



## New Aspects of Peripheral and Central Control of Micturition in the Overactive Bladder

W43, 30 August 2011 14:00 - 18:00

Start	End	Topic	Speakers
14:00	14:25	New approaches for studying sensory regulation of the bladder	<ul style="list-style-type: none"> <li>• Anthony Kanai</li> </ul>
14:25	14:40	Therapeutic targeting of peripheral afferents and the spinal cord	<ul style="list-style-type: none"> <li>• Naoki Yoshimura</li> </ul>
14:40	15:00	Discussion	All
15:00	15:30	New concepts regarding urothelial involvement in bladder function	<ul style="list-style-type: none"> <li>• Lori Birder</li> </ul>
15:30	16:00	Break	None
16:00	16:20	Urothelial-interstitial cell communication as a therapeutic target	<ul style="list-style-type: none"> <li>• Christopher Fry</li> </ul>
16:20	16:45	Discussion	All
16:45	17:15	Central regulation of micturition studied using brain imaging	<ul style="list-style-type: none"> <li>• Derek Griffiths</li> </ul>
17:15	17:35	Central targets for treating overactive bladders	<ul style="list-style-type: none"> <li>• Karl-Erik Andersson</li> </ul>
17:35	18:00	Discussion	All

### **Aims of course/workshop**

To present up-to-date information regarding investigational approaches, mechanistic concepts and therapeutic options about the peripheral and central control of micturition in normal and overactive bladders.

### **Educational Objectives**

The workshop will provide health care practitioners and scientists with the latest investigational approaches, mechanistic concepts and therapeutic options regarding peripheral and central control of micturition in normal and overactive bladders. It is anticipated that these new approaches and concepts will be valuable to facilitate further clinical and basic science research, as well as guide clinical management of OAB.

## Aims of the Workshop

To present up to date information regarding investigational approaches, mechanistic concepts and therapeutic options regarding the peripheral and central control of micturition in normal and overactive bladders.

### Summary

The first set of paired talks will cover the current knowledge on bladder afferent function and new methodological approaches to studying their activity. Altered afferent function has been implicated in a number of bladder pathologies. However, methods to study afferents have been largely limited. For example, while the measurement of multi- or single-unit afferent firing is routine, methods for measuring changes in neuropeptide release are not. Dr. Tony Kanai will present on current electrophysiological and emerging optical methods to investigate afferent function. Methods for determining whether drug treatments have a direct or indirect effect on afferent activity will be addressed. For example,  $\beta_3$ -adrenoceptor agonists, of which the Astellas compound, Mirabegron, is through phase III clinical trials, appear to reduce detrusor overactivity and afferent sensitization. However, whether the decrease in afferent function is a consequence of detrusor relaxation or direct inhibition of afferents has not been determined. In this talk, methods to address these questions will be examined. Dr. Naoki Yoshimura will present current data regarding alterations to afferent function in pathological models. There will be a discussion on potential therapeutic targets that act on primary afferents and/or higher-order spinal cord neurons for the amelioration of afferent sensitization and bladder overactivity.

The second set of talks will discuss the sensory mechanisms of the urothelium and the interactions with underlying cells in the lamina propria. Urothelial cells have been demonstrated to release an array of signaling factors such as acetylcholine and ATP in response to mechanical and chemical stimuli. The targets for these signaling factors are thought to include afferent nerve terminals and lamina propria interstitial cells, suggesting that the urothelium may be involved in regulating bladder activity. In addition, interstitial cells have been implicated as 'pacemakers' that can drive spontaneous contractions of the detrusor. Interstitial cells also demonstrate changes in connectivity and function under pathological conditions which could contribute to detrusor overactivity. Dr. Lori Birder will present current data on urothelial functions and how these are augmented in pathology. Dr. Chis Fry will follow by describing potential interactions of the urothelium with interstitial cells and how these signaling pathways may be targets for treating bladder overactivity.

