	Interstitial cystitis	TMS	Transcranial Magnetic Stimulation
ICCs	Interstitial cells of Cajal	TNP-ATP	A P2X antagonist (2',3'-O-trinitrophenyl-ATP)
IMG	Inferior Mesenteric Ganglion	trkB	A receptor for certain neurotrophins
I _A	A-type current	TRPV1	Vanilloid receptor
I _{Na}	Sodium current	TRPV2,4	Vanilloid receptor-like proteins
iNOS	Inducible Nitric Oxide Synthase	TTX	Tetrodotoxin
ISN	Inferior Splanchnic Nerves	TTX-R	Tetrodotoxin-resistant sodium channel
LCP	Lateral collateral pathway	TTX-S	Tetrodotoxin-sensitive sodium
LUT	Lower Urinary Tract	UTC	Urothelial Cells
M3	Muscarinic Receptor Type 3	UTP	Uridine triphosphate
MMHIS	Megacystis-microcolon-intestinal-hypoperistalsis syndrome	VACHT	Vesicular Acetylcholine Transferase
		VIP	Vasoactive Intestinal Polypeptide

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OUTLINE

This chapter starts with an introduction and overview before going into details on the individual components involved in the neural control of the bladder. This is to set the scene in terms of the relationships (a) between the peripheral innervation, the lower urinary tract and the spinal cord, and (b) the spinal cord, the pontine micturition centre and other higher structures in the CNS. This order reflects the overall layout of the material we have considered, and in both of these broad sections of the chapter there is reference to spinal cord injury.

First the chapter deals with the peripheral innervation and its relationships (anatomical, neurochemical, physiological, pharmacological, developmental, pathophysiological) with the urothelium and smooth and skeletal muscle. The processes of filling and voiding are considered in sequence, as are the afferent and efferent pathways and reflex connections. There has been significant new information since the last Consultation in a number of fields:

- the urothelium and its interactions with extrinsic nerves, and this area has received special attention.
- the role of mediators in the regulation of sensitivity of different type of sensory receptors
- the intramural plexuses, their relationships with smooth muscle, and intramural neural connections
- the functional integration of afferent, efferent and intramural mechanisms, culminating in the 'modular hypothesis'
- the effects of detrusor overactivity, bladder outlet obstruction, neuropathic change, ageing and inflammation

The next section is concerned with the functional relationships between the lower urinary tract (LUT) and spinal cord, and some reference is also made to interactions involving gastrointestinal and reproductive organs. The efferent pathways to the bladder and to the striated muscle sphincters and pelvic floor are considered, and the role of the spinal segments in mediating various reflexes and modulating afferent information are discussed. The importance of the monoaminergic pathways in these processes is given some prominence as these mechanisms are central to recent new therapies for stress incontinence. The anatomical and physiological basis of various clinical test of LUT function is included at this point.

There has been complete revision of the CNS section to allow for the important recent advances in PET scanning and functional magnetic resonance imaging that has had a major impact on our knowledge of the role of higher levels of the CNS on the regulation of the human LUT. These advances are laid out alongside the advances in animal neuroscience in this area.

Finally there is a section on spinal cord injury which offers some explanations as to the behaviour of the human spinal cord that is disconnected from these higher areas of CNS control. Modern methodologies of testing LUT neurological functions are again in evidence.

Neural Control

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INTRODUCTION

This chapter relies heavily on the reports of the first and second consultations publication [1,2]. Some of this work has been remodeled, reviewed, updated and extended; and the focus changed to reflect the altered title so that aspects of neural control of the bladder are the main emphasis. In addition there is increased emphasis on the study of the physiology and pathophysiology of humans. The focus of this report is predominantly on biological factors that contribute to the control of the normal and overactive bladder in humans and in animal models. This report attempts to make an integrated approach, looking at all the tissues involved, and the interactions between them: knowledge of the urothelium and its interaction with afferent nerves and the mediators involved have increased rapidly, and an extended section is devoted to this topic. It will be seen that the properties of smooth muscle and urothelium affect afferent and efferent neurones in the bladder wall, and the viability of these tissues affects reflex responses and sensation. New information is available on the ganglia present in the human bladder wall and paraurethral tissue, and on interstitial cells that have been found beneath the urothelium and around the smooth muscle bundles in the urethra and bladder.

The application of functional brain imaging methods to investigate bladder function is now being used in several different laboratories worldwide, and this is providing insight into human central nervous system control of the bladder. It is appropriate that those results should be considered in the section on central control. At the same time we have summarised and updated the previous material on neuroscience and linked it with cell biology and genetics in an attempt to produce a wider view against which the plasticity of cellular components of the lower urinary tract and

its innervation can be considered, particularly in relation to pathological disturbances.

Throughout this section we have considered pathophysiological conditions which may affect the component under discussion and explain what is known about the disordered function which may contribute to detrusor overactivity.

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 randomised 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, inadequate statistical support, 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.

A. OVERVIEW OF THE NEURAL CONTROL OF BLADDER STORAGE AND VOIDING

As indicated in Figure 1, the lower urinary tract is innervated by three sets of peripheral nerves: (1) pelvic parasympathetic nerves, which arise at the sacral level of the spinal cord, excite the bladder, and, in animals, relax the urethra, (2) lumbar sympathetic nerves, which inhibit the bladder body and excite the bladder base and urethra in animals, (3) pudendal nerves, which excite the external urethral sphincter and associated mechanisms in the pelvic floor [3-8]. These nerves contain afferent (sensory) axons as well as efferent pathways (**Table 1, Figure 1**).

Normal micturition is controlled by neural circuits in the spinal cord and brain that coordinate the activity of visceral smooth muscle in the urinary bladder and urethra with activity of striated muscle in the urethral sphincter [1,2,4-8 9,10]. The principal reflex components of these switching circuits are listed in Table 1 and illustrated in Figure 3. The circuits described have been worked out in animal models, and there may be some doubt as to whether the sympathetic component during filling and storage, and the ganglionic inhibition are present in humans. (**Figures 2 and 3**)

These circuits act as on-off switches to shift the lower urinary tract between two modes of operation: storage and elimination. In infants these switching mechanisms function purely in a reflex manner to produce involuntary voiding; however, in adults urine storage and release are subject to voluntary control as a result of connections between the forebrain and brainstem. Thus, the neural control of the urinary tract is distinct from that of most other visceral organs such as the heart, blood vessels and the intestine that are regulated exclusively by involuntary (autonomic) reflex mechanisms. Although there are some similarities between the control of the urinary tract and of the lower gastrointestinal tract, there are major differences in the mechanisms that give rise to urinary and faecal continence. Nevertheless for both systems, the regulation of striated and smooth muscle sphincters is dependent on afferent input and an ability conferred by higher levels of the central nervous system to postpone elimination, until the process is socially acceptable.

The reflex circuitry utilised by the micurition reflex in the adult includes motor nerves innervating the bladder via parasympathetic pre-ganglionic neurones originating in the sacral parasympathetic nucleus (SPN), and somatic motoneurones innervating the external urinary sphincter (EUS) which appear to have reciprocal activity compared with SPN efferent neurones. These neurones exist within the sacral cord of humans and cats, and in the lumbo-sacral cord of some other species.

Within the spinal cord, afferent neurones from the bladder and other segmentally innervated structures synapse on interneurones that mediate either local segmental connections with the motor pathways, or send their axons to the brain (**Fig.4**). These ascending neurones connect with structures in the brainstem, including the pons and periaqueductal grey matter of the midbrain to execute certain reflex functions, and with higher centres of the brain to mediate the conscious perception of sensations arising from the lower urinary tract or lower bowel. A centre of particular importance for micturition, but not for defaecation, resides in the pons, the pontine micturition centre (PMC). This is an important centre for integration and is also the origin of descending axons that communicate with the SPN and with the somatic motoneurones.

Higher brain centres are critical to delay voiding until it is socially convenient, a process achieved by inhibitory influences of the PMC arising from the prefrontal cortex, anterior cingulate gyrus and hypothalamus. The sensory information required on which to base behavioural decisions depends on conscious perception of the degree of bladder fullness. In the infant, these higher pathways are not yet functional, but social control of the bladder is gained as these connections within the brain develop over the course of childhood. These central pathways are not essential for the micturition reflex, but influence it and modulate its excitability as well as providing conscious perception of the bladder. They arise in the hypothalamus and within the forebrain, including the frontal cortex and cingulate gyrus (**Fig 5**). **Figure 6** summarises the possible connections between the forebrain and brainstem.

I. FILLING

Intravesical pressure measurements during bladder filling in both humans and animals reveal low and

Table 1. The afferents and efferent pathways and the central organisation for reflexes concerned with urine storage and for voiding.

Afferent Pathway	Efferent Pathway	Central Pathway
Urine storage		
Low level vesical afferent activity (pelvic nerve)	<ol style="list-style-type: none"> 1. External sphincter contraction (somatic nerves) 2. Internal sphincter contraction (sympathetic nerves) 3. Detrusor inhibition (sympathetic nerves) 4. Ganglionic inhibition (sympathetic nerves) 5. Sacral parasympathetic outflow inactive 	Spinal reflexes
Micturition		
High level vesical afferent activity (pelvic nerve)	<ol style="list-style-type: none"> 1. Inhibition of external sphincter activity 2. Inhibition of sympathetic outflow Reflexes 3. Activation of parasympathetic outflow to the bladder 4. Activation of parasympathetic outflow to the urethra 	Spinobulbospinal
		Spinal Reflex

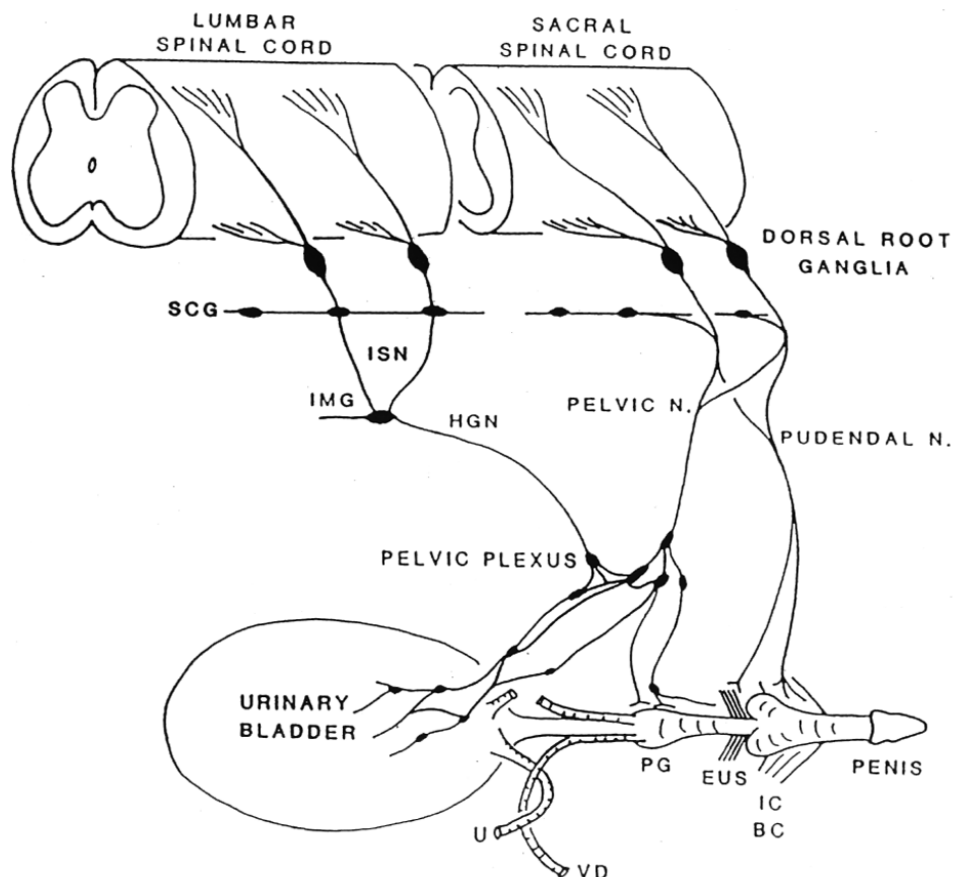


Figure 1 . Diagram showing the sympathetic, parasympathetic and somatic innervation of the urogenital tract of the male cat. Sympathetic preganglionic pathways emerge from the lumbar spinal cord and pass to the sympathetic chain ganglia (SCG) and then via the inferior splanchnic nerves (ISN) to the inferior mesenteric ganglia (IMG). Preganglionic and postganglionic sympathetic axons then travel in the hypogastric nerve (HGN) to the pelvic plexus and the urogenital organs. Parasympathetic preganglionic axons which originate in the sacral spinal cord pass in the pelvic nerve to ganglion cells in the pelvic plexus and to distal ganglia in the organs. Sacral somatic pathways are contained in the pudendal nerve, which provides an innervation to the penis, the ischiocavernosus (IC), bulbocavernosus (BC) and external urethral sphincter (EUS) muscles. The pudendal and pelvic nerves also receive postganglionic axons from the caudal sympathetic chain ganglia. These three sets of nerves contain afferent axons from the lumbosacral dorsal root ganglia. Abbreviations: ureter (U), prostate gland (PG), vas deferens (VD).

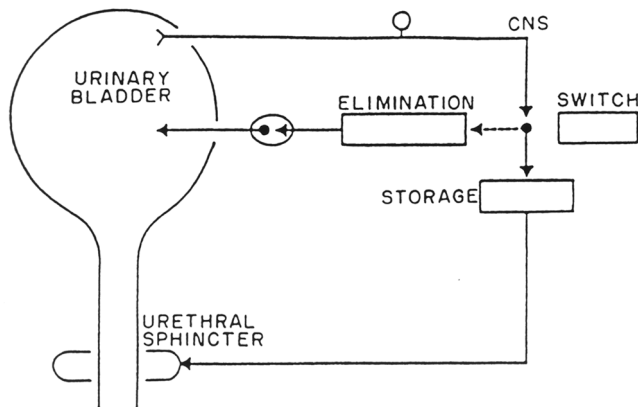
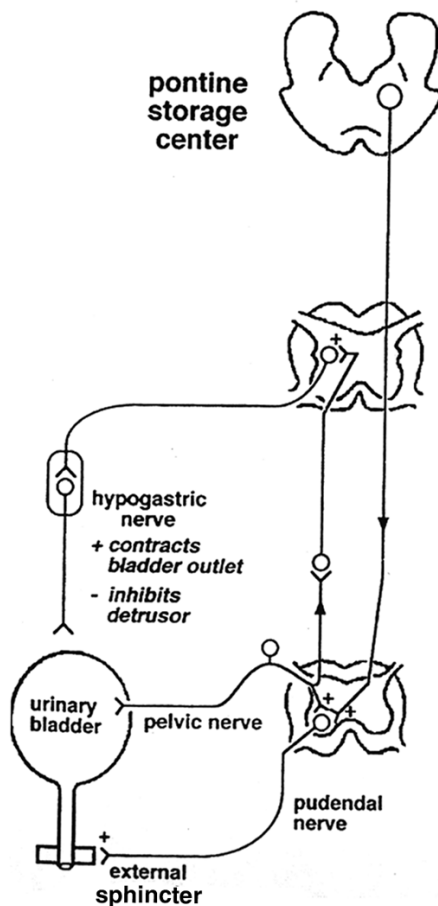


Figure 2. Diagram illustrating the anatomy of the lower urinary tract and the switchlike function of the micturition reflex pathway. During urine storage, a low level of afferent activity activates efferent input to the urethral sphincter. A high level of afferent activity induced by bladder distention activates the switching circuit in the central nervous system (CNS), producing firing in the efferent pathways to the bladder, inhibition of the efferent outflow to the sphincter, and urine elimination.

A: storage reflexes



B: voiding reflexes

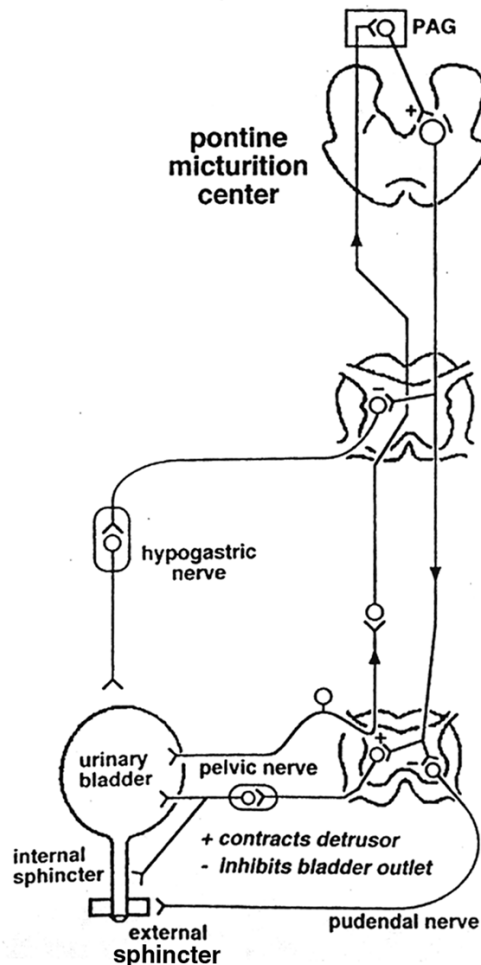


Figure 3. Diagram showing neural circuits controlling continence and micturition. (A) Urine storage reflexes. During the storage of urine, distention of the bladder produces low level vesical afferent firing, which in turn stimulates (1) the sympathetic outflow to the bladder outlet (base and urethra) and (2) pudendal outflow to the external urethral sphincter. These responses occur by spinal reflex pathways and represent «guarding reflexes», which promote continence. Sympathetic firing also inhibits detrusor muscle and modulates transmission in bladder ganglia. A region in the rostral pons (the pontine storage center or «L» region) increases external urethral sphincter activity. (B) Voiding reflexes. During elimination of urine, intense bladder afferent firing activates spinobulbospinal reflex pathways passing through the pontine micturition center, which stimulate the parasympathetic outflow to the bladder and internal sphincter smooth muscle and inhibit the sympathetic and pudendal outflow to the urethral outlet. Ascending afferent input from the spinal cord may pass through relay neurons in the periaqueductal gray (PAG) before reaching the pontine micturition center.

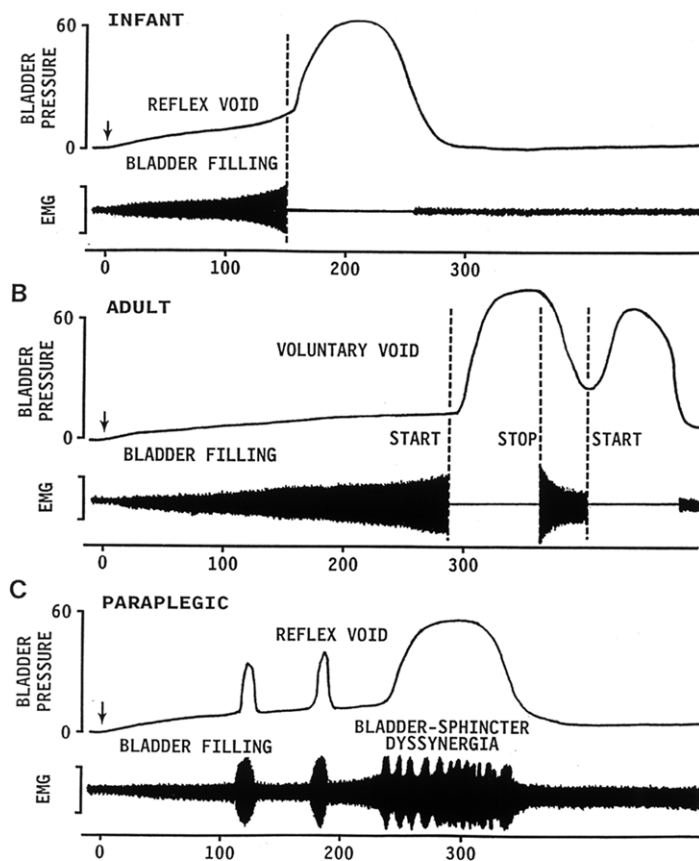


Figure 4. Combined cystometrograms and sphincter electromyograms (EMG) comparing reflex voiding responses in an infant (A) and in a paraplegic patient (C) with a voluntary voiding response in an adult (B). The abscissa in all records represents bladder volume in milliliters and the ordinates represent bladder pressure in cm H₂O and electrical activity of the EMG recording. On the left side of each trace the arrows indicate the start of a slow infusion of fluid into the bladder (bladder filling). Vertical dashed lines indicate the start of sphincter relaxation which precedes by a few seconds the bladder contraction in A and B. In part B note that a voluntary cessation of voiding (stop) is associated with an initial increase in sphincter EMG followed by a reciprocal relaxation of the bladder. A resumption of voiding is again associated with sphincter relaxation and a delayed increase in bladder pressure. On the other hand, in the paraplegic patient (C) the reciprocal relationship between bladder and sphincter is abolished. During bladder filling, transient uninhibited bladder contractions occur in association with sphincter activity. Further filling leads to more prolonged and simultaneous contractions of the bladder and sphincter (bladder-sphincter dyssynergia). Loss of the reciprocal relationship between bladder and sphincter in paraplegic patients interferes with bladder emptying.

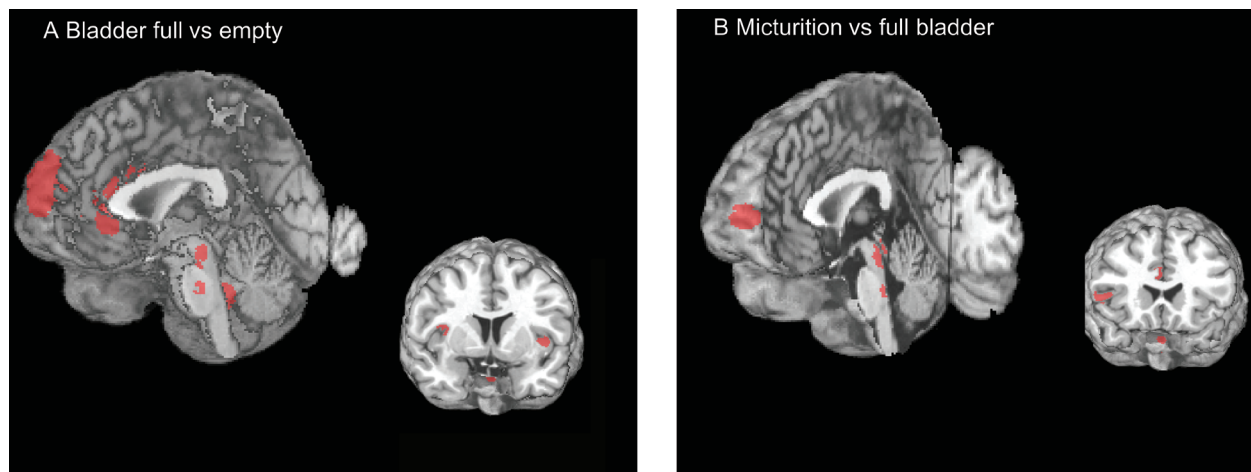


Figure 5. These images were created using MRICro software (www.mricro.com) based on the co-ordinates given in published papers (Blok et al, 1997, Blok et al, 1998, Nour et al., 2000, Athwal 2001, Matsuura et al., 2002) (A) showing sites of activation when the condition of an empty bladder was compared with bladder filling and (B) the condition of a full bladder was compared with activation during micturition (Blok et al, 1997, Blok et al, 1998). Courtesy of Dr Rajesh Kavia.

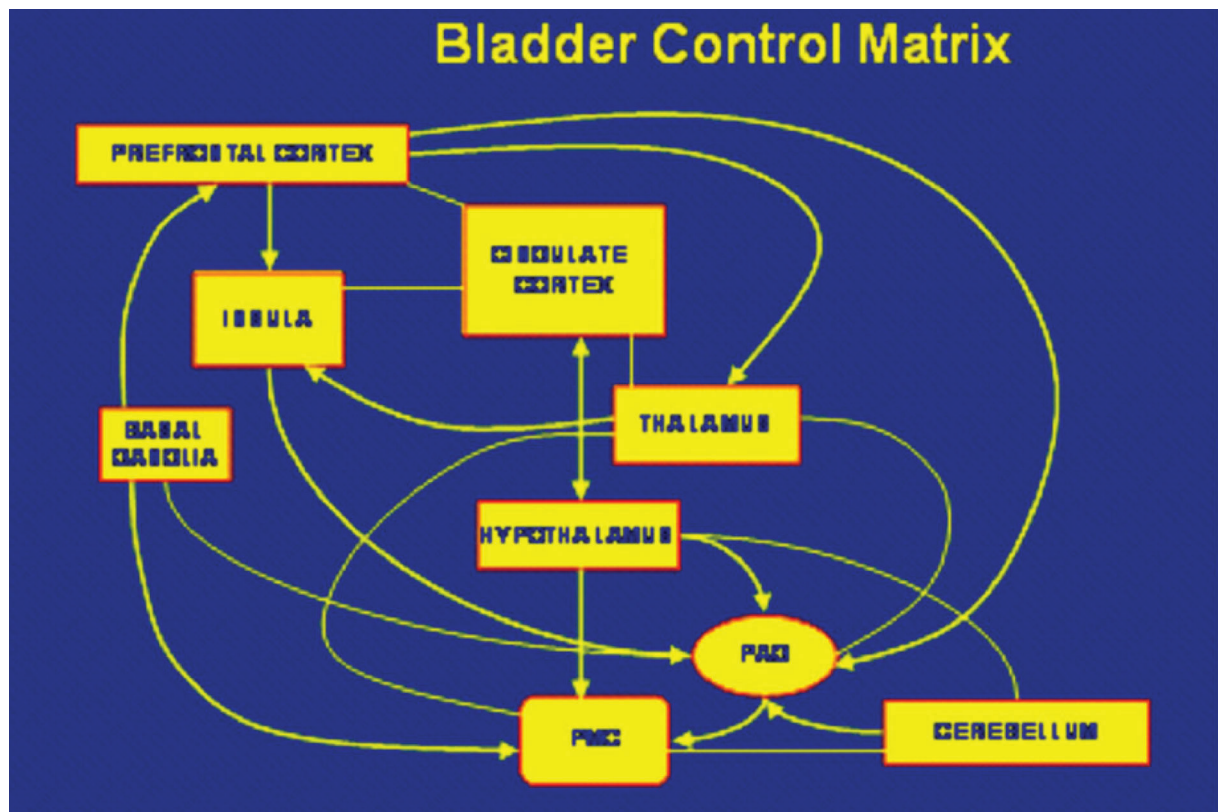


Figure 6. Diagram of the possible connections between various forebrain and brainstem structures involved in the control of the bladder and sphincters in humans. The diagram is adapted from Jones et al (2003)(Jones et al, 2003) and based on work from other groups (Blok et al, 1998, Jones, Kulkarni and Derbyshire, 2003, Holstege, 1987, Holstege et al, 1985, Vilensky and Van Hoesen, 1981, An et al, 1998, Ongur et al, 1998, Dietrichs and Haines, 2002, Critchley et al, 2003)

relatively constant bladder pressures when bladder volume is below the threshold for inducing voiding (Fig. 4). The accommodation of the bladder to increasing volumes of urine is dependent on the intrinsic properties of the vesical smooth muscle and the quiescence of the excitatory parasympathetic efferent pathway, and in some species the involvement of sympathetic nerves, and inhibitory parasympathetic pathways [4,5 6]. In addition in some species urine storage is also facilitated by sympathetic reflexes that mediate an inhibition of bladder activity, closure of the bladder neck and contraction of the proximal part of the urethra. During bladder filling the activity of the sphincter electromyogram (EMG) also increases (Fig. 4) reflecting an increase in efferent firing in the pudendal nerve and an increase in outlet resistance which contributes to the maintenance of urinary continence. Motoneurons of the external urethral sphincter (EUS) are located in a region of the sacral ventral horn in or adjacent to the nucleus of Onuf, just medial to the motoneurons of the hindlimb and lateral to those of the trunk and axial musculature.

II. VOIDING

In health the micturition pathway is switched on five to seven times per day, transiently arresting the tonic contraction of the pelvic floor necessary for urinary continence. The storage phase of the urinary bladder can be switched to the voiding phase either involuntarily (reflexly) or voluntarily (Fig 7). The former is readily demonstrated in the human infant or in the anesthetized animal when the volume of urine exceeds the micturition threshold. At this point increased afferent firing from tension receptors in the bladder reverses the pattern of efferent outflow, producing firing in the sacral parasympathetic pathways and inhibition of sympathetic and somatic pathways. The expulsion phase consists of an initial relaxation of the urethral sphincter (Fig 4) followed in a few seconds by a contraction of the bladder, an increase in bladder pressure and the flow of urine. Relaxation of the urethral smooth muscle is mediated in some animal species by activation of a parasympathetic pathway to the urethra that triggers the release of

nitric oxide, an inhibitory transmitter [11 12] and by removal of adrenergic and somatic cholinergic excitatory inputs to the urethra. Secondary reflexes elicited by flow of urine through the urethra facilitate bladder emptying [4,6,13]. These reflexes require the integrative action of neuronal populations at various levels of the neuraxis. Neural structures responsible for micturition and urinary continence are located in the rostral brainstem in all species studied including man. These have a modulating effect on other reflexes such as those mediating excitatory outflow to the sphincters and sympathetic inhibitory outflow to the bladder which are organized at the spinal level (Fig 3A). The parasympathetic outflow to the detrusor has a more complicated central organization involving spinal and spino-bulbo-spinal pathways (Fig 3B).

Interruption of the descending motor fibers from the pons to the sacral cord, for example in a transected spinal cord, abolishes normal micturition and after about 6 weeks in man, results in reflex incontinence with detrusor-sphincter dyssynergia. The duration of spinal shock differs considerable between different species. Such lesions can cause disruption of the voluntary control of micturition causing the re-emergence of reflex micturition, resulting in detrusor overactivity and incontinence (**Fig. 7**) [4-7]. Because of the complexity of the central nervous control of the lower urinary tract, incontinence can occur as a result of a variety of neurological disorders as well as changes in the peripheral innervation and smooth and skeletal muscle components. Patients with brain lesions rostral to the pons do not show detrusor-sphincter dyssynergia. However, these patients may suffer from urge incontinence, i.e. detrusor overactivity and an inability to delay voiding at an appropriate place and time.

B. SENSOR AND TRANSDUCER FUNCTIONS OF THE UROTHELIUM

The urinary bladder lumen is lined with a highly specialized tissue described as transitional epithelium composed of at least three layers [6,14,15]. These consist of an innermost basal cell layer attached to a basement membrane, an intermediate layer consisting of larger cells (containing cytoplasmic vesicles) and a superficial apical layer consisting of large hexagonal cells (umbrella cells) having an asymme-

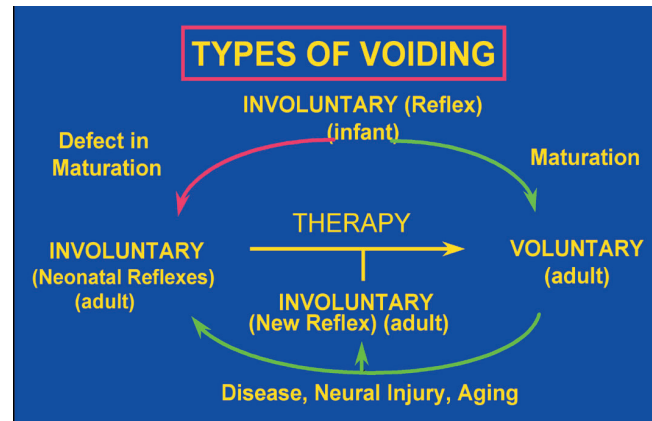


Figure 7. Influence of postnatal maturation and pathology on voiding function. In infants, voiding is initiated and coordinated by reflex circuits. In older children and adults after maturation of central neural pathways voiding is controlled voluntarily by neural circuitry located in higher centers in the brain. A defect in neural maturation (red arrow) can allow involuntary voiding to persist in adults. Diseases, neural injury or aging can disrupt the central neural pathways mediating voluntary control of micturition and lead to the reemergence of primitive reflex mechanisms that were present in the infant or that appear as the result of synaptic remodeling and the formation of new reflex circuitry. The goal of therapy is to reverse pathology-induced involuntary micturition and to reestablish normal voluntary control of voiding.

tric membrane [16]. Changes in umbrella cell surface area during bladder filling (due to increased fusion of intracellular vesicles with the cell membrane) allow the bladder to expand its epithelial surface area as urine accumulates [17]. It has been suggested that voiding triggers endocytosis in the umbrella cells, which restores the surface area [18].

I. ALTERATIONS IN UROTHELIAL BARRIER/ CELL-CELL SIGNALING MECHANISMS

The bladder urothelium functions as an effective barrier with a low permeability to H₂O, urea, ammonia and H⁺ [19-23]. Though the urothelium maintains a tight barrier to ion and solute flux, factors such as tissue pH, mechanical or chemical trauma, or bacterial infection can modulate this barrier function of the epithelium [16]. This loss of epithelial integrity following inflammation or injury could result in passage of toxic and irritating urinary constituents through the epithelium, thereby leading to changes in properties of sensory pathways. For example, alterations in bladder epithelium have been demonstrated in cats

with an interstitial cystitis – like condition (feline interstitial cystitis, FIC) [24-28], a syndrome characterized by urinary urgency, frequency and bladder pain upon filling, an innocuous stimulus. Disruption of epithelial integrity during inflammation or in IC may be due to release of a number substances such as antiproliferative factor (APF), which inhibits the proliferation of bladder epithelial cells and may adversely affect barrier function [29,30]. Alterations in release of these mediators/transmitters from the urothelium (“transducer function”) may have an influence on both urothelial integrity, as well as cell-cell signaling. Inflammation, injury or FIC, all of which increase endogenously generated levels of mediators such as nitric oxide (NO) as well as increase expression of inducible NOS (iNOS), increase permeability to water/urea in addition to producing ultrastructural changes in the apical layer [24,31]. Injury or inflammation also can lead to increased release of stress hormones (adrenal steroids and norepinephrine) which can induce a breakdown of the blood-urine barrier by disruption of the tight junctions followed by detachment and desquamation of viable urothelial cells. [31,32] Recent evidence has shown that acute injury (spinal cord transection) alters urothelial barrier function (ultrastructural changes accompanied by increased permeability). [33] These effects can be blocked by pretreatment with a ganglionic blocker, suggesting that either increased sympathetic efferent outflow, and/or increased circulating catecholamines in acute injury/inflammation may lead to urothelial dysfunction. Thus, loss of epithelial integrity following inflammation/injury could result in passage of toxic and irritating urinary constituents through the epithelium thereby leading to changes in properties of sensory pathways.

Moreover, injury or inflammation may also alter the response of urothelial cells to nociceptive/non-nociceptive stimuli. This sensitization is triggered in part by extracellular inflammatory mediators (ATP, NO, NGF, histamine, serotonin, adenosine, PGE₂) released by sensory neurons as well as non-neuronal cells (urothelial cells, fibroblasts, mast cells) in the environment. An important component of this inflammatory response is ATP release from various cell types, which can initiate painful sensations by exciting purinergic (P2X) receptors on sensory fibers. [34] Recently it has been shown that ATP can potentiate the response of vanilloids (by lowering the threshold for protons, capsaicin and heat) in a PKC dependent manner [35]. This represents a novel mechanism through which the large amounts of ATP released from damaged/sensitized cells in response to injury/inflammation might trigger the sensation of pain.

This has clinical significance, as ATP release from urothelial cells during bladder distention is significantly augmented in both animal models and in patients with interstitial cystitis (IC) [27,36,37]. Thus, alterations in ATP (or other transmitter) release could have profound impact on bladder nerves, urothelial cells, myofibroblasts as well as detrusor smooth muscle to cause detrusor overactivity. In addition, it has been demonstrated that inflammation or injury increase endogenous nerve growth factor (NGF) in the target organ, and NGF may be the link between tissue damage and hyperalgesic responses. Evidence in the FIC cat has demonstrated that both urothelium and smooth muscle express significantly larger amounts of NGF as compared to normal cat bladder [38]. NGF, acting through PKC mediated phosphorylation, can regulate the expression or activity of certain ion channels (eg., TRPV1) and may contribute to inflammation or injury induced hypersensitivity [39]. Thus, these studies also suggest that the most effective site for therapeutic intervention may not only be the cell surface receptors but also the intracellular second messengers.

II. THE POLYMODAL CHARACTER OF UROTHELIAL CELLS: DETECTORS MECHANICAL/ THERMAL/ CHEMICAL STIMULI

The urothelium may also play a role in intercellular signaling in the LUT. Urothelial cells and sensory neurons exhibit common properties including expression of ion channels / receptors (“sensor molecules”) associated with nociceptors or nociceptive functions (TRPV1; TRPV2; bradykinin; purinergic P2X) [40-42] as well as muscarinic [43], SP (NK1) [44] and protease-activated receptors or PAR 1,2,3 and 4 [45]. In addition, their involvement in the release of chemical mediators (NO, ATP; ACh; SP; PG) (“transducer” function) suggests that urothelial cells exhibit specialized sensory and signaling properties that could allow them to engage in reciprocal communication with neighboring urothelial cells as well as nerves in the bladder wall. Examples of chemical and mechanical stimuli and the associated receptors/channels in urothelium, as well as in sensory neurons, are depicted in **Table 2**.

For example, TRPV1, an ion channel protein expressed by nociceptive primary afferent neurons is activated by vanilloid compounds, acid (pH <6) and heat

Table 2 . The table shows a comparison of the properties of urothelial cells and of sensory neurones innervating the bladder to a variety of stimuli. For each stimulus(left), the receptor mechanism which mediates the response is indicated, and it can be seen that there is a considerable similarity between the mediators and receptors that influence these two types of cell.

Sensitivity/ Threshold	Conduction Velocity	Normal Stimulus	Peptides Mediators	Inflammatory	P2X3 agonist
Low Threshold	Mainly A delta (finely myelinated)	Distension and Contraction	Peptides are relatively sparse	Sensitization	Increased firing rate
High Threshold	Mainly C (unmyelinated)	Distension; Some also respond to contraction	Many peptides	Sensitization	Increased firing rate, lower threshold
Silent (including Nociceptive)	Mainly C (unmyelinated)	Insensitive to Distension unless inflamed	Many Peptides	Appearance of Mechano- sensitivity	Appearance of Mechano- sensitivity

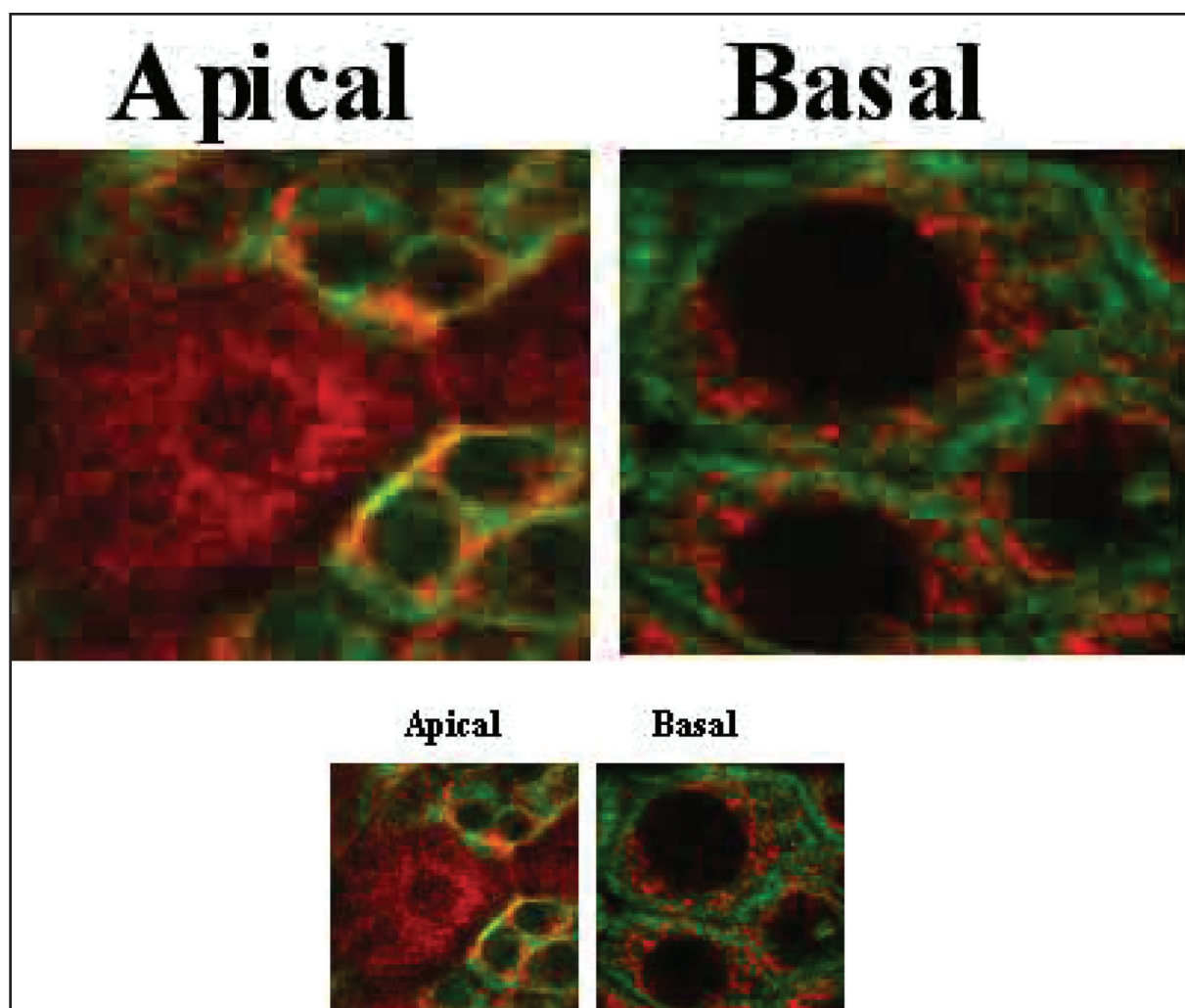


Figure 8. TRPV1 expression in urothelial cells of the urinary bladder from the rat. Confocal images of bladder urothelium in bladder whole mounts stained for TRPV1 (cy3, red) and cytokeratin 17 (FITC, green), a marker for basal urothelium. Diffuse cytoplasmic pattern of TRPV1 staining can be seen in both the apical (left panel) and underlying urothelial layers (nuclei are unstained). Right, enlarged image of basal urothelial cells expressing TRPV1 (cy3, red) and cytokeratin (FITC, green).

(>43°C). Recent data demonstrating a TRPV1 receptor in urothelial cells sensitive to capsaicin and protons, suggest that TRPV1 and other sensor molecules in non-neuronal cells may have a role in sensory mechanisms in the urinary bladder (**Figure 8**) [41]. In addition, these epithelial cells may express ion channels similar to stretch activated (mechanosensitive) channels in nervous tissue and these channels may play a role in mechanotransduction in the lower urinary tract (LUT). The epithelial sodium channel (ENaC) has been implicated in several processes including transduction of mechanical and nociceptive stimuli [46]. TRPV4 is a nonselective cation channel that is sensitive to extracellular osmolarity that has been recently found to be highly expressed in urothelial cells [47]. TRPV4 belongs to a family of ion channels that also include the recently cloned vanilloid receptor (TRPV1) and the vanilloid receptor-like protein (TRPV2). Osmotic stress, which activates TRPV4, releases more ATP from FIC cat urothelial cells as compared to normal. [36]. Mechanosensitive release of ATP from the urothelium has a number of consequences, such as activation of P2X or P2Y receptors on bladder nerves, or promotion of autocrine activation of P2Y receptors on urothelial cells. TRP channels (TRPV1) and P2X purinoceptors (P2X₂ or P2X₃) may play a role in mechanotransduction, as studies have shown that TRPV1 as well as P2X₂ or P2X₃ null mice exhibit significant deficits in stretch-evoked bladder responses [48]. These data indicate that urothelial cells display a number of properties similar to neurons (nociceptors/mechanoreceptors) and illustrate that both types of cells use a diversity of signal-transduction mechanisms to detect physiological stimuli.

III. THE UROTHELIAL-NEURAL INTERFACE

The urothelium may be actively engaged in communicating with bladder nerves, urothelial cells, smooth muscle or even cells of the immune and inflammatory systems. A number of studies have shown that afferent axons are located at the base and in close proximity to basal urothelial cells. [40,49] Urothelial cells can also release a variety of products, such as ATP, prostaglandins and nitric oxide, which can alter excitability of bladder nerves. In turn, nociceptor activation releases a number of factors, such as substance P that can activate urothelial cells [50]. This type of bidirectional chemical arrangement suggests these cells may be targets for transmitter relea-

se from bladder nerves or that chemicals released by urothelial cells may alter afferent excitability (**figure 9**). In support of this idea is evidence that ATP (released from urothelial cells during stretch) can activate a population of subepithelial bladder afferents expressing P2X₃ receptors [51]. Moreover, P2X₃ deficient mice exhibit a urinary bladder hyporeflexia, suggesting that this receptor and neural-epithelial interactions are essential for normal bladder function [52]. Thus, it is possible that activation of bladder nerves and urothelial cells can modulate bladder function directly or indirectly via the release of chemical factors in the urothelial layer. This association of epithelial cells with underlying afferent nerves is not unique to the bladder, as a similar relationship exists between hair cells of the inner ear and auditory fibers [53] as well as epithelial taste cells which are in contact with afferent fibers of the gustatory pathway [54]. These and other studies support the view that bladder urothelium serves a sensory role via expression of a number of sensor molecules and release of transmitters (NO, ATP; PG; ACh). (Figure 9)

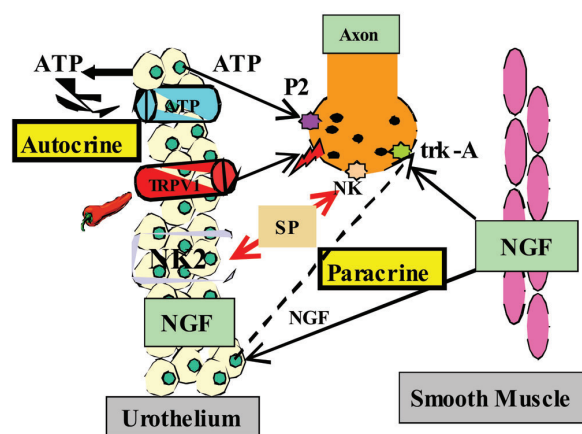


Figure 9. Hypothetical model depicting possible interactions between bladder afferents, urothelial cells (UTC) and smooth muscle (SM). Stimulation of receptor/channels on UTC can release mediators and target bladder nerves; UTC may be targets for transmitters released from nerves or smooth muscle. UTC can be activated by either autocrine (ie. autoregulation) or paracrine (release from nearby nerves or SM) mechanisms.