In the final set of talks, methods for studying central mechanisms that regulate micturition and potential therapeutic options will be discussed. Control of the bladder and urethra depends on an extensive supraspinal neuronal network that provides the ability both to postpone voiding voluntarily, even if there is a strong desire to void, and also to void when convenient, even if there is little or no sensation of bladder filling. Failure of bladder control is manifested by urine storage symptoms—urgency, increased frequency and urge incontinence. The origin of these symptoms is in some cases neurogenic or myogenic, but frequently it is idiopathic. Changes to the supraspinal network, such as deactivation in the prefrontal cortex, are possible contributory factors in idiopathic detrusor overactivity. Dr. Derek Griffiths will review the recent findings on central regulation of bladder function using functional Magnetic Resonance Imaging (fMRI). Dr. Karl-Erik Andersson will follow on by discussing pathological alterations in central regulation that have detrimental effects on urinary continence and possible therapeutic options.

# 1) New approaches for studying sensory regulation of the bladder.

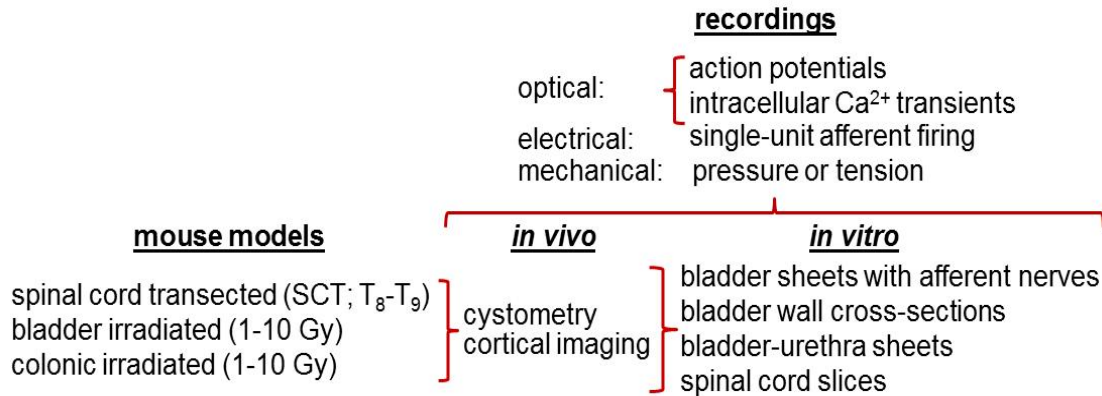
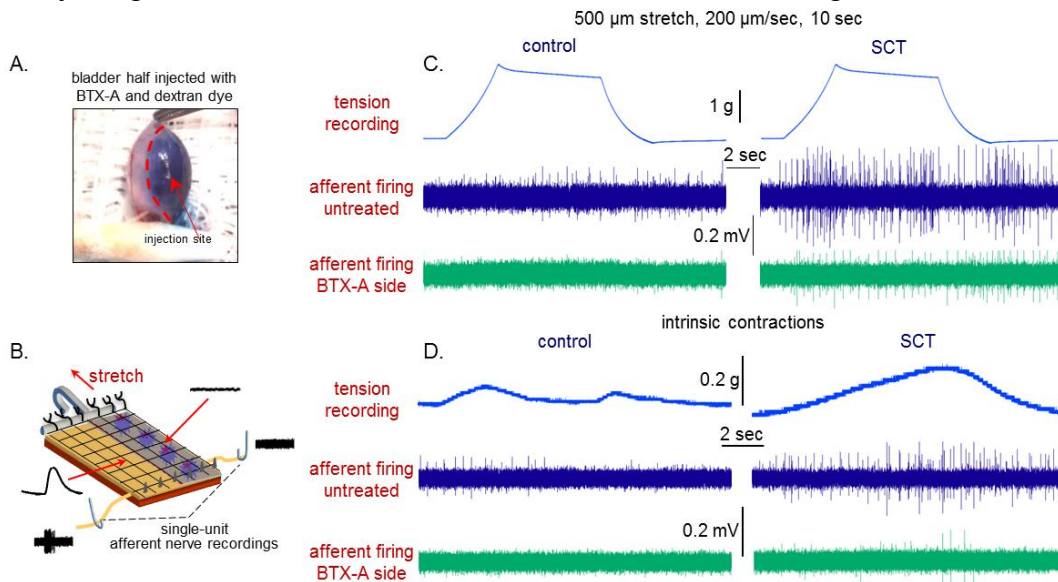