Recently a layer of cells that have characteristics of myofibroblasts has been described that lie in the sub-urothelial space and make close contact with nerves, that have characteristics of myofibroblasts [55,56]. These cells stain intensively for vimentin and the gap junction protein connexin [43] and furthermore gap junctions can be observed under the electron microscope. The latter observation implies that these cells

can form a functional syncytium. These cells can be isolated from the surrounding tissue and can respond to ATP by generating a transient increase of intracellular Ca^{2+} and generate spontaneous depolarizations [57]. ATP binds to P2Y receptors to initiate these responses [58] as they can be mimicked by ADP and UTP, and thus represents a separate mechanism to the P2X₃ activation of afferents. The rise of intracellular Ca^{2+} initiates a Ca^{2+} -activated Cl^- current, that reverses at about -25 mV and thus is inward, and hence depolarizing, at the resting potential of about -60 mV [58]. Thus exposure to ATP will secondarily generate depolarization that could propagate through the network forming an intermediate stage in sensations of bladder filling. ATP released from the urothelium would activate these cells to generate a feed-forward action on afferents, or a feed-back action on the urothelium. Either way they have the capacity to modulate bladder sensations. The hypothesis is given added interest by the fact that many nerve fibres terminate on these cells [59], thus providing a means to modulate the sensitivity of the bladder filling sensations. Understanding the mechanisms contributing to and maintaining these types of urothelial cell-cell interactions may provide important insight into development of novel targets for clinical management of a number of bladder disorders.

C. PERIPHERAL NERVES THAT INNERVATE THE LOWER URINARY TRACT

I. AFFERENT NEURONES

1. PROPERTIES OF BLADDER AFFERENT NEURONS.

Afferent axons in the pelvic, hypogastric and pudendal nerves transmit information from the lower urinary tract to the lumbosacral spinal cord [60, 61, 4] 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 large 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' [62] because they are excited by bladder wall tension caused either by distension or by contraction, and neurones with this range of conduction velocities are less likely to contain peptides. These 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 spino-bulbosinal 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 [5]. It is not clear whether they also contribute to normal voiding. However in the rat, C-fibre afferents control the micturition reflex in anaesthetised animals. 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. They 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 blad-

der 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 [56]. 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 3**).

Table 3 : Properties of bladder afferents

Stimuli	Urothelium	Sensory Neurones
ATP	P2X/P2Y	P2X/P2Y
Capsaicin	TRPV1	TRPV1
Heat	TRPV1; TRPV2; TRPV4	TRPV1; TRPV2; TRPV4
Cold	TRPM8; TRPA1	TRPM8; TRPA1
H ⁺	TRPV1	TRPV1; ASIC; DRASIC
Resiniferatoxin	TRPV1	TRPV1
Osmolality	in part TRPV4	in part TRPV4
Substance P	NK1; NK2	NK1; NK2; NK3
CGRP	CGRP	CGRP
Bradykinin	B1;B2	B1;B2

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 became 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 had 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 [63]. 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 [64-67]. There is also recent evidence that nerves cross the basal lamina and enter the basal layers of the human urothelium [68]. (**Figure 10**)

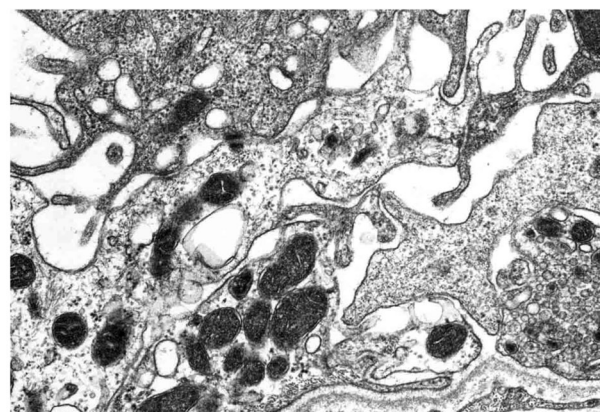


Figure 10. Naked axonal varicosities containing both CV and DCV were occasionally observed to penetrate the epithelial basal lamina to lie between the basal processes of the epithelial cells. (from Wiseman et al, 2002)

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 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 (see **Figure 11**).

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.

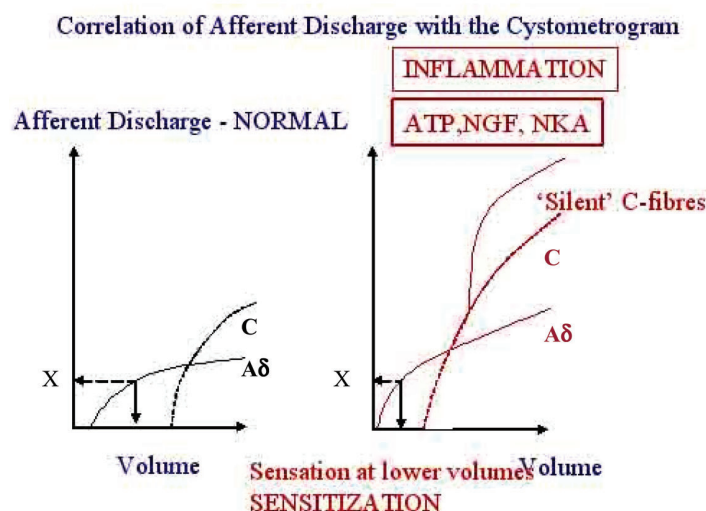


Figure 11. Correlation of afferent discharge with the cystometrogram. Left: Diagram of afferent discharge at different volumes for (A) A-delta fibres, and (B) mechanosensitive C-fibres. Right: Diagram showing the increase responses when the afferent endings are sensitized by inflammation or by administration 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. Note that the afferent discharge at point X is the same in both diagrams, but the effect of sensitization is that the sensory message occurs at a lower volume, throughout the full range of activities.

4. ROLE OF ATP AND P2X3 RECEPTORS

Recent studies of mice have shown the P2X2/3 receptor, is present in small sensory neurones inner-

vating 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 [69,70]. 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.

All groups of bladder afferents (low and high threshold as well as 'silent' 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 have been studied intensively in the rat and mouse using protocols designed to avoid sensitization of the afferents [71-74]. 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 [73] 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.

5. ROLE OF 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 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 [75].

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 [76]. 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 [77], 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 [78], 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 [79,80]. 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 [81]. 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 [82], 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 [83].

6. 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 analgesic 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 [84], (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 [85-88], 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 [89-90]. More recently it has been shown that rat dorsal root ganglion neurones are excited by NK2 agonists, but are inhibited by NK-3 agonists [91]. 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.

7. ROLE OF VANILLOID (TRPV1) AND OPIATE RECEPTOR-LIKE (ORL1/OP4) RECEPTORS

Mention has already been made of the actions of capsaicin on the urothelium and nerves. The release of NO and the increase in intracellular Ca^{2+} induced by capsaicin are blocked by the TRPV1 antagonist capsazepine. 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 [92,93] and may act on peripheral perivascular sensory terminals in a manner that is antagonised by the capsaicin antagonist capsazepine. 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 [94]. Both anandamide and PEA have been found to attenuate bladder hyper-reflexia induced by intravesical NGF [95-97].

The TRPV1 (capsaicin) receptor is a cation channel expressed by nociceptive neurones and can also be activated by protons or temperature greater than 43 degrees C [98,99]. 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). Stimulation of the TRPV1 receptor with capsaicin causes the release of CGRP [100].

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 [101]. Nociceptin produced 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 [101]. 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.

In humans nociceptin elicits a strong acute inhibitory effect on the micturition reflex in patients with a neurogenic bladder [102]. 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 from the bladder to high pressure distension of the urinary bladder [103]. 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.

8. ROLE OF NEUROTROPHINS

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 [104], interstitial cystitis and bladder cancer [105], in rats with inflamed bladders [106], spinal cord injury or chemically induced cystitis [107] or bladder outlet obstruction [108], in diabetic rats [109] and a number of other states. This protein is known to sensitize myelinated and unmyelinated afferents from the bladder [110,111] and it is involved in the production of

referred pain in bladder inflammation [112] (see **Figure 12**). It also appears to stimulate the expression of the vanilloid receptor TRPV1 [100].

Brain Derived Neurotrophic Factor (BDNF) levels in the urinary bladder and some other epithelia are higher than those found in the brain or skin [113]. 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]) were not present on the urothelium but were present in neurons of the peripheral nervous system. Hence it is thought that in the bladder the neurotrophin is produced by the urothelium and can act on the nerves. The mRNAs for NGF, BDNF and neurotrophin-3 all increase within 2 hours of bladder inflammation in the rat, and this increase expression may contribute to sensory and reflex hyperactivity [106].

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) [110].

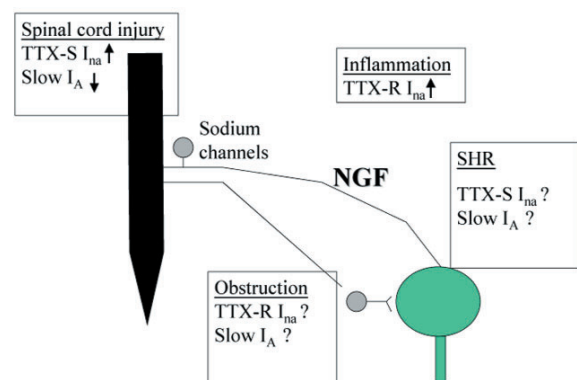


Figure 12. Following inflammation and cystitis a rise in TTX-R Na currents is measured in bladder DRG. After spinal cord injury or in SHR increased TTX-S Na currents is seen. A reduction in slow inward delayed rectifier K currents may reduce hyperpolarization and cause increased neuronal excitability. Thus, elevated NGF may act to influence a subunit expression which affects the excitability of neurons. This excitability may be manifested in a variety of ways such as a reduction in threshold, spontaneous or burst firing of afferents. Such an increase in afferent excitability could lead to a reduction in volume threshold for micturition in OAB conditions. (from 2001)

TTX-resistant Na channels (Nav 1.8 and Nav 1.9) have been found in SP/CGRP immunoreactive small DRG giving rise to C-fibers supplying the bladder [114,115]; these also express the trkA receptor, which binds NGF and is necessary for its action. Plasticity of TTX-sensitive and TTX-resistant Na⁺ channels (Nav 1.8 and Nav 1.9) occurs in these neurones after spinal cord injury, and a decreased expression of Nav 1.8 channel immunoreactivity and a small increase in Nav 1.9 channel immunoreactivity in bladder DRG neurons can be observed [116,117].

The dependence of the sensitization of these afferent neurones and the occurrence of overactivity on NGF and its actions on the Nav 1.8 channels has been shown in experiments using immunoneutralisation of NGF or antisense oligonucleotide treatment to reduce the expression of these channels in sensory neurones [114,118].

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 [119-124] with variable degrees of success.

II. PERIPHERAL GANGLIA

The section below concentrates on papers published since the 2nd International Consultation on Incontinence and some topics covered in less detail therein. The peripheral ganglia within the pelvis 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 [125]. There is considerable heterogeneity in organization and neurochemistry of pelvic ganglion cells and their spinal inputs in different species. Large mammals, including humans, have a plexus of interconnected pelvic and intramural ganglia, containing cytologically complex multidendritic neurones. They are mixed autonomic ganglia, containing both sympathetic and parasympathetic neurones that receive synaptic inputs from preganglionic neurones located in the lumbar and sacral spinal cord, respectively. Within the pelvic plexus there is topographical representation of the pelvic organs. In the female dog, neurones supplying different pelvic organs are located in separate ganglia, which possess a distinctive composition of neurone types and different preganglionic supply [126]. Neurones retrogradely label-

led from the urinary bladder mainly occur in ganglia located at the vesico-ureteric junction. They comprise catecholaminergic calbindin neurones and noncatecholaminergic neurones containing calbindin or NOS, with relatively sparse pericellular varicose nerve fibres. 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. A recent study described the structural and chemical properties of pelvic ganglion cells and their axonal projections in male mice [127]. The major pelvic ganglia are located 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 main outputs are the penile (cavernous) nerve, and fine bundles of axons, some containing ganglion cells, supplying the urogenital organs. Cellular subtypes are apparent within the major pelvic ganglion (**Table 4**). Tyrosine hydroxylase (TH) is expressed by one-third of neurones, 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 [127]. Neurones immunostained for choline acetyl transferase (ChAT) have a complementary distribution to noradrenergic neurones. 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 [127]. Neurones with NPY are numerous and apparently randomly distributed throughout the ganglion, with marked variation between mouse strains. All noradrenergic neurones contain NPY, but many NPY neurones are not noradrenergic. Many of the cholinergic NPY neurones also contain VIP. ChAT is seen in varicose axon terminals closely associated with ganglion neurones. Neither NPY nor VIP are present in preganglionic terminals, except for a small number of individual neurones. The latter may arise from viscerofugal neurones in the myenteric plexus of the lower bowel [128].

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 [129], while the rat bladder contains the post-synaptic innervation alone [130]. The ganglia are found throughout the bladder wall and vary considerably in size [64,131]. They show immunoreactivity 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 somatostatin (Som) [65], 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.

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 [132]. Histochemical and immuno-histochemical examination of the ganglia and nerves again show a heterogeneous population. 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 [133]. Synergic co-ordination of bladder and urethra is required for normal voiding, for which the fundamental role of supraspinal mechanisms is well recognized. One study suggests that synergic lower urinary tract function may be a feature of the peripheral innervation independent of CNS co-ordination. In the female

minipig, pre-ganglionic pelvic nerve stimulation reproducibly evokes a pressure increase in the bladder and a pressure decrease in the urethra [134]. This observation was taken to indicate one of two possible anatomical facets of the organisation of the peripheral innervation:

- a) motorneurones to the bladder and the urethra are separate; the urethral motorneurones are inhibitory and have lower thresholds than bladder motorneurones,
- b) postganglionic motorneurones send branches which supply both the bladder and the urethra. Thus, the synergic behaviour of the lower urinary tract depends on release of different neuromuscular transmitters from branches of the same motorneurone. This is circumstantially supported by the observed co-localisation of acetylcholine- and nitric oxide-related enzymes [135] (**Table 4**).

Little information is available on the subunit composition of the nicotinic receptors within the pelvic plexus and the intramural ganglia of the bladder. The clinical condition megacystis-microcolon-intestinal-hypoperistalsis syndrome (MMHIS) [136] could give some guidance, as patients have reduced or no alpha-3 nicotinic receptor subunit [137]. Selective gene knockout mice lacking the alpha-3 nicotinic receptor subunit alone or the beta-2 and beta-4 subunits in combination [138], 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 [139], indicating the potential importance of alpha-3, beta-2, and beta-4 nicotinic receptor components in control of

Table 4. Comparison of chemical classes of pelvic neurons in two mouse strains (Wanigasekara, Kepper et al. 2003).

Peptide	Quackenbush-Swiss (n =4)		C57BL/6 (n =2)	
	Mean +/-SE (%)	Range	Mean +/- SE	Range
TH (total)	31 +/- 3	23-26	34 +/- 4 3	0-37
NPY (total)	93 +/- 1	88-92	67 +/- 7	60-73
VIP (total) 5	5 +/- 4	47-64	43 +/- 3	40-45
TH/NPY	31 +/- 3	23-36	33 +/- 3	30-37
TH/VIP	<1	0-0.2	<1	0-0.3
TH/-	0	0	<1	0-0.3
NPY/VIP	50 +/- 4	43-60	24 +/- 6	18-30
NPY/-	13 +/- 4	9-24	18 +/- 1	18-19
VIP/-	5 +/- 1	4-6	25 +/- 2	22-27

voiding, but not their functional location. Nicotinic receptors have been identified on intramural nerve cell bodies within the bladder [139], but there are no reports suggesting that the detrusor muscle expresses any form of nicotinic receptor. In the isolated whole guinea pig bladder, exogenously applied nicotine has no detectable effect [140]. However, the nicotinic ligand lobeline enhances spontaneous activity at low concentrations. With higher concentrations, lobeline-evoked activity comprises phasic pressure changes whose frequency is dependent on the agonist concentration, and which are insensitive to atropine, tetrodotoxin (TTX) and hexamethonium. In contrast to the effects of carbachol in the isolated guinea pig bladder, no tonic basal change in intravesical pressure is elicited by lobeline [140].

1. POSTGANGLIONIC EFFERENT INNERVATION

The presence of different axon types in the bladder wall based on immunohistochemical criteria has been extensively documented. The majority of nerves running in the detrusor stain positively for acetylcholinesterase and for vesicular acetylcholine transferase (VACHT) [141,142] and are thought to be parasympathetic. Many also contain NPY and VIP, while some contain NOS or Gal. Putative postganglionic sympathetic fibres immunoreactive for TH or NPY are rare in the detrusor, although they are moderately frequent in the suburothelium [66]. In the human bladder, markers for sensory nerves (SP, CGRP and neurokinin A) occur infrequently in nerves running in the detrusor but are moderately frequent in the suburothelial layer [64-67]. 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 [127]. Cholinergic axons are prevalent in muscle and mucosa, with similar relative prevalences 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 [127]. Cholinergic nerves are also present in the suburothelium; most of them in addition contain NPY and some contain NOS [66]. Their function is uncertain, although in other organs they may be secretomotor [141].

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 [143]. The nerve supply is distributed by a series of dichotomous branchings, illustrated schematically in **Figure 13**.

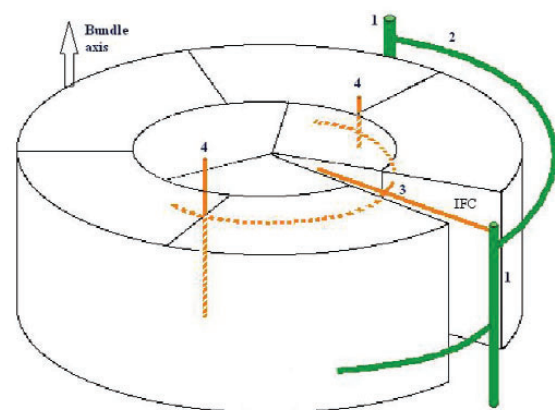


Figure 13. Muscle bundle nerve supply A A schematic representation of the midpoint of a muscle bundle, made up of 8 fascicles separated by connective tissue planes, with an interfascicular cleft between some of the fascicles penetrating to the centre of the bundle. The peribundle nerve trunks are shown in green and the interfascicular ramifications within the bundle are shown in orange. From the latter arise the preterminal and terminal intrafascicular innervation (not shown). 1= Longitudinal peribundle nerve trunk, 2= Circumferential peribundle nerve branch, 3= Transverse interfascicular branch, entering bundle in a connective tissue cleft. 4= Axial interfascicular branch, source of the intrafascicular terminal innervation. IFC = interfascicular cleft. Figure from (Drake et al., 2003a).

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.

Responses to stimulation of peripheral nerve trunks are complex. Direct electrical nerve stimulation (DENS) in the isolated whole guinea pig bladder elicits a frequency-dependent increase in intravesical pressure, increasing in amplitude up to 30 Hz frequency for short stimulus durations [140]. DENS using longer stimulus durations elicits somewhat complex responses **Figure 14**.

At low frequency, the pressure response is phasic, the pressure fluctuations occurring at a lower frequency than the DENS frequency. Based on the pressure waveform and visual analysis of contraction activity in the bladder wall, each pressure fluctuation elicited by DENS is seemingly derived from summation of several components [140]. Two distinctions must be drawn between these observations and those elicited by electrical field stimulation (EFS) in isolated muscle strips. Firstly, DENS responses are entirely inhibited by atropine, in contrast to EFS responses in isolated muscle strips. Secondly, it is difficult to establish incontrovertibly what is being stimulated. DENS presumably applies stimulation to a mixed population of pre- and post-ganglionic nerve

fibres, possibly with an antidromic component via afferent fibres. EFS aims primarily to act on post-ganglionic fibres, but could also affect sensory fibres, and there is a variable element of direct muscle stimulation.

2. INTEGRATIVE PHYSIOLOGY

The term 'integrative physiology' refers to whole organ/ tissue behaviour, in contrast to the study of components in isolation, such as individual cell types. While such projects are complex and may be largely qualitative, they are an essential part of understanding tissue properties, since it is often difficult to anticipate interactions purely from observations of components acting in isolation.

a) Peripheral afferent; urothelium/ volume sensing organ hypothesis. This has been discussed in the sections on urothelium and afferents above.

b) Peripheral autonomous activity

Voiding requires generation of sufficient contractile force in the bladder wall to expel the contained urine.

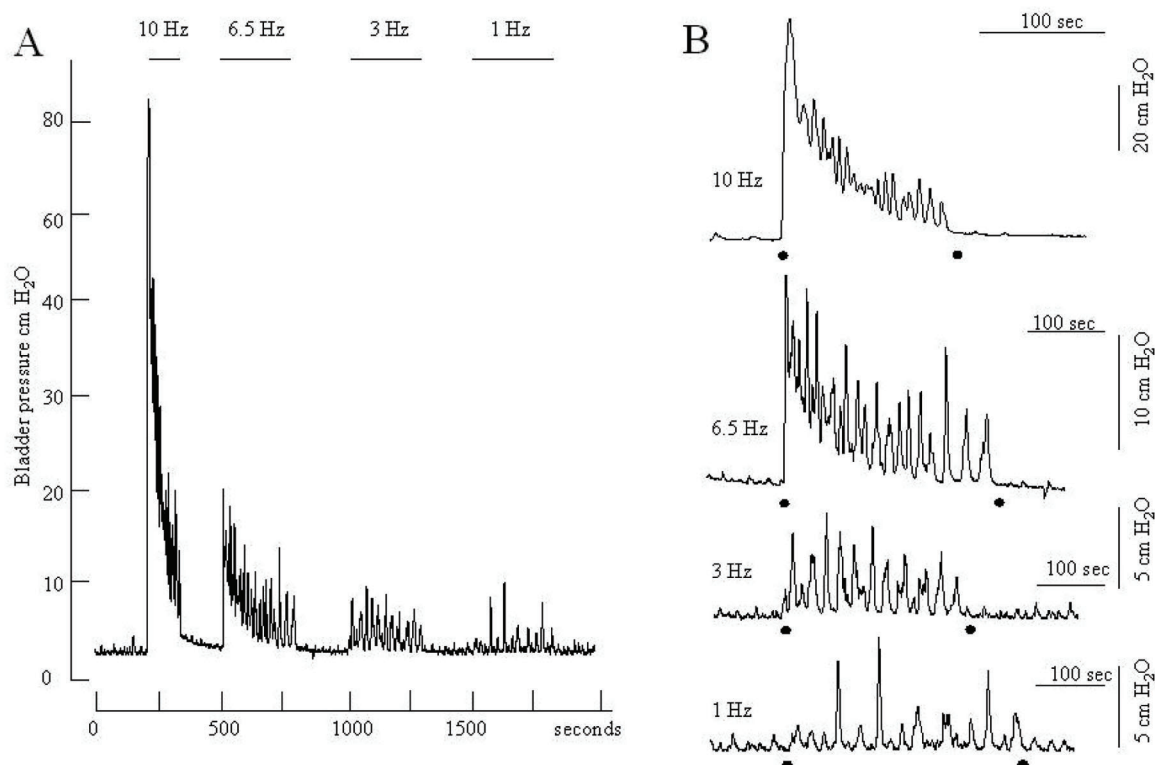


Figure 14. The effects of prolonged direct electrical nerve stimulation on intravesical pressure in the isolated whole guinea pig bladder. *A* the entire pressure trace from a frequency-response experiment. Where indicated by the horizontal bars, the nerves to the bladder were stimulated at the frequencies shown. Ordinate, intravesical pressure; abscissa time in seconds. *B*, illustrates sections of the record in *A* on an expanded time scale. Nerve stimulation was applied in the period denoted by the filled circles. Note the phasic nature of the response. Figure from (Gillespie et al., 2003).

The 'classical' mechanism responsible is based on efferent excitation, relayed synaptically via nicotinic receptors in the peripheral ganglia, leading to local release of acetylcholine, directly eliciting smooth muscle contraction via postjunctional muscarinic receptors. Based on this model, the predicted response of the isolated whole bladder on exposure to non-specific and M3-selective muscarinic agonists must be a generalised tonic detrusor contraction. Since the M3 receptor is expressed throughout the detrusor, the global increase in wall tension would be expected to make the shape of the bladder more spherical and substantially raise intravesical pressure. In fact the response of the whole organ, at least from the guinea pig [140,144] and rat [145] is regionalised; within the same bladder it is possible to see areas of shortening as anticipated, but at the same time other areas maintain a constant length while others actually elongate, despite the presence of a contractile agonist [140]. These effects are dynamic, such that the bladder is constantly in motion and changing shape, with commensurately complex phasic pressure fluctuations, with a baseline tonic component. 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 [140].

Preliminary studies using optical imaging methods and calcium-sensitive and voltage-sensitive dyes in whole bladder preparations from the neonatal rat have detected electrical activity moving in a co-ordinated manner from localised regions over the entire bladder [146]. In the neonatal rat, considerable activity arises in the bladder wall, which is apparent only when inputs from the lumbosacral spinal cord are disrupted [147]. Selective spinal cord and root lesions indicate that intrinsic bladder activity of the neonatal rat is tonically inhibited by parasympathetic efferent outflow [147]. 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 [147]. 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. Spontaneous activity is not apparent in

bladder strips from neonatal rats [148], but subsequently emerges, so that at one month, high-frequency spontaneous contractions occur in conjunction with high-amplitude, low frequency contractions. Such activity is apparent in only 20% of bladder dome strips, but occurs in a higher proportion of strips taken from the bladder base. Several species show differences in contractile activity according to the region of the bladder from which the muscle strip is taken [149]. TTX does not affect the spontaneous activity of bladder strips, while atropine reduces the amplitude of spontaneous contractions, without influencing frequency (see **Figure 15**). Cholinergic agonists induce expression of the fast component of spontaneous activity in some strips that were quiescent prior to agonist exposure, additionally altering the frequency of the slow component. A differential effect between the bladder base and the dome is apparent, depending on developmental age. Adult animals of several species also show autonomous activity when freed of central nervous input, including the rat [145], guinea pig [140,144] and pig [150]. Autonomous activity has been studied in most detail in the guinea pig bladder, revealing a surprisingly complex mix of micromotion phenomena, including localised microcontractions, microstretches and propagating waves [140,144].

The above findings indicate three likely components regulating detrusor contractility; 1. CNS efferent excitation, 2. CNS efferent inhibition, 3. Peripheral excitatory mechanisms. The existence of peripheral inhibitory mechanisms remains a fourth possibility. The role of peripheral excitatory mechanisms in the rodent neonate appears to be to subserve the effector mechanism of voiding induced by parental stimulation of the perineum, prior to establishment of mature control by the higher micturition centres [147,148]. On the other hand, elimination of this co-ordinated activity during postnatal development could be linked with the emergence of the storage functions of the mature bladder smooth muscle [148]. The physiological role of such activity in the adult is not known, but could include; 1. Optimisation of the bladder wall configuration for volume contained, to ensure efficient voiding regardless of volume [151], 2. Stimulation of 'in series' receptors for signalling bladder volume [150], 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 [152].

Autonomous activity in the bladder bears some

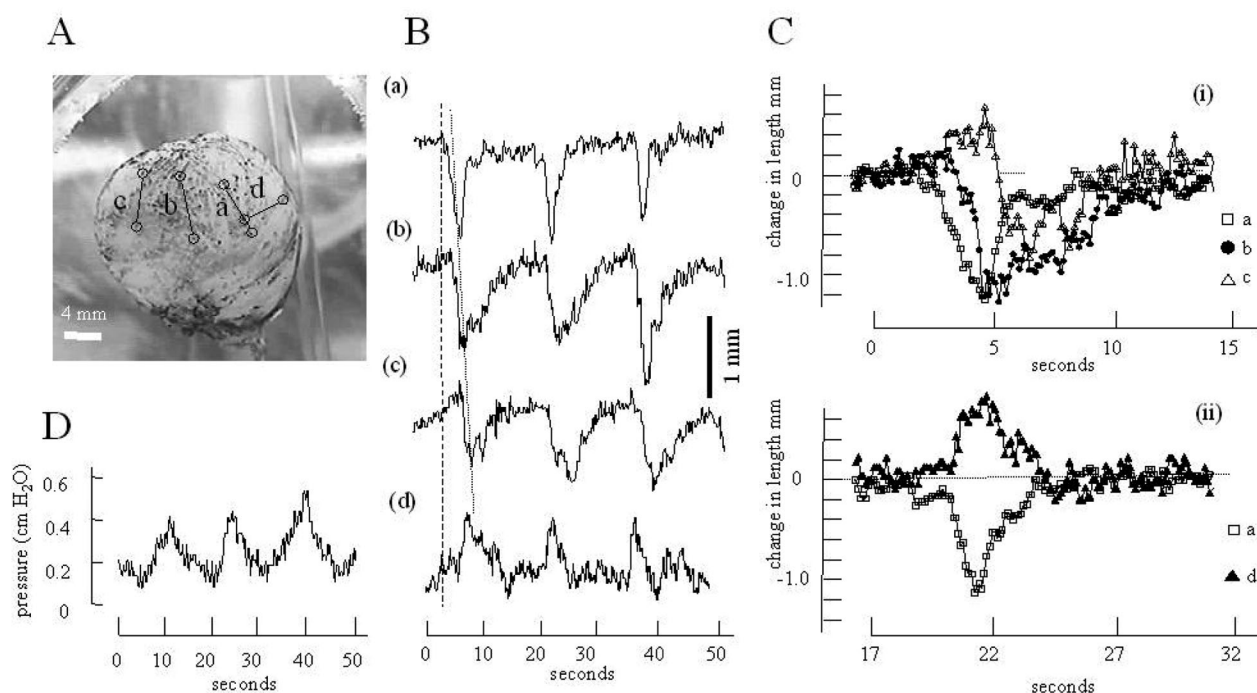


Figure 15. A spontaneous propagating wave of contraction over the surface of an isolated guinea pig bladder. **A and B** In this sequence, 4 regions of interest were identified (a-d). The displacements of identified pairs of carbon particles are illustrated in panel B. Vertical dotted lines are shown to indicate the delay in the initiation of the contraction in successive regions a-c. The oblique dotted line is drawn to demonstrate that the peak of each episode of shortening occurs later in regions a-c respectively. **C** shows superimposed records from different areas. (i) shows regions (a), (b) and (c) illustrating the progressive time delay in the initiation of shortening. (ii) superimposes traces from regions a) and (d). The stretch in (d), commensurate with the shortening in (a) is clearly seen. Panel D shows the intravesical pressure during the micromotion activity. Figure from (Drake et al., 2003b).

resemblance to smooth muscle contractility in the gastrointestinal tract. The gut possesses both extrinsic (sympathetic and parasympathetic) and intrinsic (enteric) innervation, the latter distinguished by the intramural location of the cell bodies. In addition, there are several subclasses of myofibroblasts, the interstitial cells of Cajal (ICCs). These structures interact in the generation of peristalsis, which serves to mix the luminal contents (unco-ordinated segmentation contractions) and move food boluses in the anal direction (co-ordinated propulsion contractions). Purely enteric circuits play a major role in generation and co-ordination of these reflexes [153], since the sensory limb, interneurons and motoneurons are all contained within the gut wall, and remain active when the organ is isolated from extrinsic autonomic input. The hardwiring of the enteric circuitry has been studied in several species. Subclasses of enteric neurones can be identified on the basis of morphology, neurochemistry and connectivity [154]. Each differs in respect of their presynaptic inputs, axon course and ganglionic contacts. Some subclasses of enteric neurones are polarised

[155], and target specific coding has also been identified [156]. Several enteric circuits regulating muscle activity have been well characterised, while further enteric circuits have been proposed to mediate gastric accommodation.

The above qualitative observations on autonomous bladder activity can be synthesised into a schematic model of the functional arrangement of the bladder wall as a modular structure, by reference to the better characterised mechanisms in the gut [151]. The modular hypothesis states that the detrusor is functionally arranged into circumscribed areas, each capable of contracting independently, similar to the gastrointestinal tract [157], or the skeletal muscle motor unit (**Figure 16**).

Within each module, an integrative mechanism is proposed, globally termed the 'myovesical plexus', comprising a functional interaction between innervation circuitry and interstitial cells akin to the myenteric plexus of the gut. This summates inputs from various sources, both inhibitory and excitatory, with contraction of the muscle of the module occurring

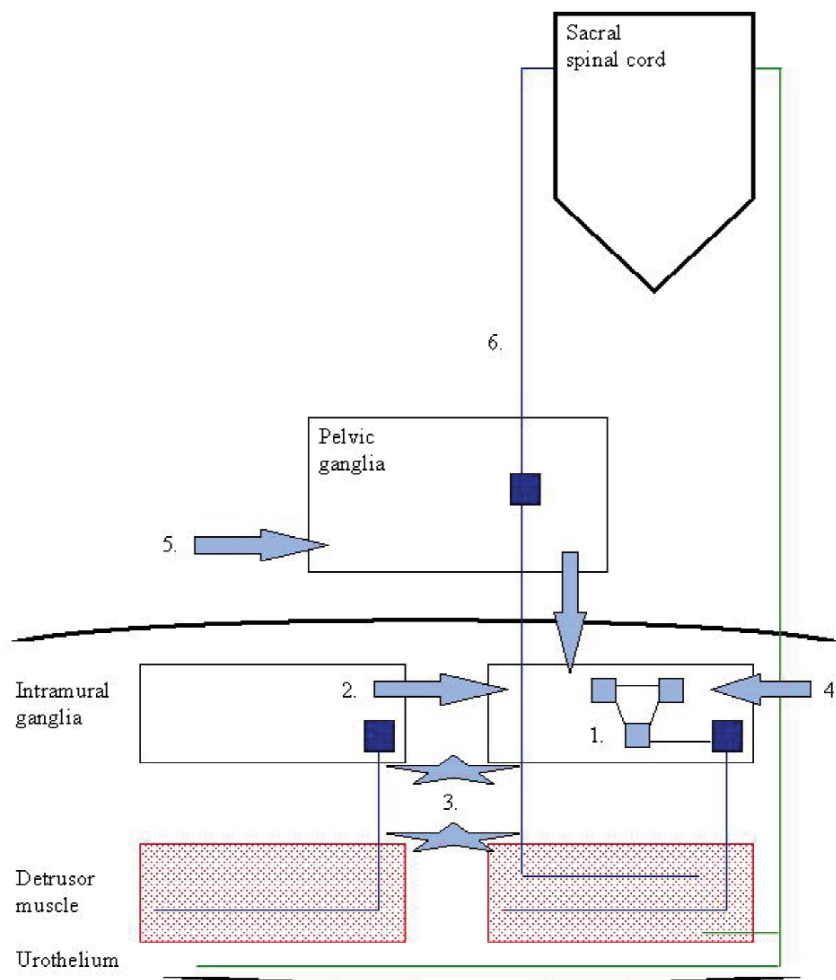


Figure 16. Inputs to the detrusor module. The module is a circumscribed region of smooth muscle possibly controlled by an intramural ganglion. Within the ganglion is an integrative circuit (1), receiving inputs from some or all of; neighbouring modules (2), interstitial cells (3), afferent collaterals (4) and other pelvic organs (5). These summate to give a level of excitation, affecting the likelihood of contraction of the module. A module can thereby show autonomous activity, regardless of the primary sacral efferents (6). Figure from (Drake et al., 2001).

when the balance of excitatory inputs exceeds inhibition. Possible inputs into the integrative mechanisms include; neighbouring modules, afferent collaterals, other pelvic organs and interstitial cells (see below). In addition, the local hormonal and cytokine milieu may influence the regulatory mechanisms, or the responsiveness of the associated smooth muscle; for example, locally-released ATP [72], or prostaglandins [158] and circulating testosterone [159]. Wider propagation of excitation through the bladder wall would also occur through the myovesical plexus, which could explain the transformation of localised micromotion activity into propagating contraction waves on increased physical (stretch) or pharmacological (muscarinic and nicotinic) stimulation [144,145].

The modular hypothesis is able to explain observations of localised contractions in several species, viz

phasic shortening in a circumscribed part of the bladder, while neighbouring areas remain unchanged in length or elongate. This has been reported in rat [145,160], guinea pig [145,160] and pig [150], with preliminary evidence supporting their occurrence in normal humans [161]. The bladder does have distinct functional zones (dome, trigone, bladder neck). Furthermore, voiding proceeds across an anatomically complex outlet, comprising several structures (bladder neck, prostate, urethra and pelvic floor) which require integrated activity for effective function. Differing functional properties of strips from separate regions of the bladder might be linked with the different functions of the base (bladder neck closure and urine storage) and dome (urine elimination) [148]. The relevance of the modular hypothesis to detrusor overactivity and sensory urgency is discussed later.

III. CLINICAL STUDIES AND ANIMAL MODELS OF CLINICAL CONDITIONS

1. PERIPHERAL CHANGES IN 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 or in animal models:

- 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 unstable bladders there is reduced contractile response to intrinsic nerve stimulation, along with supersensitivity to muscarinic agonists and potassium solutions [162-165]. 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 [166]. 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 [66]. 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 [167]. 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 [168,169]. A common ultrastructural feature of the overactive detrusor is the emergence of protrusion junctions and ultra-close abutments between the smooth muscle cells [170].

Overall, the cells may be better coupled electrically in detrusor overactivity, perhaps allowing spontaneous activity to propagate over a wider area. Bladder biopsies from unstable detrusor shows a patchy denervation; some muscle bundles may be completely denervated, whilst neighboring ones appear normal and in other areas sparser innervation is also seen [66,166,167,171]. A similar pattern is seen in animal models [163,172]. 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 [162,173].

2. BLADDER OUTLET OBSTRUCTION

Bladder outlet obstruction is a recognised predisposing factor for detrusor overactivity, and exogenously-induced partial bladder outlet obstruction is a common animal model for study of the condition. Studies in the rat have shown that the bladder becomes more sensitive to the inhibitory effects of atropine, resulting in an increased inhibition of nerve-mediated contraction [174]. Unlike several other models of DO, functional denervation was not apparent. Accordingly, these authors suggested that the cholinergic and purinergic components of neuromuscular transmission can be modulated independently, and that outlet obstruction may cause proliferation of cholinergic nerve fibres [174].

In the peripheral modular model ([151], see above), any change causing a shift in the balance of excitation and inhibition towards excitation will predispose to contraction of the muscle within a module. If, in addition, some alteration in the bladder wall increases the likelihood of propagation of activity through the myovesical plexus, a larger part of the bladder wall will contract. From there it is logical to surmise that detrusor overactivity may result from exaggerated symptomatic expression of peripheral autonomous activity, arising without central control. Conversely, a shift in the balance of excitation and inhibition towards the latter will have therapeutic potential in detrusor overactivity. One study examined the influence of partial bladder outlet obstruction on modular autonomous activity in the isolated whole rat bladder [145]. In sham operated controls, baseline contractile activity took the form of localised microcontractions in a focus near the vesicoureteric junction. In obstructed animals, multifocal microcontractions were seen in two to six areas of approximately two to four mm diameter. Small incremental increases in intravesical volume precipi-

tated the onset of co-ordinated contraction waves. Subsequent increments significantly increased the frequency of contraction waves, but in a disorganised manner which had little effect on the fluctuations apparent in the intravesical pressure trace. In contrast, volume increases apparently enhanced co-ordination of the intramural contraction waves in the obstructed bladders, so that contraction wave frequency fell with increasing volume and the associated pressure fluctuations were more pronounced [145]. These observations appear to indicate that the changes in obstructive overactivity derive from a combination of increased propensity to localised muscle contraction, along with a tendency towards wider propagation of this activity (see **Figure 17**).

Enhanced propagation of excitation might reflect enhanced transmission through the muscle itself, perhaps through gap junctions between muscle cells. Levels of the gap junction protein connexin-43 and its mRNA are low in the normal rat bladder, but they increase following partial urethral obstruction [175,176]. The contrasting contractile activity between the co-ordinated waves in the BOO bladders and the 'chaotic' activity of the normal adult mirrors developmental changes. In the neonatal rat, electrical activity moves in a co-ordinated manner over the entire bladder [146]. In contrast, chaotic activity originating at multiple sites is apparent in adult bladders [148]. Overall, several peripheral factors may determine intravesical pressure, including the number and size of modules active in the bladder wall, their co-ordination and the overall intramural tension in the remainder of the bladder wall [145]. Similar changes could also underlie clinical sensory urgency [150], and studies in the human indicate changes in localised contractile activity correspond with reported sensations [161] and chronic pelvic pain [177].

3. PERIPHERAL BLADDER CHANGES IN NEUROPATHIC DISEASE

Bladder decentralisation leads to widespread degeneration of intrinsic axons and muscle cells. In the longer term, reversal of degeneration leads to restitution of cholinergic axon terminals, increased adrenergic and copeptidergic axons and muscle cell regeneration. A recent ultrastructural study examined the human bladder in chronic lower or upper motor neurone disease [178]; most axon profiles had features of axonal degeneration, while 20% had features of regeneration (axoplasmic mitochondria and large dense cored vesicles, with or without increased neurotubules and neurofilaments) and 16% had normal

ultrastructural morphology. Axonal degeneration and regeneration coexisted in several biopsies. Axonal degeneration generally far exceeded muscle cell degeneration concomitantly observed in the same biopsies. Regeneration where observed was generally limited to small populations of axon profiles, and had features suggesting initial regeneration that subsequently regressed. The regressed form was dominant in meningocele and spinal cord injury, and degenerated axons were much more numerous than normal axons in spinal cord problems. In contrast, axons in biopsies from people with upper motor neurone lesions had stable regenerated or normal morphology. This distinction may confer the ability to distinguish the nature of the neurological lesion based on peripheral bladder biopsy [178].

Partial denervation has been observed in several studies into detrusor overactivity, typically with a 'patchy' distribution. The studies describing this have generally employed standard histological techniques, but one paper described a serial sectioning study evaluating the pattern of denervation in consecutive sections in the neuropathic bladder. This study established that patchy denervation is cross-sectionally consistent throughout the long axis of affected muscle bundles [143]. The major innervation deficit was at the level of the terminal intrafascicle innervation. Despite the close proximity of innervated muscle, there was no obvious nerve sprouting into denervated areas (see **Figure 18**).

The ability of the bladder to mount substantial phasic rises in pressure is retained in a large proportion of patients with neuropathic disease, indicating that widespread co-ordinated contraction can occur despite partial denervation. Putatively, several mechanisms could facilitate the co-ordinated participation of denervated areas in a global bladder contraction [143]; 1. if an ostensibly denervated fascicle receives any nerve supply at any point along its length, the innervation could trigger contraction synchronous with the rest of the bladder, with excitation propagating myogenically in the denervated part of the muscle fascicle. 2. Contraction could be co-ordinated by some other excitable structure, for example smooth muscle cells or myofibroblasts. Some evidence in favour of the muscle cells is suggested by the observation that not all are longitudinally aligned within the muscle bundle, a small number deviating perpendicular to the axis and running between fascicles [143]. 3. purely physical stimuli, i.e. localised stretch, could mediate widespread contraction of the detrusor.

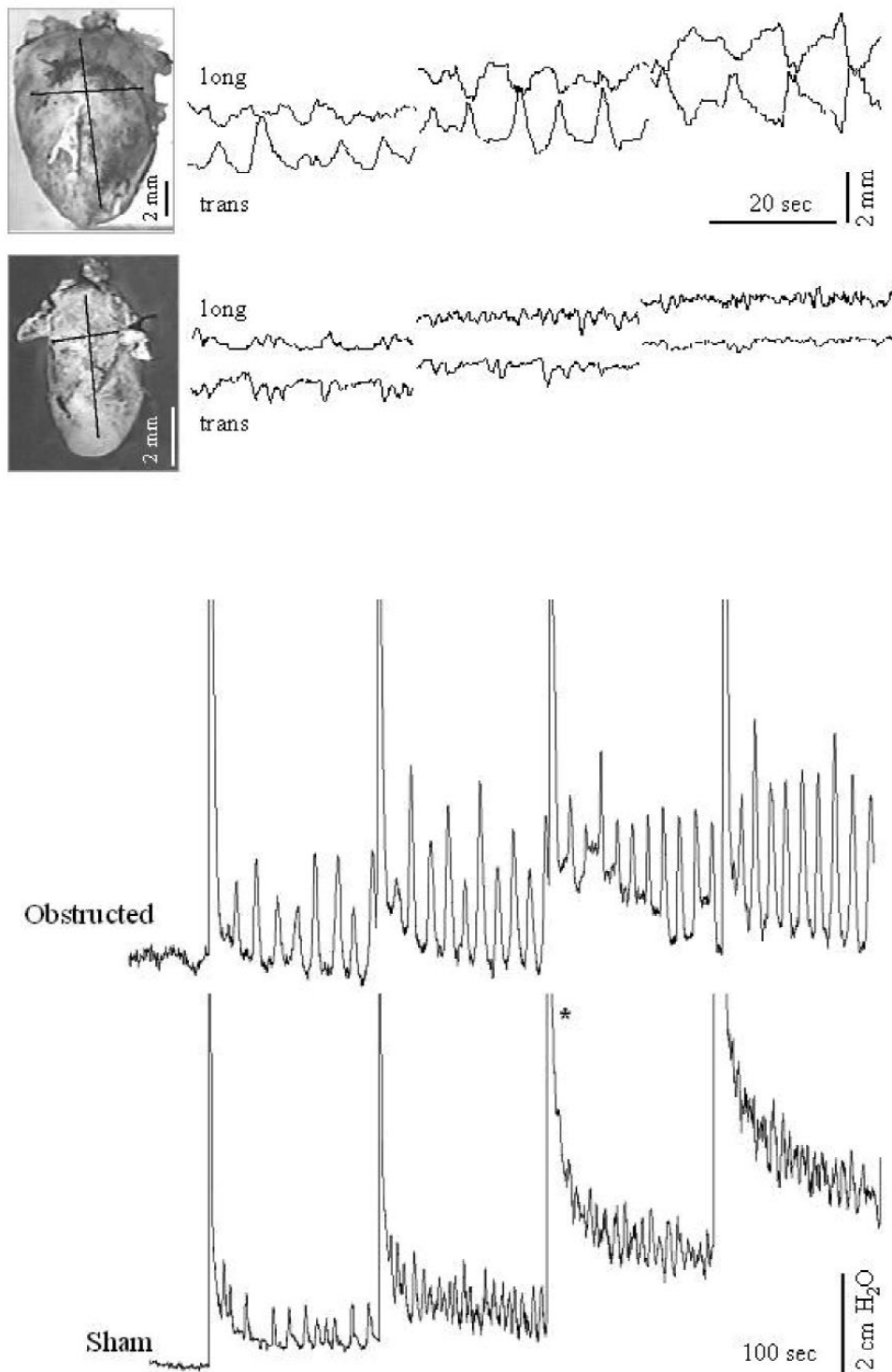


Figure 17. Response to increasing intravesical volume in the isolated rat bladder. The upper panel shows micromotions during three incremental increases in intravesical volume in a rat bladder following a period of partial outlet obstruction (above) and a sham operated bladder (below). Stretch elicited propagating waves of contraction, which were assessed by measuring separation of points on the longitudinal and transverse axes of each preparation, as indicated on the photographs. Micromotion frequency was lower in the obstructed bladder, showing increased co-ordination and a fall in frequency with stretch, which contrasted with the sham-operated specimen. The lower panel shows intravesical pressure traces during stretch response experiments, in which each bladder received four rapid volume increments of 0.2ml. Both preparations showed increasing frequency of pressure fluctuations with filling, but only the obstructed bladder showed increased amplitude. long = longitudinal, trans = transverse. Figure from (Drake et al., 2003c).