Table 1 lists new and established methods that are used for studying the consequences of afferent sensitization on the urinary bladder, spinal cord and cerebral cortex and that will be discussed in the first talk. Presentation two will expand on studies in the spinal cord and presentation 5 on those in the cerebral cortex. The utility of these approaches for determining therapeutic relevance and sites of action of a number of agents will be discussed including botulinum toxin type-A (BTX-A),  $\beta_3$ -receptor agonists and TRPA1 and TRPV1 receptor antagonists.

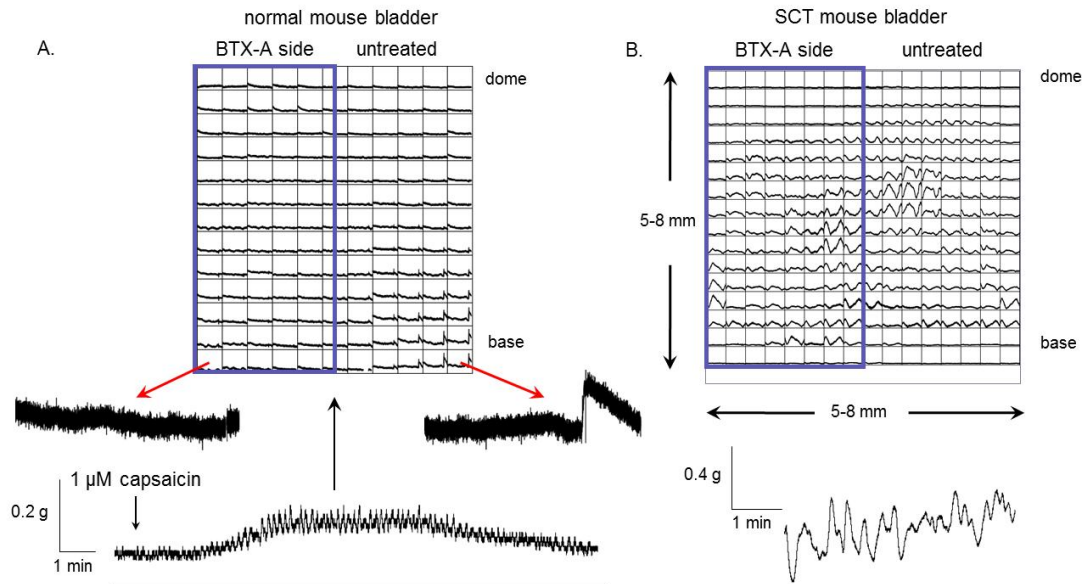
There are a number of key questions that will be addressed regarding sites of action of BTX-A. 1<sup>st</sup>) Is there a direct action on afferent nerves to decrease their excitability and/or neuropeptide release; 2<sup>nd</sup>) Does BTX-A stay near the injection site or does it diffuse across and throughout the bladder wall; and 3<sup>rd</sup>) Does BTX-A inhibit ATP release from the urothelium? Attempts to genetically engineer a botulinum toxin that contains features of different serotypes to preferentially target it to afferent nerves will be discussed. Figure 1A demonstrates an



approach where BTX-A is injected into one half of a mouse bladder by combining it with blue dextran dye of comparable molecular weight (150 KDa) to track its distribution. Both control and spinal cord transected (SCT; T<sub>8</sub>-T<sub>9</sub>) mice were studied. Transected mice were used two weeks after surgery and BTX-A was injected 48 hours prior to bladder isolation. In this way, whole