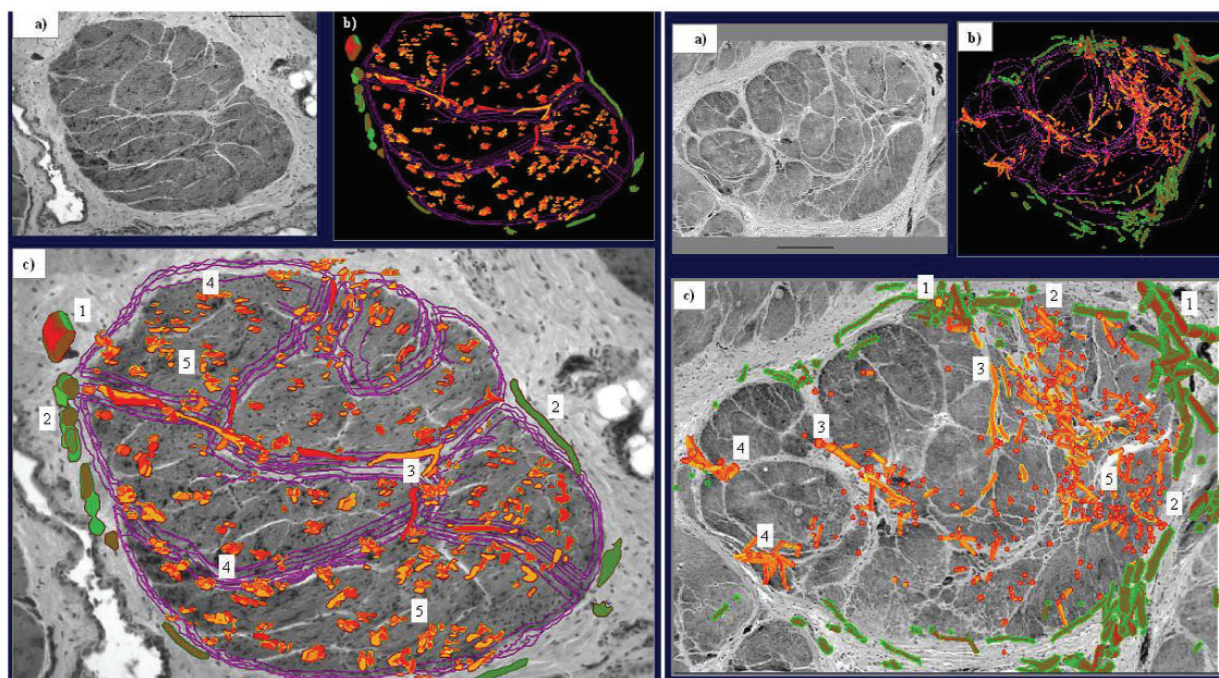


Figure 18. Detrusor muscle bundle innervation. Images derived from a serial sectioning of a bundle from a control specimen (left) and a person with neurogenic detrusor overactivity (right). For each specimen, the 3 images provided represent; a) a low resolution image of the fascicle studied; b) a 3-D reconstruction of the local innervation, showing outlines of fascicles (purple), nerve trunks in interbundle connective tissue planes (green) and nerve fibres making close approach to muscle (yellow/orange); c) a reconstruction in which the low resolution image is merged into the 3-D reconstruction image stack. 1= Longitudinal peribundle nerve trunk, 2= Circumferential peribundle nerve branch, 3= Transverse interfascicular branch, 4= Axial interfascicular branch, 5= Intrafascicular axial innervation (preterminal and terminal segments). Patchy denervation is clearly seen in the neuropathic specimen, which mainly affects the perterminal and terminal innervation. Figure from (Drake et al., 2003a).

Bladder outlet obstruction in rats causes hypertrophy of bladder afferent and efferent neurons [179,180]. Conversely, relief of obstruction is associated with the reduction of urinary frequency and reversal of these neural changes [181], except in those animals that fail to revert to a normal voiding pattern after relief of obstruction. The afferent plasticity involves nerve growth factor (NGF), the content of which is increased in obstructed bladders prior to the enlargement of bladder neurons and the developmental of urinary frequency [181]. Blockade of NGF action prevents the neural plasticity and urinary frequency following obstruction. Mechanical stretch, denervation and ischemia are all capable of inducing NGF. These findings suggest a cause and effect relationship between NGF-mediated changes in bladder afferents and an enhanced spinal micturition reflex and urinary frequency associated with obstruction.

4. PERIPHERAL BLADDER CHANGES WITH AGEING

Ageing studies are often difficult to interpret. Particular care is needed not to confuse demonstration of

association with a causative role, i.e. the finding of a change in an ageing group should not lead to spurious assertion that the finding is responsible for clinical changes. Furthermore, the wide range of changes that occur can confound interpretation of findings. For example, 'innervation density' may appear to fall if simply assessed as a number of axon profiles per unit area, yet the number of axons for the muscle cell mass may actually be unchanged if there has been connective tissue infiltration. With these caveats in mind, it does appear that the ageing bladder shows a reduced density of innervation [182]. However, frequency-response curves for electrically evoked bladder contractions are similar in young and old rat bladders [183], indicating that the release of neurotransmitters from pre-synaptic nerve endings is not altered during ageing. An immunohistochemical survey of a group of young adult rats and an ageing group evaluated sensory neuropeptides in the rat bladder [184]. Pituitary adenylate cyclase activating polypeptide (PACAP) was present in numerous suburothelial varicosities. The lamina propria showed similar axons, but fewer in number. In the muscle layers, most of the smooth muscle bundles were

innervated by axons containing PACAP. The density of these axons was higher in the base than in the rest of the bladder. The density of PACAP-containing nerves was lower in aged animals, while innervation density for SP, CGRP or VIP were unchanged. Accordingly, loss of PACAP has been put forward as a possible explanation for the observation that distension-sensitive afferents are less able to monitor bladder volume in the aged rat [184]. In the aged rat there is a slight reduction in the density of NOS-immunoreactive nerves [185]. Some observations suggest a diminution in the sympathetic control of the urinary tract in aged rats [186].

5. INFLAMMATION OF THE BLADDER

Inflammation results in neuroplasticity of the sensory nerves supplying the bladder [110]. Repeated inflammatory stimuli lead to enlargement of bladder dorsal root ganglion neurons [187]. Following chemical or mechanical inflammation, increased expression of nitric oxide synthase occurs in bladder afferent neurons [188]. Bladder overactivity induced by inflammation can be inhibited by a fusion protein that prevents interaction between NGF and its receptor [111]. Other substances including neurotrophins, prostaglandins, and tachykinins may also contribute to altered afferent excitability [189].

D. SPINAL CORD

I. SPINAL PROJECTIONS OF LOWER URINARY TRACT PRIMARY AFFERENT NEURONS

This section is concerned with the central projections of the primary afferent neurones. Axonal tracing experiments have been performed in many animal species [60,190-192] 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 (Figure 19). The dorsal commissure (DCM), superficial dorsal horn and sacral parasympathetic nucleus (SPN) all contain interneurons with rostral projections that are activated during noxious [193,194] or non-noxious stimulation [195] of the rat bladder and the urethra. These neurones 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 [196,197]. 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 [198].

Visceral afferent fibers of the pelvic [199] and pudendal [191] 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 (Figures 19 and 20)

1. EFFECTS OF AFFERENTS FROM THE URETHRA, BOWEL AND GENITAL ORGANS ON PARASYMPATHETIC ACTIVITY.

Studies have demonstrated that electrical stimulation of urethral afferent fibres when the bladder is full can evoke strong detrusor contractions sufficient for voiding in intact cats [200,201] as well as acute spinalized cats [202]. 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 [2] but these do not seem to produce efficient bladder emptying.

The excitability of the micturition reflex can also be influenced by other sacral afferent pathways [200,203-205], including facilitatory effects resulting from stimulation of urethral afferents, and of inhibition of bladder activity by stimulation of the dorsal nerve of the clitoris which is in keeping with known interactions from the vagina and colon [4,206]. Stimulation of urethral afferents by flowing fluid through the urethra can facilitate the micturition reflex; however contraction of the urethral sphincter resulted in inhibition of bladder motility [13].

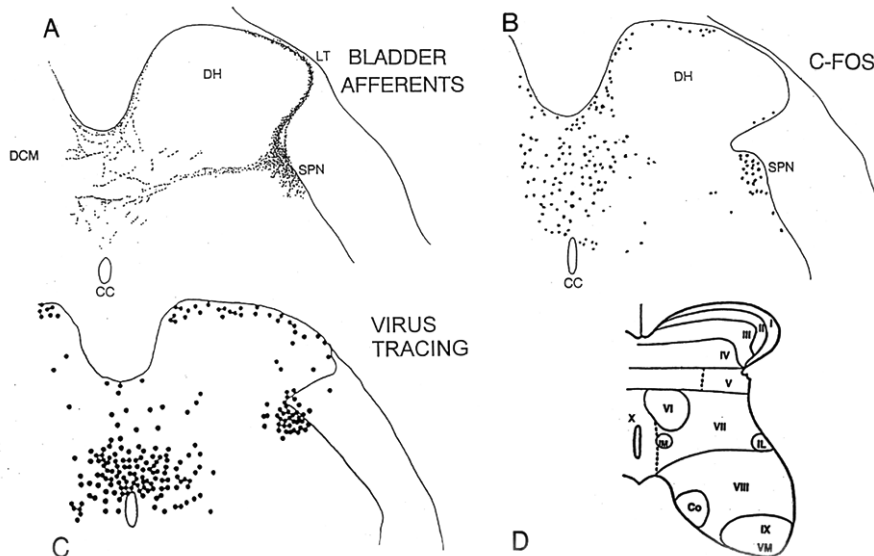


Figure 19. Comparison of the distribution of bladder afferent projections to the L6 spinal cord of the rat (A) with the distribution of c-fos positive cells in the L6 spinal segment following chemical irritation of the lower urinary tract of the rat (B) and the distribution of interneurons in the L6 spinal cord labeled by transneuronal transport of pseudorabies virus injected into the urinary bladder (C) Afferents labelled by WGA-HRP injected into the urinary bladder. C-fos immunoreactivity is present in the nuclei of cells. DH, dorsal horn; SPN, sacral parasympathetic nucleus; CC central canal. (D) Diagram showing the laminar organization of the spinal cord.

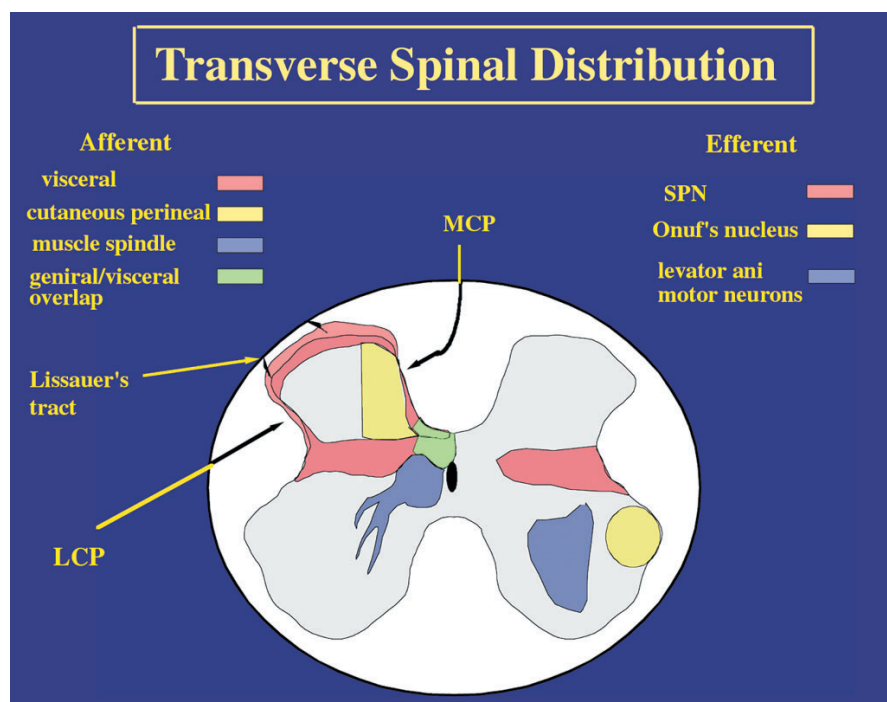


Figure 20. Neuroanatomical distribution of primary afferent and efferent components of storage and micturition reflexes within the sacral spinal cord. For purposes of clarity, afferent components are shown only on the left, while efferent components are shown only on the right. Both components are, of course, distributed bilaterally and thus overlap extensively. Visceral afferent components (pink and green regions) represent bladder, urethral, and genital (glans penis/clitoris) afferent fibers contained in the pelvic and pudendal nerves. Cutaneous perineal afferent components represent afferent fibers that innervate the perineal skin contained in the pudendal nerve. Muscle spindle afferent components represent Ia/b afferent fibers contained in the levator ani nerve that innervate muscle spindles in the levator ani muscle. SPN sacral parasympathetic nucleus LCP lateral collateral projection MCP medial collateral projection

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 [207-209].

These afferent neurons contain a number of peptidergic neurotransmitters, and the central distribution of bladder afferent terminals and peptidergic immunoreactive fibers is quite similar [60,208]. There has been considerable interest in the role of tachykinins in micturition [1]. Intrathecal treatment of adult rats with intrathecal capsaicin caused a reversible block of the micturition reflex [210]. This and other studies [211-213] suggest that substance P may play a part in transmission at the first synapse in the micturition reflex. Intrathecal administration of the NK-1 antagonists, RP 67580 and CP 96345, increased bladder capacity in conscious rats without changing micturition pressure; whereas NK-2 antagonists were ineffective [214].

II. EFFERENT PATHWAYS AND REFLEX CONTROL OF THE LOWER URINARY TRACT

In many species, afferent pathways terminate on interneurons in the spinal cord that relay information to the brain or to other regions of the spinal cord including the preganglionic and motor nuclei. Bladder, urethral and sphincter reflexes are mediated by disynaptic or polysynaptic pathways, and interneurons play an essential role in the coordination of detrusor and sphincteric activities.

The following paragraphs outline the properties of efferent neurones that regulate the lower urinary tract: (a) the parasympathetic preganglionic neurones, (b) the sphincteric motoneurons and (c) the levator ani motoneurons.

1. PARASYMPATHETIC PREGANGLIONIC NEURONS.

Bladder parasympathetic preganglionic neurons (PGN) are located in the lateral part of the sacral intermediate gray matter in a region termed the sacral parasympathetic nucleus (SPN) (**Figure 20**) 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 com-

missure. Their axons pass through the ventral roots to peripheral ganglia where they release the excitatory transmitter, acetylcholine [5]; recently they have been divided into tonic and phasic types [215], depending on the activity of their potassium channels. In some species they also release opioid peptide transmitters and express nitric oxide synthase [216]; there is also recent evidence of involvement of Pituitary adenylate cyclase activating peptide (PACAP), a peptide present in visceral afferent neurones, and of prostaglandins within the spinal cord [217-220].

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. Most of our knowledge about the functional role of parasympathetic pathways in humans comes from stimulating these roots through implanted electrodes designed principally for bladder emptying in spinal cord injury [221]. Stimulation of the sacral anterior roots, particularly S3, elicits two principal responses (**Figure 21**); at low levels of stimulation, the external urethral sphincter, external anal sphincter and pelvic floor muscles are contracted whereas, at much high levels of stimulation (10-15 times higher), parasympathetic activation causes good contraction of the detrusor muscle leading to very efficient emptying of the bladder when the sphincter muscle relaxes [222]; this contraction is dependent upon acetylcholine [223].

There was some expectation that by using magnetic stimulation over the spinal cord, parasympathetic pathways could be stimulated non-invasively [224]. This would have many benefits for diagnostic investigations of otherwise inaccessible nerves. However, recent studies of functional magnetic stimulation in both patients with spinal cord injury and healthy volunteers has shown that these stimulators do not have the power to activate the small parasympathetic neurones at the level of the lumbar-sacral roots [225, 226].

2. EXTERNAL URETHRAL SPHINCTER MOTONEURONS

The external urethral sphincter (or urethral rhabdosphincter; striated muscle intrinsic to the urethra) is innervated by the motoneurons of the pudendal nerve, which are located along the lateral border of the sacral ventral horn in Onuf's nucleus (Fig. 6) [191]. Within the nucleus, urethral motor neurons occupy a ventrolateral position and anal motor neu-

rons occupy a dorsomedial position. Sphincter motoneurons exhibit tightly-bundled dendrites that run rostrocaudally within the confines of the nucleus, and transversely into the lateral funiculus, and dorsally towards the SPN and central canal; this is similar to that of bladder PGN and very different from that of limb motoneurons, suggesting that EUS motoneurons and PGN receive inputs from similar regions of the spinal cord. Although textbooks often indicate that the pudendal nerve innervates the pelvic floor muscles, recent studies in humans [227], squirrel monkeys [228], and rat [229] indicate that the levator ani muscles have a distinct innervation that does not include pudendal nerve branches.

3. PELVIC FLOOR MOTOR NEURONS

The innervation of the levator ani muscle is described in textbooks as being innervated by the “S3-S5 spinal roots” and the pudendal nerve. Since the S3-S5 spinal roots innervate many structures in addition to the levator ani muscles, it was felt that this nomenclature needed revision and that a detailed study of the innervation in various species was warranted [227-229]. In humans [227], the levator ani muscles are innervated by a nerve that originated from the S3 to S5 foramina (30% S4 alone, 40% from S3 and S4, 30% from S4 and S5), crossed the superior surface of

the coccygeal muscle (3.0 ± 1.4 cm medial to the ischial spine, traveled on the superior surface of the iliococcygeal muscle innervating it at its approximate midpoint, and continued on to innervate both the pubococcygeal and puborectal muscles at their approximate midpoint (**Fig. 22**). Despite specific attempts to locate pudendal branches to the levator ani, none could be demonstrated in human, monkey or rat [228,229].

Retrograde tracing studies involving injection of cholera toxin B (CTB) into the levator ani muscle and fast blue into the anal sphincter muscle of squirrel monkeys [230] and rats (Bremer et al, in preparation) show that levator ani motor neurons are located in the sacral ventral horn (fig.22) in a longitudinal column. In contrast to the very dense packing of sphincter motor neurons in Onuf’s nucleus, the levator ani motor neurons are more diffusely distributed; and furthermore Onuf’s motoneurons are all of a uniform intermediate size, in contrast to the levator ani motor neurons, which show a bimodal distribution of large neurons (presumably alpha motor neurons) and small neurons (presumably gamma motor neurons). These findings are in keeping with the lack of muscle spindles in rhabdosphincter muscle and their presence in levator ani muscle; consequently levator ani can exhibit stretch reflexes, whereas the EUS does not.

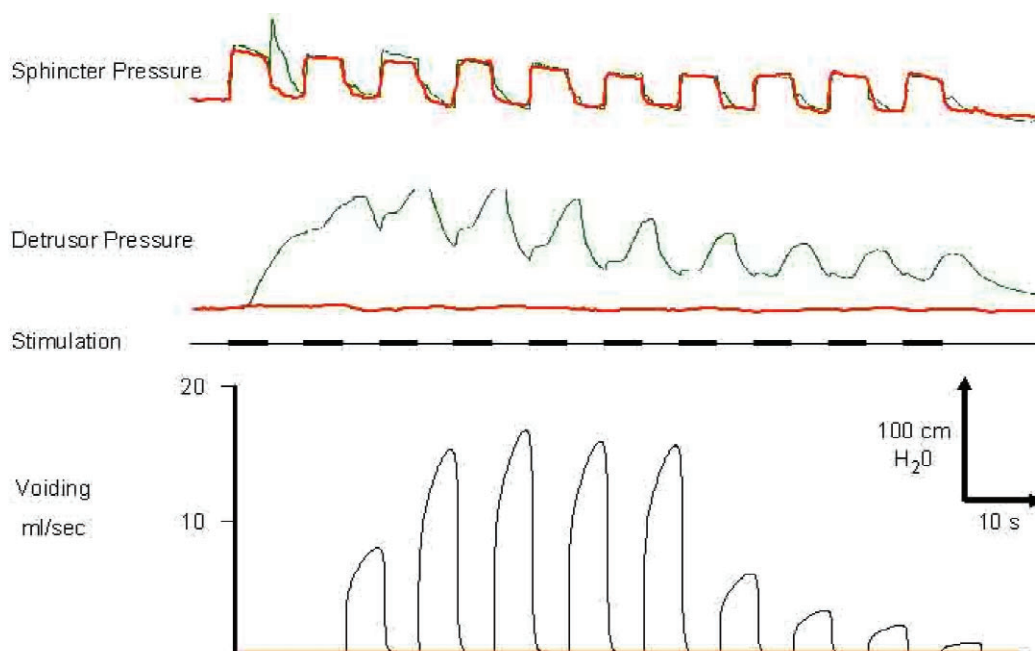


Figure 21. Responses to S3 sacral anterior root stimulation through a Finetech-Brindley implant. The red lines are the responses to low level stimulation eliciting only sphincter responses whereas the black lines show maximal effects on both the sphincter and detrusor to generate efficient voiding in the interval between the bursts of stimulation. Compared to the sphincter responses alone, the detrusor responses to give voiding required about 10 times greater current.

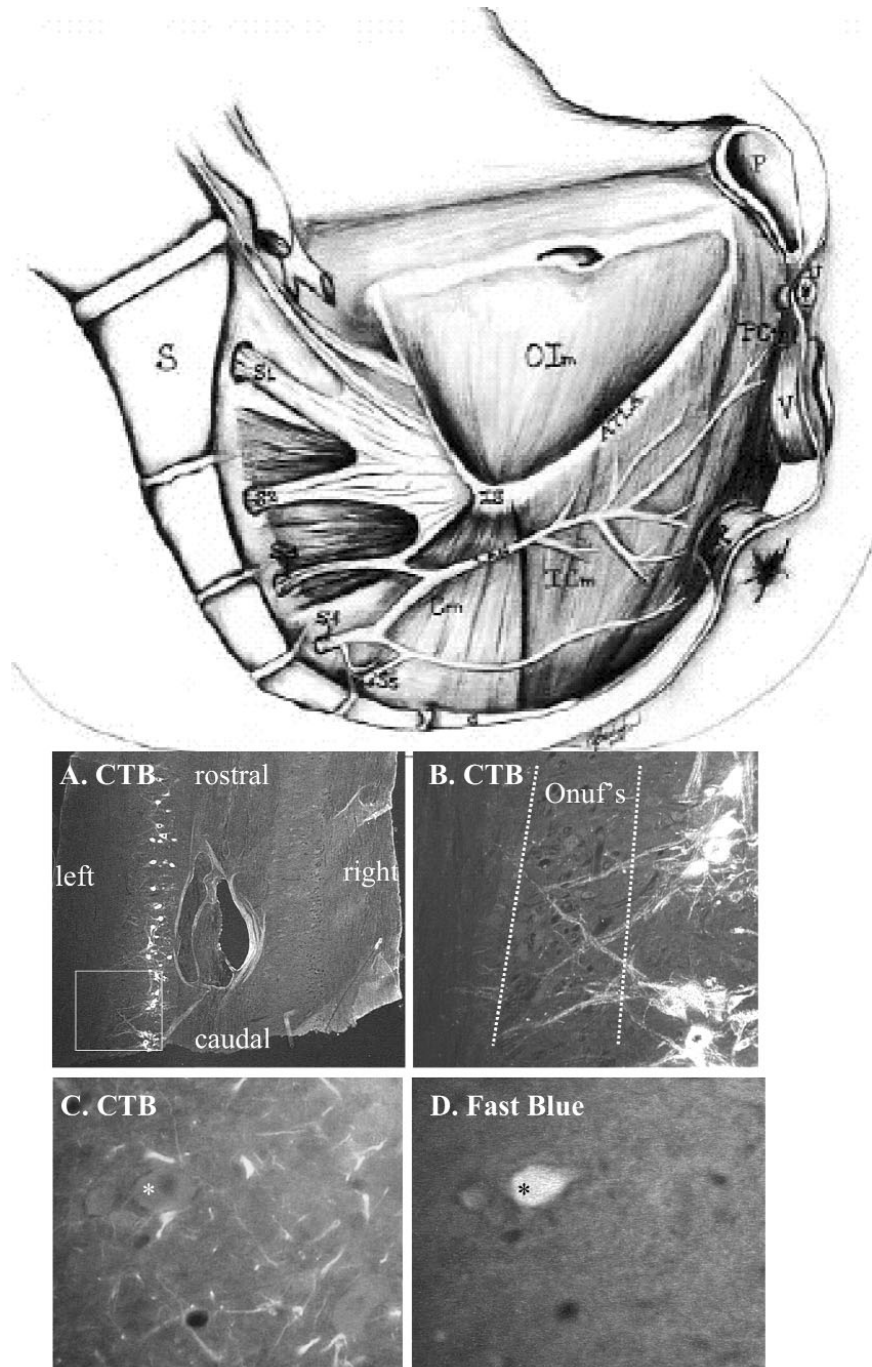


Figure 22 Top – Illustration of the levator ani nerve in human female (sagittal view of left hemipelvis). S (sacrum), S1-S5 (sacral foramina), Cm (coccygeal muscle), LAN (levator ani nerve), IS (ischial spine), ICm (iliococcygeus muscle), OIm (internal obturator muscle), PCm (pubococcygeus muscle), PRm (puborectal muscle), ATLA (arcus tendineus levator ani), C (coccygeus), V (vagina), U (urethra), R (rectum). Adapted from Barber et al., (2002)

Bottom: Location of levator ani motor neurons labeled with CTB in relationship to Onuf's nucleus anal rhabdosphincter motor neuron labeled with fast blue in a longitudinal section from the S1 spinal cord of squirrel monkey. A. Note diffuse distribution of levator ani motoneurons along the longitudinal plane. B. Close up of boxed area in panel A. Note lateral projection of dendrites into area identified as Onuf's nucleus. C and D. High power photomicrographs of the same area viewed under fluorescein or fast blue filters, respectively, showing close apposition of CTB labeled processes (either dendrites or axon collaterals) of levator ani motor neurons with an anal sphincter motor neuron labeled with fast blue.

Bottom: Location of levator ani motor neurons labeled with CTB in relationship to Onuf's nucleus anal rhabdosphincter motor neuron labeled with fast blue in a longitudinal section from the S1 spinal cord of squirrel monkey. A. Note diffuse distribution of levator ani motoneurons along the longitudinal plane. B. Close up of boxed area in panel A. Note lateral projection of dendrites into area identified as Onuf's nucleus. C and D. High power photomicrographs of the same area viewed under fluorescein or fast blue filters, respectively, showing close apposition of CTB labeled processes (either dendrites or axon collaterals) of levator ani motor neurons with an anal sphincter motor neuron labeled with fast blue.

A potentially important finding is that the dendrites of levator ani motor neurons project into Onuf's nucleus to form close appositions with sphincter motor neurons (Figure 23) in both monkey (Pierce et al., in preparation) and rat [229]. Presumably, these appositions reflect a neuroanatomical substrate for coordination of the rhabdosphincter and the pelvic floor muscles during micturition and defecation [231,232].

Analysis of the urethral closure mechanisms during sneeze-induced stress conditions in urethane anesthetized female rats has revealed that pressure increases in the middle portion of the urethra are mediated by reflex contractions of the external sphincter as well as the pelvic floor muscles [233]. Transection of the pudendal nerves reduced urethral reflex responses by 67% and this was increased by an additional 25% by transecting the nerves to the iliococcygeus and pubococcygeus muscles. 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 pathways. However in conscious chronic spinal cord injured female rats transection of the hypogastric nerves reduced residual volume and maximal voiding pressure and increased voiding efficiency indicating that sympathetic pathways to the bladder neck and proximal urethra contribute to functional outlet obstruction and voiding dysfunction after spinal cord injury [234].

4. EMG STUDIES OF THE INNERVATION OF STRIATED MUSCLE OF THE URETHRA, ANUS AND PELVIC FLOOR

In man, the motor innervation of the external urethral sphincter (EUS), as well as that of the external anal sphincter and pelvic floor muscles, has been explored extensively especially by concentric needle electromyography (CNEMG) which permits isolation of sphincter activity from adjacent perineal musculature (see Clinical Neurophysiology Chapter).

Although testing of sphincter function can be done during voluntary contractions it is almost impossible to contract the external urethral sphincter, or indeed any other individual pelvic floor muscle, individually. Indeed, the external anal sphincter (EAS), being more accessible is often used as a surrogate for the EUS [235]. More reliable and consistent activation of the EUS motoneurons can be achieved by direct electrical stimulation of the muscles themselves through their terminal pudendal motor nerves [236], or at the level the sacral anterior nerve roots with

non-invasive magnetic stimulation and indirectly through transcranial magnetic stimulation (TMS) to facilitate excitability in the anterior horn motoneurons supplying the pelvic sphincters [237]. Selective stimulation of the pudendal afferent nerves, for example via dorsal penile or clitoral nerves [238], is also a reliable way to reflexly activate the sphincters and is often used to assess the integrity of sacral segmental pathways (see S7A and Chapter on Clinical Neurophysiology). However, such reflexes do not necessarily differentiate activity in the pelvic floor muscles and so DPN or DCN stimulation often elicit pudendo-anal, pudendo-urethral and bulbo-cavernosus reflexes simultaneously. Perhaps this is not surprising in the human as, unlike many other mammals, the anterior horn cells for the pudendal nerves to these muscles are closely organised in Onuf's nucleus [239].

III. MODULATION OF THE MICTURITION REFLEX BY SACRAL INTERNEURONES

The micturition reflex can be modulated at the level of the spinal cord by interneuronal mechanisms activated by afferent input from cutaneous and muscle targets as well as inputs from other visceral organs [3,189,197,206,240-242]. A potential site for modulation is at the first synapse in the reflex pathway between the bladder primary afferent and the second order projection neurons in the LCP, lamina I and/or the intermediate gray matter [241-243]. Stimulation of afferent fibers from various regions (anus, colon/rectum, vagina, uterine cervix, penis, perineum, pudendal nerve) can inhibit the firing of sacral interneurons evoked by bladder distension [197,206,243]. This inhibition may occur as a result of presynaptic inhibition at primary afferent terminals or due to direct postsynaptic inhibition of the second order neurons. Direct postsynaptic inhibition of bladder PGN can also be elicited by stimulation of somatic afferent axons in the pudendal nerve or by visceral afferents from the distal bowel [244]. There is recent evidence that sacral preganglionic neurons in the neonatal rat cord receive inputs from the lateral funiculus, the dorsal commissure and from local interneurons, and that glutamate is a major neurotransmitter at these synapses. The interneurons in the SPN and DCM regions make direct synaptic excitatory contacts with parasympathetic preganglionic neurons, and some have inhibitory effects on the motoneurons innervating the external urethral sphincter [192,209,245,246].

Interneurons dorsal and medial to the SPN receive primary afferent inputs and make excitatory connections mediated by glutamatergic receptors [192,209]. However a minority of the medial group produce inhibitory synaptic responses in the PGN mediated by the release of glycine and gamma amino-butyric acid (GABA) which inhibit distension-induced firing [247 241,246]. Individual neurons release both transmitters. Clinical studies have revealed that intrathecal administration of a GABA-B receptor agonist (baclofen) increased the volume threshold for inducing the micturition reflex [189].

Neurophysiological [240,242,248-250] and neuroanatomical techniques [195 245] including trans-synaptic tracing of pathways with pseudorabies virus and the expression of the immediate early gene, c-fos (Figure 19) have identified interneurons that receive input from mechanoreceptor and nociceptor afferents from the bladder. There is agreement between these techniques that interneurons concerned with bladder and/or urethral sensation and reflexes are located in the region of the sacral parasympathetic nucleus (SPN), the dorsal commissure (DCM) and the superficial laminae of the dorsal horn (Figures 19 and 20).

The superficial DH interneurons appear to be related to nociception rather than to distension with low volumes, and it should be emphasized that the vast majority of bladder-responsive interneurons identified also respond to other stimuli.

In the rat electrical stimulation of the pelvic nerve triggers changes in the sacral cord including c-fos expression and alterations in neuropeptide immunoreactivity [251 252] which is likely to be the basis of the effect of neuromodulation seen in man. Suppression of overactive bladder and of urge incontinence in patients by pudendal nerve or sacral root stimulation may reflect in part activation of the afferent limb of these viscerobladder and somatobladder inhibitory reflexes [253-255].

A phenomenon that cannot be studied in the experimental animal but which is important in man is the voluntary inhibition of voiding when urgency is sensed but voiding deemed to be inappropriate, by contraction of the striated sphincter and pelvic floor. This effect is thought to be mediated by urethral afferents and it has been shown in animals that it is the contraction of the striated muscle that is necessary to achieve this inhibition [13].

IV. REFLEX INFLUENCES ON THE STRIATED MUSCLE OF THE SPHINCTERS AND PELVIC FLOOR IN HUMANS

In man, most muscles of the pelvic floor, including the urethral and anal sphincters, can be reflexly excited by stimulating pudendal afferent nerves at various sites in the perineum. However, probably more interesting is the way in which pudendal nerve reflexes interact with autonomic pelvic reflexes of the bladder to control continence.

For example, experimental studies in cats has demonstrated a group of reflexes operating during bladder filling and storage whose function is to maintain continence through spinal reflex pathways involving the pontine micturition centre in the brain stem [189].

As the bladder slowly fills, low-level vesical afferent activity in the pelvic nerves increases progressively to enhance external urethral sphincter tone to provide continence. This supra-spinal involuntary reflex may also be associated with an inhibitory spinal mechanism which simultaneously suppress pre-ganglionic parasympathetic activity to the bladder to further assist continence (Figure 23).

That man possesses a similar reflex mechanism which can sometimes become aberrant, as for example in complete spinal cord injury, has been known for many years [256]. The “guarding reflex” as it has become known, is a concept originally proposed by Garry et al [257]. (again from experimental studies on cats) to mitigate against stress incontinence. More recently however, Park et al have described this reflex as a defence against the ‘inherent tendency’ for the bladder to ‘empty itself during filling and it is necessary that (this) tendency is harnessed by inhibitory neural circuits to maintain low-pressure storage and continence’ [258].

V. MODULATION OF MICTURITION AND SPHINCTERIC REFLEXES BY DESCENDING PATHWAYS

The sacral cord also receives inputs from multiple descending supraspinal systems; e.g. from the pontine micturition center [49], paraventricular hypothalamus [259], and serotonergic raphe spinal neurons

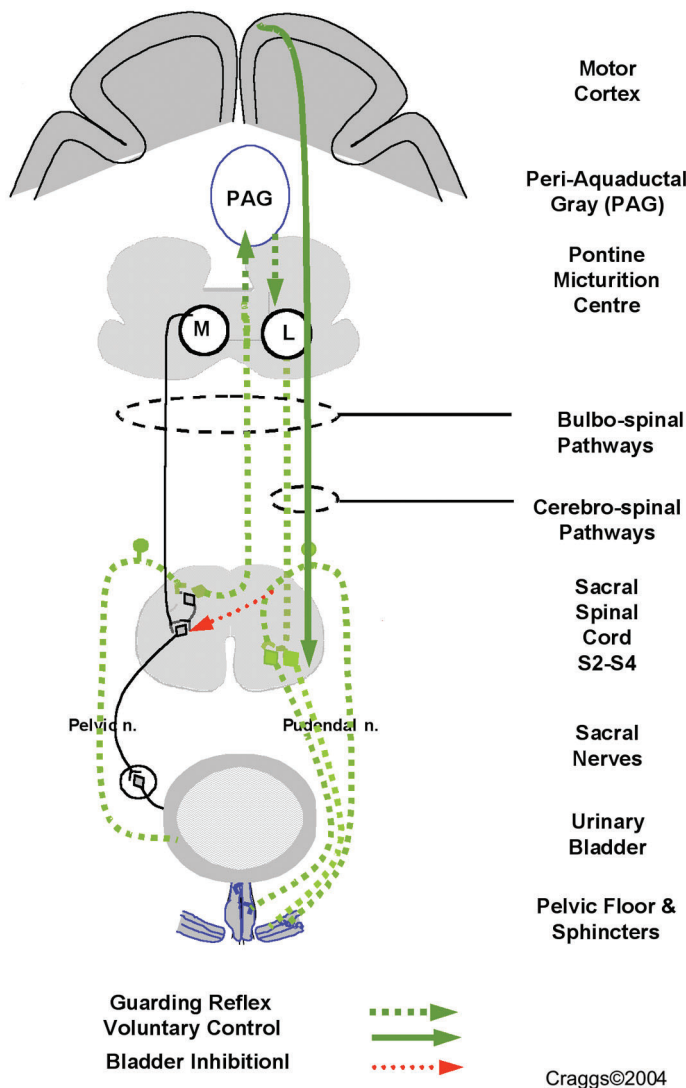


Figure 23. Pathways of the Guarding Reflex.

[260,261]. This convergence of numerous pathways in the LCP suggests that the processing of sensory input from the lower urinary tract is likely to be subjected to complex regulation and therefore may be susceptible to various neurological diseases and thus play a critical role in the emergence of neurogenic bladder disorders.

1. DESCENDING AMINERGIC PATHWAYS

The sacral dorsal horn, SPN and Onuf's nucleus are densely innervated by serotonin (5-hydroxytryptamine, 5-HT) immunoreactive terminals [262,263] and also exhibit moderate to high densities of various 5-HT receptor subtypes [264]. These pathways appear to be closely involved in transmission in Onuf's nucleus [264] and the activation of 5-HT₂ and α_1 -adrenergic receptors enhance contraction of the EUS, probably via interactions within the sacral

cord. The origin of the **serotonergic** pathways is in the brainstem and these seem to be important in regulating voiding function. Neurophysiological studies have located rostral medullary raphe (i.e. nucleus raphe magnus, NRM) cells that respond to bladder distension [4,5]. Chemical or electrical stimulation of the raphe nucleus [265] or administration of serotonergic drugs [266] inhibits reflex bladder activity. Intrathecal administration of 5-HT blockers decreased the threshold volume for perception of fullness in unanesthetized cats [267], and depletion of noradrenaline or 5-HT in the spinal cord increased micturition volume in conscious rats [250]. These observations imply that descending serotonergic pathways tonically modulate afferent information at the sacral spinal level.

The **noradrenergic** system in the brain stem has also been implicated in the control of lower urinary tract function [4-6,268,269]. Norepinephrine (NE)-containing terminals are prominent in the sacral dorsal horn, SPN and Onuf's nucleus [270,271]. Adrenergic neurons in the A5 region and in the ventral portion of locus coeruleus and subcoeruleus as well as in the Kollicker-Fuse nucleus (A7 noradrenergic cell group) were labeled after injection of pseudorabies virus into the bladder or urethral sphincter [195,245] (Fig. 10). Electrophysiological studies showed that bladder distension activates adrenergic neurons in locus coeruleus [272]. The results of experiments using α_1 and α_2 adrenergic antagonists vary with anaesthesia, and species, and are difficult to interpret because of concomitant effects on micturition pressure and residual urine volume [1,273-275]. Intrathecal administration of a receptor subtype selective antagonists reveal that α_{1A} or α_{1B} receptors inhibit the frequency of bladder contractions on CMGs in anesthetized rats [276]. However, some α_{1A} receptors facilitate the descending limb of the micturition reflex pathway.

Dopaminergic terminals which are thought to arise from dopamine-containing neurons of the A11 cell group in the diencephalon have been identified in the sacral spinal cord in the regions of visceral afferent projections, the SPN and Onuf's nucleus [277].

2. SPINAL OPIOID MODULATION

An inhibitory modulation by opioid peptides of the afferent limb of the micturition reflex at a spinal level has been suggested [1]. However the effects of morphine and its metabolites are dependent on species, anaesthesia and mode of administration, and the

available data [214,278] suggest that spinal opioid mechanisms may be able to influence the afferent limb of the micturition reflex in rats. This may be important in man.

Opioid peptides have an inhibitory action on reflex pathways in the spinal cord. It is clear that activation of spinal δ - and probably μ -opioid receptors but not kappa-opioid receptors inhibits isovolumic contractions evoked by suprathreshold distension of the bladder [279-282]. Inhibition of reflex bladder activity is mediated by δ -receptors (cat) or μ - and δ -receptors (rat); whereas inhibition of sphincter activity is mediated by kappa-receptors [5,189]. Dynorphin-B, a kappa-opioid antagonist, had no effect on micturition even at 10 times its analgesic dose [279].

VI. SELECTIVE PHARMACOLOGICAL MODULATION OF SPHINCTERIC ACTIVITY

There are certain drugs acting on monoaminergic and opioidergic receptors that elicit a selective modulation of the EUS reflex pathway and offer the hope of new therapeutic approaches.

1. MONOAMINERGIC MECHANISMS

Reflex activation of urethral striated muscle in chloralose-anesthetized cats is modulated by monoamines acting via α_1 and α_2 adrenoceptor [283-285]. α_1 adrenoceptor stimulation facilitates sphincter reflexes, while α_2 adrenoceptor stimulation inhibits sphincter reflexes. Stimulation of 5HT₂ serotonin receptors also facilitates sphincter reflexes [264].

2. OPIOID MECHANISMS

There appear to be some effects of selective opioid receptor agonists that can influence the reflex activity of the EUS or the bladder but not both. A κ -opioid agonist has been shown to inhibit pudendal nerve reflex firing in chloralose anesthetized cats and leave hindlimb reflex and bladder activity unaffected [286]. In rats, K_2 opioid agonists disrupt the spinal pattern generator responsible for high frequency oscillations of the urethral rhabdosphincter that occur during voiding in this species [287], and the effects are reversed by appropriate doses of naloxone. In contrast, a δ -opioid agonist (DSLET), abolished bladder activity and reduced the sphincter reflex to about 60% of control, leaving a hindlimb reflex unaffected [286]. Thus the spinal opioid modulation of the external urethral sphincter has characteristics quite different from those regulating the bladder.

VII. TESTING OF LUT REFLEX PATHWAYS IN HUMANS

Stimulating somatic afferent nerves in the perineal region usually elicits reflex contractions of the sphincter muscles of the urethra and anal canal and muscles of the pelvic floor [288], and are used to assess the integrity of sacral reflex pathways [289]. After standard neurological testing of the sacral dermatomes such as perineal (eg peri-anal) skin stimulation with a blunt needle to assess sensation and reflex effects on the anal sphincter, these electrophysiological tests including the pudendo-anal (PAR) and bulbo-cavernosal (BCR) reflexes are the easiest to elicit and record and therefore the most frequently used (**Figure 24 A**). The pudendo-urethral reflex (PUR) on the other hand requires more skill as the external urethral sphincter is small and less accessible and hence recordings need to be more precise using needle electrodes to avoid artefact from other large adjacent muscles.

Selective electrical stimulation of pudendal afferents is possible at the level of the dorsal penile or dorsal clitoral nerves (DPN/DCN), and single, paired pulses or trains of pulses can be used to elicit both sensory effects and reflex effects on the sphincters and pelvic floor muscles. Paired electrical pulses to the DPN or DCN (2-4 milliseconds apart) usually give the most reliable anal sphincter reflex responses (**Figure 24 B**) [290]. Electrical stimulation threshold levels for sensation are normally lower than those for reflex effects but disease or trauma in the sensory, motor or sacral segmental pathways can all markedly affect these levels.

Stimulating other sites in the perineum, particularly in the anal canal, vagina or on perineal skin, also elicits relatively low threshold sacral reflexes of the pelvic muscles and sphincters; but with higher levels of cutaneous stimulation, discomfort or pain or direct stimulation of pelvic floor muscles can limit the usefulness of these sites in normally sensate subjects. Interestingly, the use of relatively low levels of intra-vaginal electrical stimulation, can inhibit bladder overactivity experimentally [291] and clinically [292] (see also the report of the Committee on Conservative Management).

Recently, low levels of ano-genital electrical stimulation have been used successfully to treat children with urge incontinence [293]. DPN/DCN stimulation with low frequency electrical pulses (10-20Hz) not only inhibits detrusor contractions [294] but interestingly, delays the desire to void in healthy men [295].

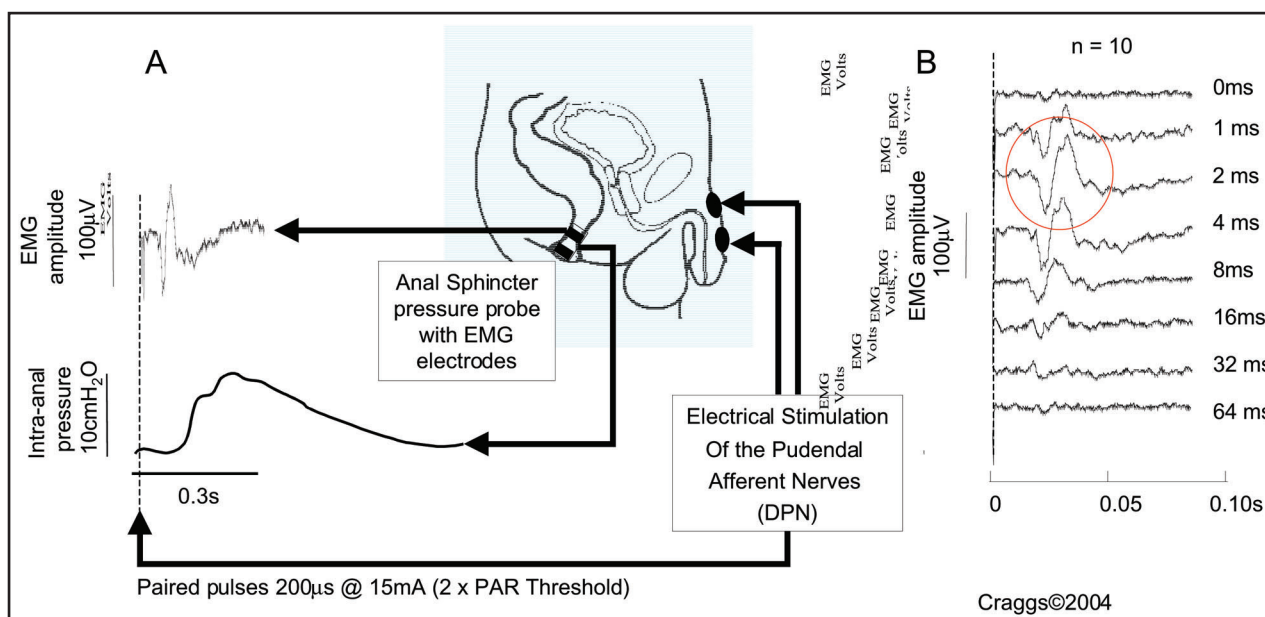


Figure 24. The optimised Pudendo-Anal Reflex

Measured sensations of bladder filling and urge in patients presenting with urge incontinence are also suppressed by selective pudendal afferent stimulation (electrical neuromodulation), prolonging continence and increasing bladder capacity [296].

Neuromodulation of this sort can also be achieved with electrical stimulation of the pudendal afferent nerves lying in the mixed sacral nerves using electrodes in the sacral foramen [297] or alternatively with non-invasive magnetic stimulation of the mixed sacral nerve roots [298].

Clearly, sacral somatic afferent activity whether generated naturally or artificially with stimulation has important effects on the neural control of continence both by reflex activation of the sphincters and inhibitory effects on the excitatory autonomic pelvic reflexes of the urinary bladder. Furthermore, stimulating the somatic afferents has a profound attenuating effect on the sensations of bladder fullness and the desire to void.

E. CENTRAL PATHWAYS THAT CONTROL THE BLADDER

Injuries or diseases of the nervous system in adults are amongst some of the causes of disruption of the voluntary control of micturition. These conditions may cause the re-emergence of reflex micturition, resulting in bladder hyperactivity and incontinence [4-7]. It should be noted that the complexity and the extent of central nervous control of the lower urinary tract, makes it particularly vulnerable to disruption by a wide range of neurological disorders (Figure 25).

Prior to the development of functional brain imaging methods our understanding of the supraspinal pathways involved in bladder control in man was based on clinical case descriptions correlating clinical findings with pathology at specific sites, and extrapolation from the results of animal experiments. Functional brain imaging using positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) has provided a window into the brain and in recent years these techniques have been usefully applied to the analysis of cerebral control of the bladder. However, it should not be forgotten that although the pictures produced by these experiments give a striking visual result they are the result of extensive mathematical manipulation of data. Neither fMRI nor PET show neural activity directly but detect a signal of vascular and metabolic origin res-

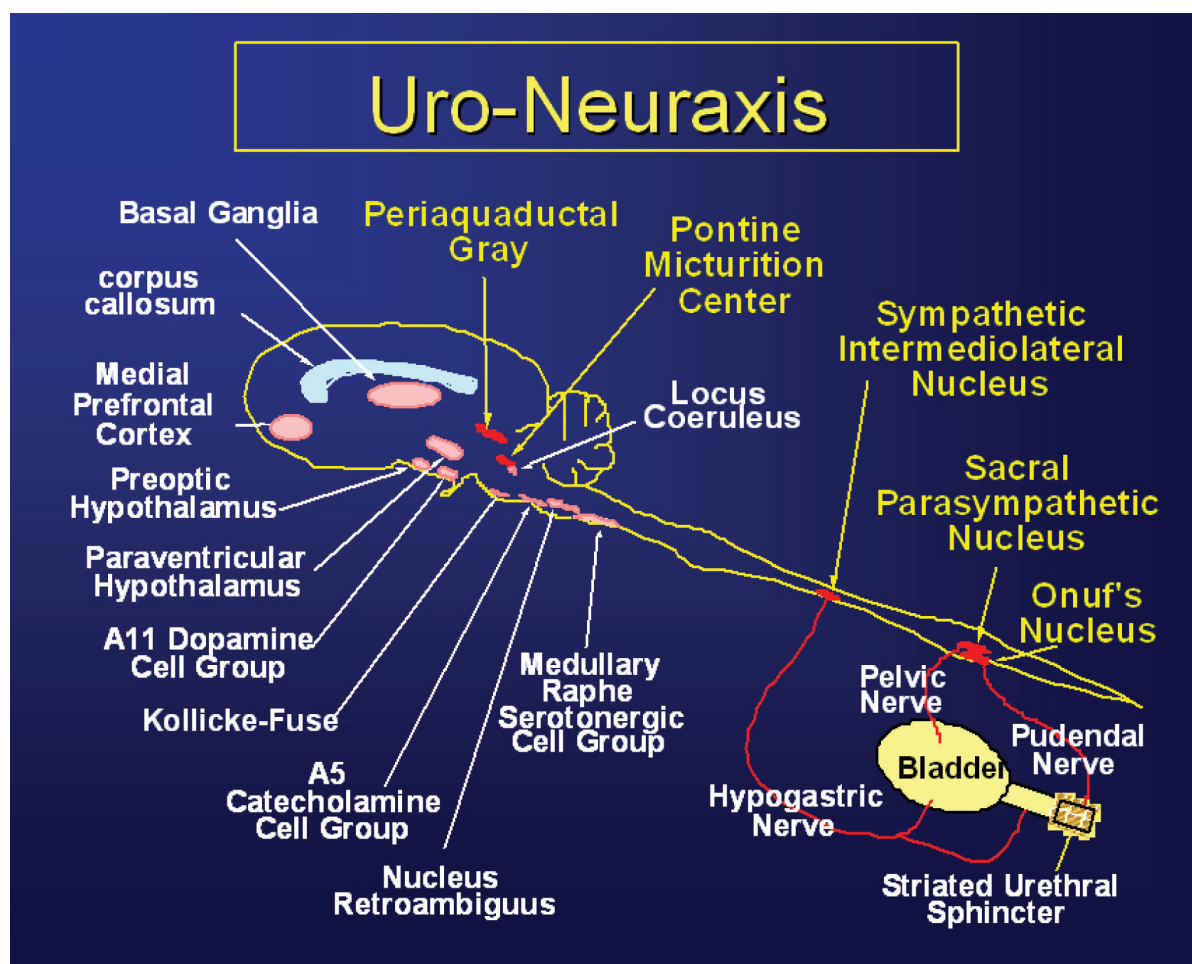


Figure 25. Diagram showing neuroanatomical distribution of brain and spinal cord nuclei involved in CNS regulation of lower urinary tract function. The primary components of the storage and micturition reflexes are shown in red and labeled in gold. The secondary (or modulatory) components are shown in pink and labeled in white.

pectively; as a result the excitatory or inhibitory nature of the neurotransmitters release by increased activity and metabolism cannot be distinguished.

I. SUPRASPINAL PATHWAYS

1. PONTINE MICTURITION CENTER (PMC).

The dorsolateral pontine tegmentum is firmly established as an essential control center for micturition in normal subjects. First described by Barrington in the cat [299] it has subsequently been called “Barrington’s nucleus”, the “pontine micturition center”, or the “M region”[300-49]. In the cat neurons in the brain stem at or below the level of the inferior colliculus have an essential role in the control of the parasympathetic component of micturition, whereas areas of the brain above the colliculus appear to have predominantly inhibitory effects.

Neurons in the PMC in the cat provide direct synaptic inputs to sacral parasympathetic preganglionic neurones (PGN) [301] (Fig. 26), as well as to neurons in the sacral dorsal commissure (DCM). The former neurons carry the excitatory outflow to the bladder, while the latter are thought to be important in mediating an inhibitory influence on EUS motoneurons [302]. As a result of these reciprocal connections the PMC can promote bladder-sphincter synergy. The PMC projection also overlaps with the projections of bladder (and other, e.g. pudendal) primary afferent neurons in the cat and the primate [191,303]. This may be an anatomical substrate for primary afferent depolarization, and thus suppression, of urethral/perineal afferent inputs during micturition but it is expected to be mediated through an interneuron [304,305].

Because of its close anatomical relationship with noradrenergic cells in the locus coeruleus in cats [306], some studies have mistakenly identified the locus coeruleus as the essential region for controlling

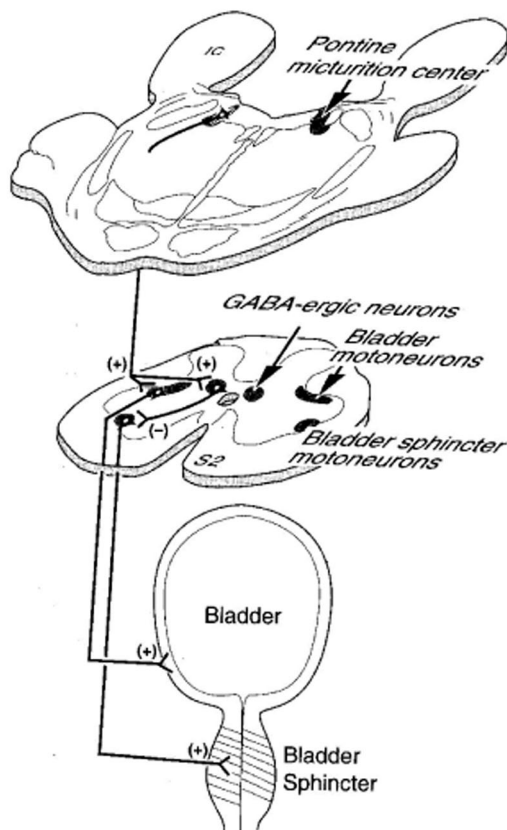


Figure 26. Coordination of voiding with external urethral sphincter. Descending glutaminergic input to sacral spinal cord excites bladder neurons in sacral parasympathetic neurons (SPN). Simultaneous activation of GABA neurons inhibits sphincter neurons in Onuf's nucleus. Conversely, during attempts at urine storage activation of GABA neurons providing input to bladder neurons while providing excitatory input to sphincter neurons.

micturition [268]. The presence of reciprocal connections between the PMC and locus coeruleus [306-308], and the existence of neurons in the PMC that project to both the sacral spinal cord and the locus coeruleus via axon collaterals [308] (Fig. 27), may explain some of the difficulties distinguishing these two cell groups. In addition to providing axonal inputs to locus coeruleus and the sacral spinal cord [307-309], neurons in the PMC also send axon collaterals to the paraventricular hypothalamic nucleus (Fig. 27), which is thought to be involved in the limbic system modulation of visceral behavior [309]. Some neurons in the pontine micturition center also project to the PAG, which regulates many visceral activities as well as pain pathways [310]. Thus, neurons in the PMC communicate with multiple supraspinal neuronal populations that may coordinate micturition with other functions of the organism.

Modulatory influences on the PMC

Dopamine and its agonists have mixed effects in bladder function in experimental animals depending on the site and mode of application. Bladder hyperactivity is a feature of parkinson's disease-like models in experimental animals [5,311]. In cats and monkeys, microinjection of dopamine into the PMC facilitated the micturition reflex [6], whereas intracerebroventricular administration of dopamine or a D-1 dopamine receptor agonist (SKF38393) inhibited the micturition reflex [312]. These findings imply that dopaminergic regulation of micturition may occur at several levels but the details are not yet clear. Some patients with Parkinson's disease may exhibit bladder overactivity [5,189] but the contributions of disease duration and treatment with anti-parkinsonian agents have not been satisfactorily delineated.

The role of opioid peptides in modulating micturition has been studied using focal microinjections as well as icv injection of morphine-like agonists and antagonists [278,282,313]. Both μ - and δ -opioid but not kappa-opioid receptors can mediate inhibition of micturition [282,314], but apparently by different mechanisms.

The first PET studies of bladder function to be carried out looked primarily at brain activation during voiding. In the pioneering studies of Blok et al., it was shown that in both male and female volunteers who were able to void in the scanner, there was increased blood flow in an area in the right dorsomedial pontine tegmentum close to the fourth ventricle, presumed to be the PMC [315,316], (Figure 27). Those who could not void under these experimental conditions had brainstem activity more ventrally (see below) (Figure 27). These findings were interpreted as showing the neurological organization of bladder control in man was essentially similar to that which had been elucidated in the cat and what data there is from the clinical literature supports the view that, in humans, bladder control resides in the rhombencephalon.

The brain stem is the site of many critical neural functions such that extensive brainstem disease is often not compatible with survival. However there are a few cases histories of discrete pontine lesions presenting with urinary retention and a systematic study of brainstem vascular lesions established that it was those dorsally situated that were associated with bladder dysfunction, whereas ventral lesions were not [317].

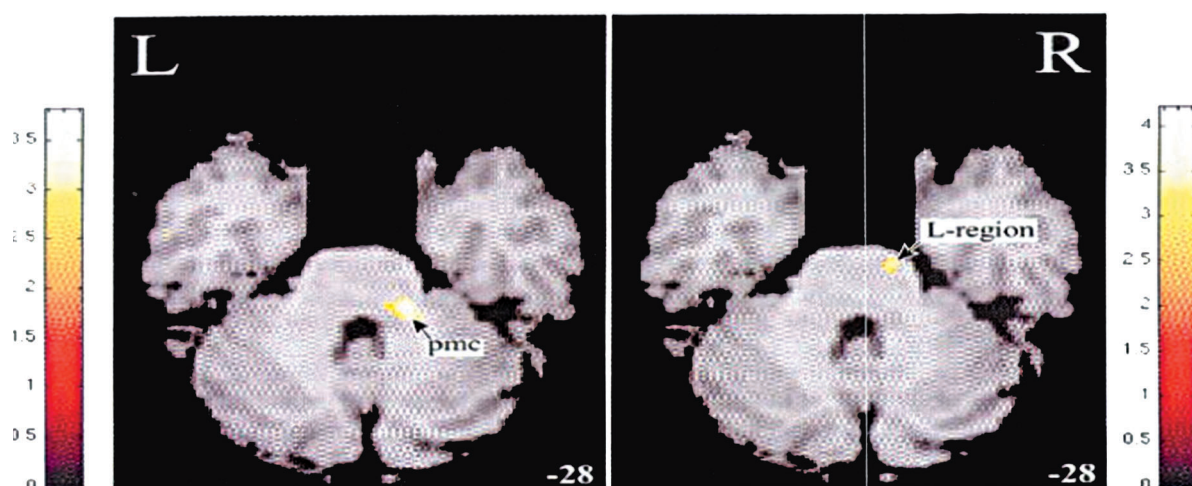


Figure 27. PET images of brainstem. Left: significant differences in rCBF in the right dorsal pontine tegmentum (indicated by pmc) after comparison between conditions 'successful micturition' and 'empty bladder'. Right: significant difference in rCBF in the right ventral pontine tegmentum (indicated by L-region) after comparison between the conditions 'unsuccessful micturition' and 'empty bladder' (Blok, Sturms and Holstege, 1998)

2. PONTINE L-REGION

In the cat pons a region located laterally to the PMC (hence the term “L region”) (Fig. 27) and just ventral to the brachium conjunctivum contains neurons that send a prominent input to EUS motoneurons in Onuf’s nucleus as well as a projection to the thoracolumbar PGN (Fig 6) [318]. Bilateral lesions of the L region in some cats produced bladder hyperreflexia and incontinence [319], but a recent reappraisal of the experimental findings proposes that whereas the M-region in cat is small, specific and necessary to voiding, the L-region is part of a larger less specific area that probably serves sphincter control in various circumstances when increased activation is required such as during respiration, coughing, sneezing, or sexual activity [320].

In the PET experiments in healthy male and female volunteers it was found that in those unable to micturate during scanning, brainstem activation was more ventrally located in the pons, in a location somewhat comparable to that of the L region in cats. It was suggested that the volunteers who were unable to void, probably did so because they did not feel themselves in a safe environment, contracted their urethral sphincter and withheld urine despite wanting to void [315]. That exact experimental paradigm has yet to be repeated but if activity in this more ventral location were to be essential in withholding urine, its activation would be expected in those functional imaging experiments that have looked at particularly at the bladder storage phase and this is not inevitably the case [321,322].

The proposal that there is an area in the pons that is active during bladder storage lent itself readily to the hypothesis that the “switching” mechanism originally proposed by de Groat [300], instead of switching the PMC “on” and “off”, switched between the “L” and the “M” regions during storage and voiding respectively. However it is well established that the mechanism for bladder storage is organised at a spinal level and alternative switching in the pons between a “micturition” and a “storage” centre may not be correct – input from the “L” region being involved in enhancing sphincter contractions rather than determining the process of storage.

3. PAG (PERIAQUEDUCTAL GREY MATTER)

The PAG is part of the “emotional motor system”, crucial for survival of individual and species and is involved in the control of many uncomplicated primary reactions such as aggression, defensive, maternal, reproductive behaviour [323-325].

There are extensive projections from various lamina in the the lumbosacral spinal cord to the PAG [194,326,327]. In the cat, the direct spinal projection to the PMC is very sparse [328], but there is a substantial projection from the ventrolateral PAG to the PMC [328,329]. This spinal cord – PAG – PMC pathway has been proposed to be the micturition reflex pathway, but there is as yet little evidence that the afferent path carries information on the state of the bladder [330]. Clearly the many demonstrated roles played by the PAG would dictate a diversity of afferent input. The PMC in the rat receives neural

projections from the lumbosacral spinal cord directly [331] and from the ventrolateral PAG. In this species, neurotransmission blocking experiments have confirmed the importance of the caudal ventrolateral PAG to bladder function [322]. Thus, although a role for the PAG in micturition is plausible based on anatomical connections and functional data in the rat, it remains to be confirmed as a major component of bladder control in other species.

The results of functional imaging experiments in man are supportive of the view that the PAG is an important relay station of bladder afferent activity. Although in the first PET imaging studies PAG activation was seen in the withholding phase prior to micturition in men but not women [315,316], subsequent studies focussing on the storage phase in healthy male volunteers showed increasing activation in the PAG with increasing bladder volumes [321] and a study which looked at activation during natural filling to capacity showed activation of the PAG with the same brain co-ordinates [322] (**Figure 28**).

4. CEREBELLUM

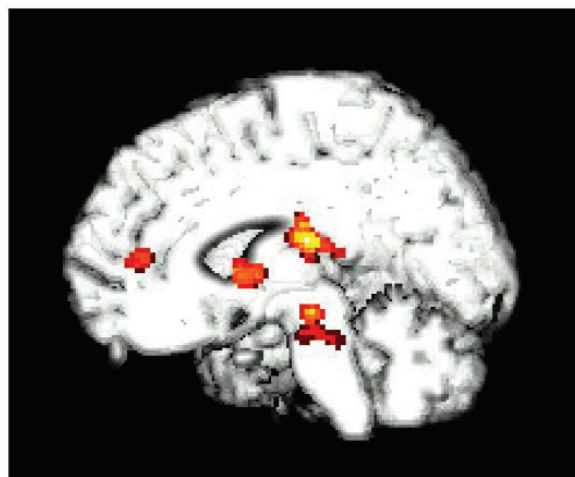
From observations of the effect of cerebellectomy on reflex micturition in the decerebrate dog it was proposed that the cerebellum has an inhibitory role in storage and a facilitatory role during emptying [332]. Reciprocal connections have been demonstrated between the cerebellum and the hypothalamus raising the possibility of a significant influence of cerebellum on autonomic function [333-336].

In functional imaging studies activation of the cerebellum has been seen during storage [321,322] and micturition [337]. Other functional imaging experiments examining cortical processing of human somatic and visceral sensation by stimulating the proximal and distal oesophagus found the greatest difference in stimulation-evoked brain responses to be in the cerebellum [338], with activation most marked with the more distal visceral stimulation. Cerebellar activation is thought to be brought about by activation of C fibres but not A delta fibres [338] and the sensation of non-painful distension of the bladder is thought also to have a significant C-fibre mediated component [321].

5. HYPOTHALAMUS

In animals there is a robust spinohypothalamic projection, especially from the sacral segments [339,340]. That bladder afferents connect with this pathway is implied by virus tracing experiments

Matsuura et al, 2002



Athwal et al, 2001

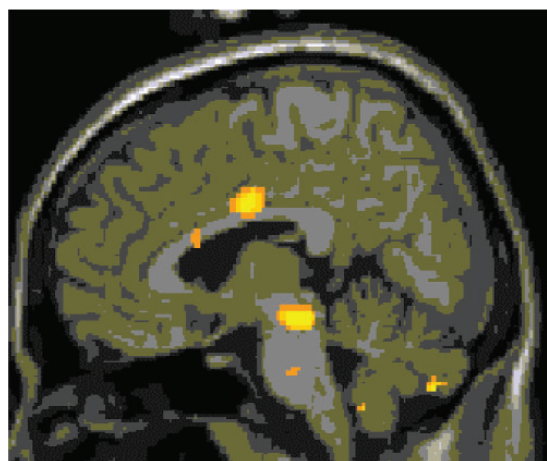


Figure 28. PET images of activation seen with a full bladder compared to empty (a) and increasing volumes (b). Note differences in activation in cingulate cortex (see text for details)

[341]. Lumbosacral spinohypothalamic cells lie predominantly in the deep dorsal horn [339] and a significant proportion respond to visceral as well as somatic stimuli, although bladder distension was not tested [342]. Stimulation in the hypothalamus can produce a variety of visceral responses, including bladder contraction or inhibition depending on the site stimulated [343-346]. The hypothalamus projects widely to the brainstem and spinal cord [359], including to supraspinal areas directly related to bladder control, PAG and PMC [347]. Virus tracing experiments also verify a multisynaptic connection between the hypothalamus and bladder and external

sphincter [245-348]. Thus there are plausible pathways through which bladder responses could be elicited from the hypothalamus. Given the variety of responses achievable from various sites in the hypothalamus and its extensive projections to the brainstem and spinal cord, the modulatory role of the hypothalamus on bladder control is likely to be complex and requires substantially more study.

The paraventricular hypothalamus is one of the richest sources of neuropeptide neurotransmitters in the brain. Thus, it is possible that some of the peptidergic transmitters found in the SPN arise from the paraventricular nucleus. Oxytocin containing projections from the brain, presumably the hypothalamus, are found in the sacral spinal cord [349]. Intrathecal oxytocin reduced the volume threshold for micturition and increased micturition pressure on awake cystometrograms in female rats and this was blocked by oxytocin receptor antagonist implying that the effect was receptor mediated [350]. However the lack of an effect by the antagonist alone makes a role for oxytocin in normal micturition uncertain. Whether this transmitter and hormone acts in the dorsal horn on afferent input, interneurons or directly on autonomic preganglionic neurons is uncertain. It should be noted that the paraventricular nucleus is also implicated in other autonomic functions, for example, the control of penile erection [349,351], so care must be exerted in ascribing roles to hypothalamic pathways.

The medial preoptic region (MPO) of the hypothalamus is important for reproductive behaviour in rats [352]. It projects extensively to hypothalamus and brainstem, particularly to PAG and PMC [259,353-355]. (Figure 6) and stimulation of MPO elicits bladder contraction [344,356].

Further linkage between the medial preoptic nucleus and micturition has emerged from functional brain imaging studies which showed increases in regional blood flow in this region of the hypothalamus during micturition [315] as well as with increasing bladder filling [321] (Figure 29).

6. CORTICAL PATHWAYS

Although the forebrain is not essential for the micturition reflex in experimental animals, the voluntary control over micturition necessary for social continence in man and cats, dogs and other domesticable species is likely to reside in centres within the cerebral cortex. Studies on animals suggest that the prominent facilitatory effect of decerebration in animals with an intact neuraxis seems to be due in large part to removal of tonic inhibition originating in the cerebral cortex [4,5]. Various cortical areas are labelled following injection of pseudorabies virus into the lower urinary tract [195,245,348]; thus, cortical control of voiding is likely to be complex. The prefrontal cortex of the rat is considered a visceromotor area, while the insular cortex is considered a viscerosensory area. In rats subjected to infarction of the middle cerebral artery on one side a prominent decrease in bladder capacity was seen, implying that there is tonic cortical inhibition of bladder function [357]. However, several types of synaptic changes related to both inhibitory and facilitatory mechanisms were observed, attesting to the complexity of cortical influences on bladder control [358-360]. In the cat, stimulation of forebrain structures, such as anterior cingulate gyrus, hypothalamus, amygdala, bed nucleus of the stria terminalis and septal nuclei, can elicit bladder contractions [344].

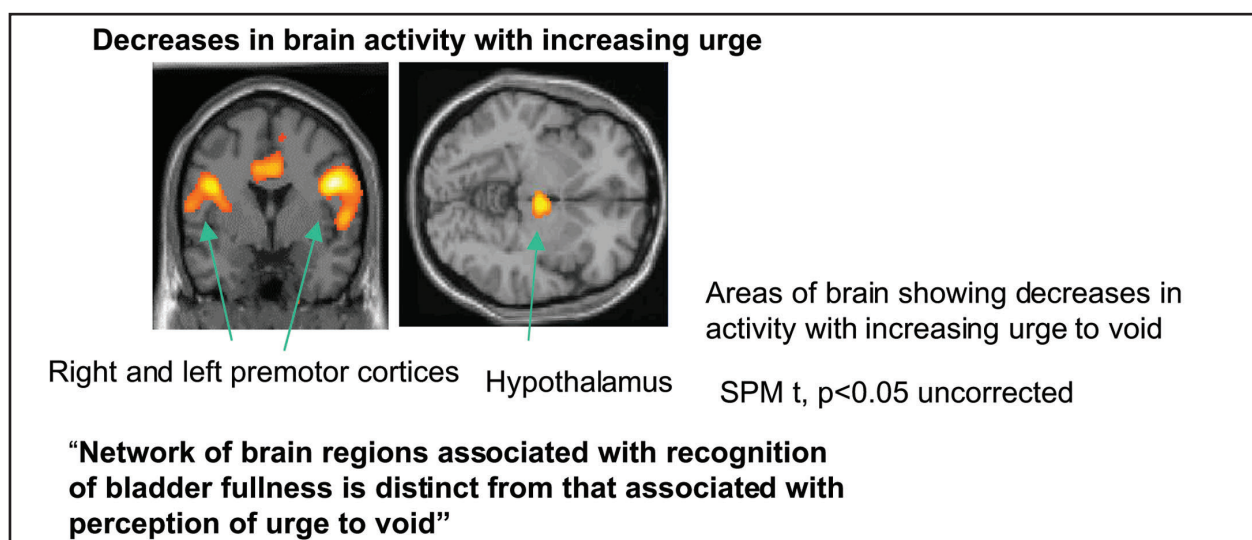


Figure 29. PET images showing decreases in brain activity with increasing urge to void. (Athwal et al, 2001)

That the medial part of the anterior regions of the frontal lobes is critical for bladder control in man was most clearly delineated by the study that described a series of patients with various frontal lobe pathologies. These included intracranial tumours, damage following rupture of an aneurysm, penetrating brain wounds and leucotomy which were correlated with the clinical histories. "The feeling of gradual distension of the bladder is lost and the only warning that the patient has that his bladder is full is the sensation associated with the imminence of micturition" [361]. The typical clinical picture of frontal lobe incontinence is of a patient with severe urgency and frequency of micturition and urge incontinence but without dementia, the patient being socially aware and embarrassed by their incontinence. It is only when the frontal damage is more severe that patients become disinhibited and unconcerned about their incontinence. Micturition is normally coordinated. Using SPECT imaging in the elderly, frontal cortex under perfusion was shown to be associated with urge incontinence and reduced bladder sensation [362]. Subsequently others have also reported the occurrence of urinary incontinence in patients with frontal lobe lesions due to a number of different pathologies, and there have been a small number of case histories of patients with frontal lobe pathology who had urinary retention and in whom there was restoration of voiding when treatment of the frontal lobe pathology was successful [363].

Following stroke a proportion of patients develop urinary incontinence and the general conclusion of various studies is that voiding is normally coordinated, and that the commonest cystometric finding is of detrusor overactivity. Although it has not been possible to demonstrate a correlation between any particular lesion site and urodynamic findings, anterior brain lesions are much more likely to be associated with incontinence than posterior, occipital ones [364].

The cingulate gyrus functions as part of the limbic system [365] and is an area that is very commonly activated by the wide range of tasks performed during functional imaging. It is thought to be involved in response selection, attention and subjective emotional experience and activity in this structure has been reported in a number of brain imaging studies of visceral sensation, particularly visceral pain [366,367]. There is differential activation of the cingulate depending on the nature of the task: more anterior regions showing activation with emotional and dorsal regions with cognitive tasks (Fig 28) [368].

In the first studies of voiding, cerebral blood flow in the cingulate gyrus was significantly decreased during voluntary withholding of urine and during the urge to void [316], whereas in the study of increasing filling [321] and of fullness to capacity [322] activation of the cingulate gyrus was also seen, albeit in a different part of the cingulate.

In the first studies of voiding, activation was seen in the right inferior frontal gyrus when micturition took place as well as during involuntary withholding of urine [316,337,369]. During the bladder filling experiment bilateral activation in the prefrontal cortex was seen with increasing fullness.

It may be that activation of the prefrontal cortex and the anterior cingulate gyrus do not reflect specific involvement in micturition, but more a general mechanisms, such as attention and response selection. The midbrain may be central in processing afferent activity about bladder volume whereas the cingulate and prefrontal cortex play a crucial role in restraint and inhibition, and making the decision whether or not micturition should take place at a particular time and place.

In the original PET studies there was a striking lateralisation of activation of brain structures to the right side, extending down to the level of the brainstem. The nature of these studies is that the image used to illustrate the findings is usually chosen to be that which shows the areas of highest statistical significance rather than all areas involved and it is likely that left sided structures were also activated but at a less significant level. Although those original studies were carried out in exclusively right-handed subjects subsequent studies used mixed volunteers and the same degree of lateralisation for bladder function was not observed.

Types of sensation

A new area of investigation that functional brain imaging in man has opened is the opportunity to study cortical activation in response to different types of sensation arising from the bladder. It appears that the network of brain regions associated with the recognition of bladder fullness is distinct from that associated with the perception of urge, urinary urge being associated with a decrease in activity in the hypothalamus, bilaterally in the premotor regions and the cingulate regions but no activity in the PAG. Activation of the primary somatosensory cortex was not seen with either changes in bladder volume or the urge to void but sensorimotor activity was observed during voiding in the presence of a

catheter [337]. A difference was also seen between natural bladder filling to capacity and filling with ice water in that there was no overlap between the areas activated in these two conditions, there being no PAG but marked somatosensory cortex activity when filling with ice water [322].

PET studies were conducted in adult female volunteers to identify brain structures involved in the voluntary motor control of the pelvic floor during four conditions: 1) rest; 2) repetitive pelvic floor straining; 3) sustained pelvic floor straining; and 4) sustained abdominal straining [315,370]. The results revealed that the superomedial precentral gyrus, the most medial portion of the motor cortex, is activated during pelvic floor contraction and the superolateral precentral gyrus during contraction of the abdominal musculature. In these conditions, significant activations were also found in the cerebellum, supplementary motor cortex, and thalamus. The right anterior cingulate gyrus was activated during sustained pelvic floor straining. No activation was found in the subcortical structures that have been shown to be involved with bladder storage or voiding.

F. SPINAL CORD INJURY

Acute spinal cord injury disrupts the normal connections between the sacral cord and the supraspinal circuits that control urine storage and release. Following days to weeks of urinary retention, hyperactive voiding develops. Electrophysiological data reveal that this detrusor hyperreflexia is mediated by a spinal micturition reflex that emerges in response to a reorganization of synaptic connections in the spinal cord [193,246,300]. In addition, bladder afferents that are normally unresponsive to low intravesical pressures become more mechano-sensitive leading to the development of detrusor overactivity. Normal micturition is associated with a spino-bulbo-spinal reflex mediated by lightly-myelinated A- δ afferents [5,300]. These fibers represent only 20% of bladder afferents in some species. Compared to A- δ fibers, the more prevalent unmyelinated C-fibers are relatively insensitive to gradual distention of the urinary bladder, at least in the cat [371]. Most C-fibers in this species remain silent during normal filling of the bladder although in the rat some studies indicate that C-fibers can fire at low pressures [372]; whereas other studies [373] showed firing at higher intravesical pressures of approximately 30 mm Hg. After spi-

nal cord injury, a capsaicin-sensitive C-fiber-mediated spinal reflex develops (Fig 12). These C-fiber afferents are thought to play a role in the development of bladder overactivity after spinal cord injury. Capsaicin sensitive C-fibers have also been implicated in detrusor overactivity following upper motoneuron diseases such as multiple sclerosis and Parkinson's disease [374,375].

Insight into the mechanism underlying the increased mechano-sensitivity of C-fibers after spinal cord injury has been gained by examining the dorsal root ganglion (DRG) cells supplying the bladder. Plasticity in these afferents is manifested by enlargement of these cells [376] and increased electrical excitability [116]. A shift in expression of sodium (Na⁺) channels from a high threshold tetrodotoxin (TTX)-resistant type to a low threshold TTX sensitive type occurs after spinal cord injury.

Plasticity in bladder afferents after spinal cord injury may involve the retrograde transport of substances from either the spinal cord or bladder to the DRG neuron. Bladder DRG neurons are responsive to a variety of neurotrophins, especially NGF which has been associated with hypertrophy of bladder DRG cells in a variety of conditions including obstruction and inflammation). Exposure of cultured DRG neurons to exogenous NGF promotes expression of TTX sensitive channels expressed following spinal cord injury [377]. Moreover, DRG from mice overexpressing NGF exhibit predominately TTX-S Na currents. Other trophic substances such as basic fibroblast growth factor (bFGF), brain-derived neurotrophic factor (BDNF), glial derived neurotrophic factor (GDNF), and neurotrophins-3-4 (NT-3-4) may trigger morphological and electrophysiological alterations in DRG cells after spinal cord injury. GDNF may be especially important because a small population of DRG neurons giving rise to C fibers are non-responsive to NGF, but respond to GDNF [378,379]. It is worth noting that the response of other neurogenic disorders associated with urge incontinence respond to intravesical capsaicin therapy suggesting that plasticity in C-fiber afferents could form the neurogenic basis for bladder overactivity [116,376]. The emergence of a spinal reflex circuit activated by C-fiber bladder afferents represents a positive feedback mechanism (Figures 7 and 12) that may be unresponsive to voluntary control by higher brain centers and thereby be able to trigger involuntary voiding.

The chronic effects of a spinal cord injury on the voluntary control of sacral reflexes in man can be

very variable. At one extreme, when the injury is supra-sacral and complete (neurologically defined as ASIA A (American Spinal Injuries Association) there is usually no modulation of pelvic floor reflexes such as the pudendo-anal (or urethral) reflex, whereas in incomplete injuries (ASIA B-D grades) the reflex is variably facilitated by volition depending on the extent of the lesion (**Figure 30**) [380]. Thus, when voluntary control over the sphincters and pelvic floor muscles is weak or absent, then unlike in healthy volunteers [238], there will be little or no volitional suppression of bladder reflex activity, resulting in neurogenic detrusor overactivity and incontinence. However, the level of such incontinence will depend on the extent of detrusor sphincter dyssynergia and its obstructive effects (see chapter on Neurogenic Patients).

As described earlier, normal bladder and sphincter function depend on the integrity of supra-spinal pathways to and from the brainstem. Such pathways include those that voluntarily control the pelvic floor and the striated urinary sphincter as well as brain-stem reflexes that coordinate the bladder and sphincters to enhance continence and produce efficient voiding. Collectively these comprise, among others, the so-called “guarding reflex”[257]. The normal guarding reflex functions to enhance sphincter closure and inhibit unwanted activity of bladder detrusor muscle as the bladder fills and so help to prevent incontinence. The peripheral afferent limb of the reflex is conveyed by the pelvic sensory nerves from the bladder to the sacral cord with an ascending pathway to the peri-aqueductal gray in the brain-stem and an efferent limb from the lateral nucleus in the pons

via the pudendal motor nerves to the pelvic floor and sphincters from Onuf’s nucleus in the sacral anterior cord [239]. Activity generated in the pudendal afferents from pelvic muscles and organs is said then to inhibit the bladder to enhance its capacity.

That the bladder can be profoundly inhibited by stimulation of pudendal afferents in the sacral spinal nerves [381-383] or more peripherally in the dorsal penile (clitoral) nerves [384-386] has been shown in patients with spinal cord lesions. In complete spinal cord injury this suggests that although local sacral segmental reflexes may be aberrant leading to incontinence, they can be reliably suppressed through implanted sacral posterior root stimulators to facilitate some restoration of normal bladder filling function [387].

Synthesis of the guarding reflex requires the involvement of the pontine micturition centre, where normal bladder-sphincter coordination takes place, and the integrity of supra-sacral pathways. Siroky and Krane [256] found that the guarding reflex (GR) was absent in over 85% of patients with complete (ASIA grade A) cord lesions, but it was nearly always present in patients with either incomplete (ASIA B-D) or suprapontine lesions. Therefore, the preservation or loss of the guarding reflex in response to bladder filling correlates well with the completeness of spinal transection.

Recent studies of neuro-urological function in spinal cord injury [388] have now confirmed that at bladder end fill volume (efv – defined as the volume at which voiding or neurogenic detrusor overactivity (NDO) occurs) the guarding reflex, as measured by the optimised [389] ‘pudendo-anal reflex’ (PAR), is robust in healthy volunteers, that is, as the bladder slowly fills the PAR increases significantly as the EFV is approached (**Figure 31 A**). In contrast, the guarding reflex is absent or weak in most patients with a neurologically defined complete supra-sacral spinal cord lesion (cSCI) (**Figure 31 B**).

In incomplete lesions (iSCI) the guarding reflex is often preserved but very variable across this group of patients (**Figure 31 B**). A weak guarding reflex seems to be usually associated with a small bladder capacity (**Figure 31 C**), that is, NDO often occurs at low bladder volumes presumably because pudendal inhibition of the bladder is weak. In contrast to healthy volunteers, who have a very low level pudendo-anal reflex during voiding consistent with properly coordinated voiding, in most patients with cSCI, the pudendal reflex is abnormally enhanced as expected

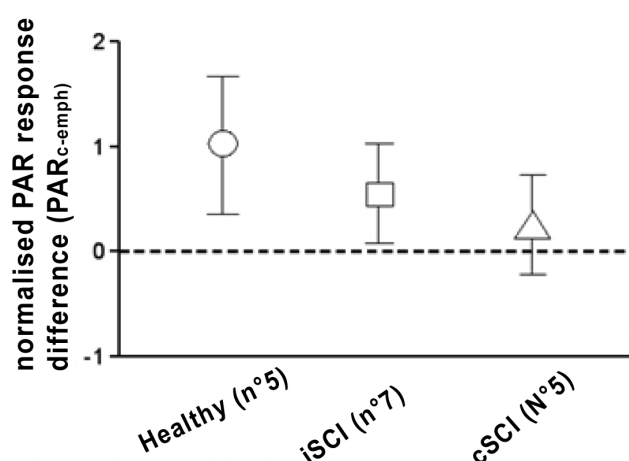


Figure 30 Facilitation of the normalised Pudendal Anal Reflex during voluntary effort in healthy subjects and patients with SCI. (Mean \pm SD).

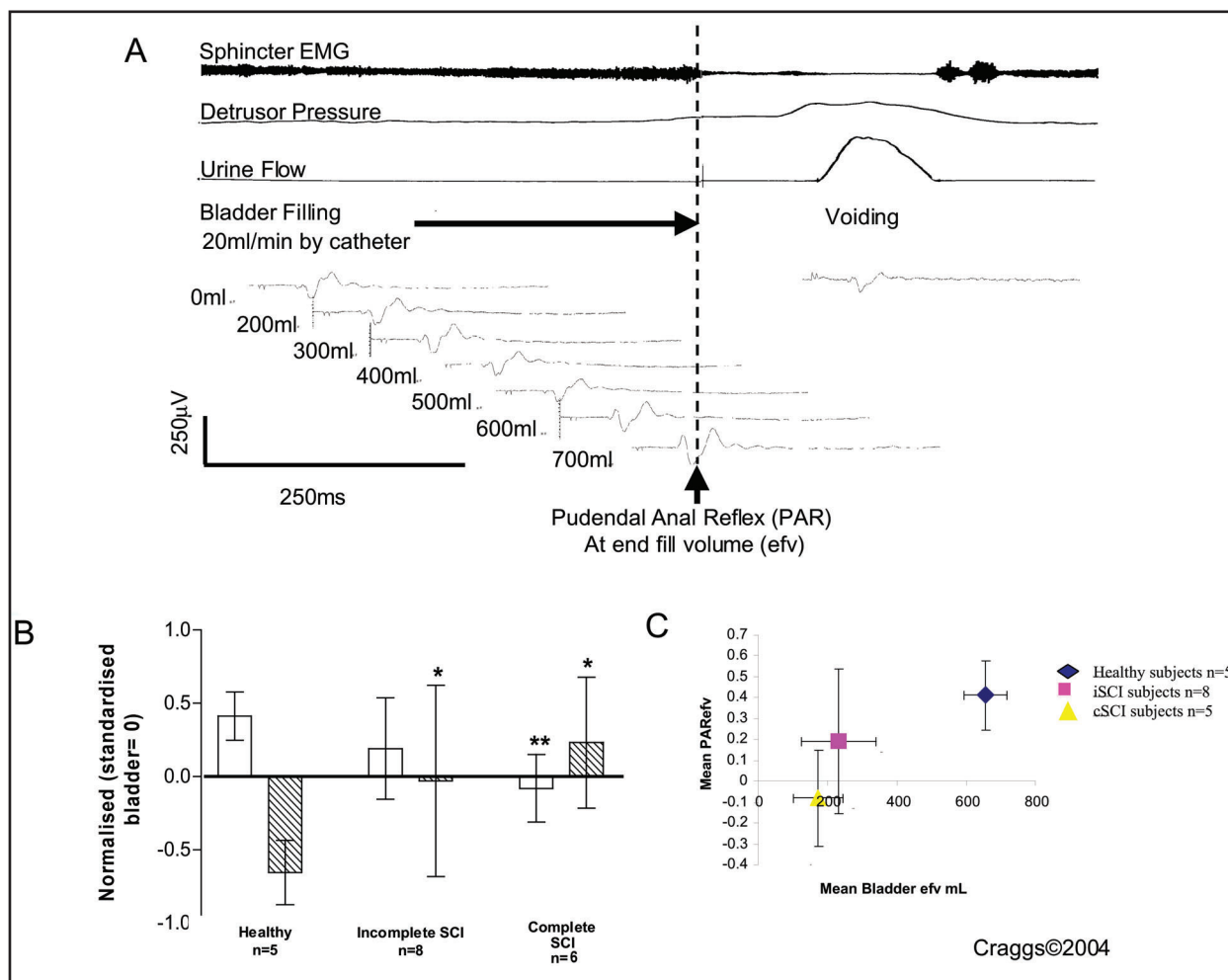


Figure 31. Interaction of normal bladder function with optimised PAR in healthy volunteer.

B. The normalised Pudendal Anal Reflex representing the Guarding Reflex at bladder end fill volume (open), and during voiding (Healthy) or neurogenic detrusor overactivity (SCI) (Hatched). Mean \pm SD. **C.** Volumes and PAR - Correlated values of PARefv and bladder efv in the 3 subject groups. Data expressed as mean \pm SD for both parameters. Significance between the bladder efv of the healthy and SCI subjects was denoted by *** $p < 0.0001$ along the x-SD bar; and significance between the PARefv of the healthy and SCI subjects was denoted by $p < 0.05$ denoted by * along the y-SD bar.]

during NDO and vesico-sphincter dyssynergia [390,391]. In iSCI patients the reflex is very variable ranging from near normal to grossly aberrant as in cSCI [388].

Overall there is some agreement between the experimental findings of aberrant neural control of the lower urinary tract in animals and man following spinal cord damage, however, we still know rather little about the plastic changes taking place in the brain and spinal cord following upper motor neuron lesions. Although most of the neural circuits involved in the normal control of the lower urinary tract in man are autonomic and involuntary just like other animals, micturition and storage are under very pre-

cise volitional control through cerebro-spinal activation. They appear to involve a 'guarding reflex' [258] which has both voluntary and involuntary components depending on the perceived sense of bladder fullness and desire to void. Interestingly, voluntary contractions of the sphincters and other pelvic floor muscles have the potential to suppress undesirable bladder contractions through facilitation of the guarding reflex not only in healthy subjects but also in some patients with incomplete spinal cord injury. In this context, pelvic floor muscle training (see chapter on Adult Conservative Management) could be a promising strategy to help some of these patients regain motor recovery by promoting increased cortical drive to surviving corticospinal neurones.

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