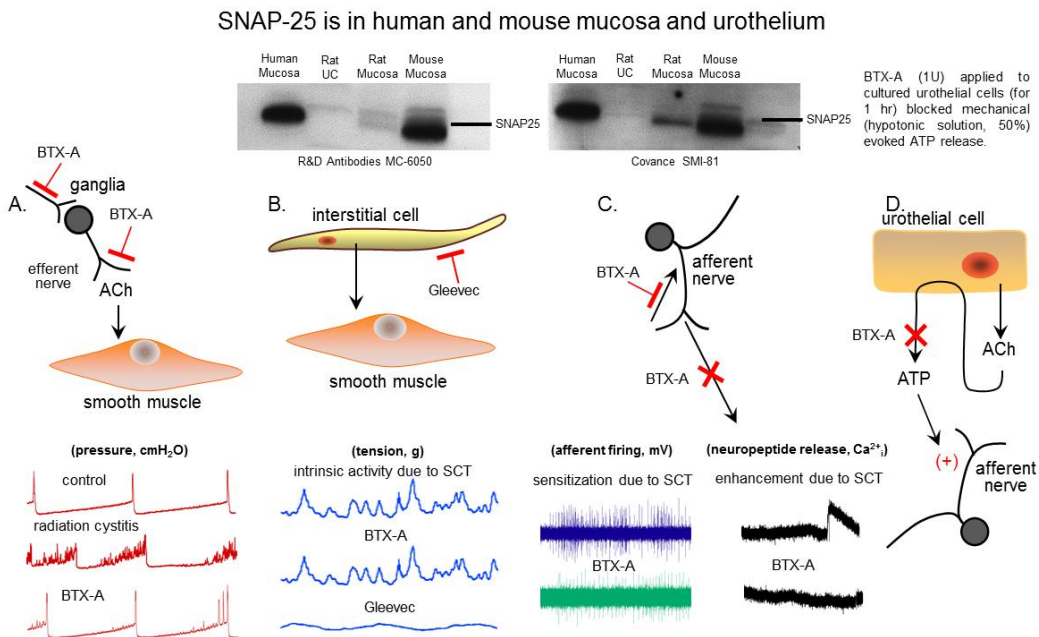
bladder sheets (1B) can be studied where there is a built-in control in the untreated half. Sheets were isolated with the pelvic and/or hypogastric spinal nerves to record single-unit afferent nerve firing in response to stepper-motor controlled stretches (1C). Alternatively, the effects of intrinsic bladder contractions on afferent firing were studied (1D).

Figure 2A demonstrates the effects of BTX-A on neuropeptide release in response to the

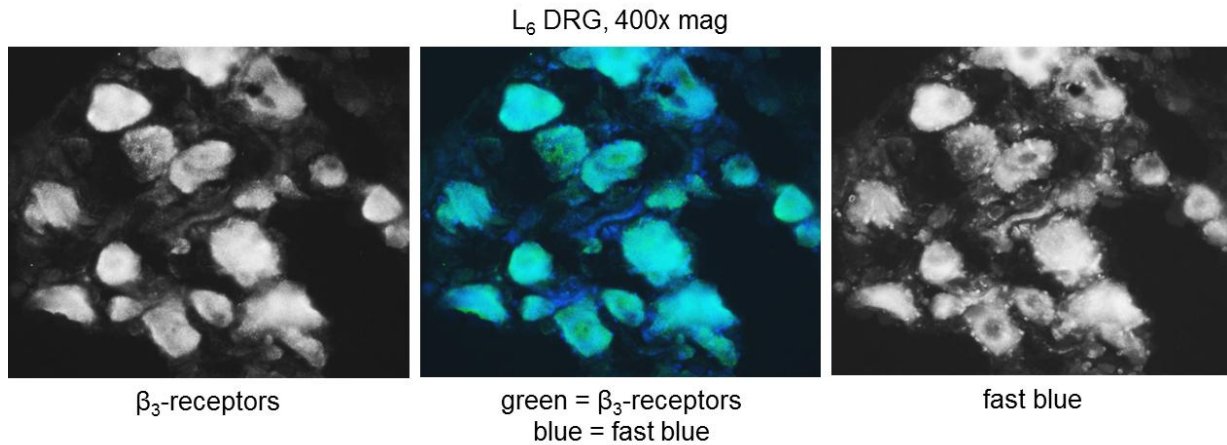


exogenous administration of capsaicin to whole bladder sheets from control mice. The area inscribed by the blue rectangle was injected *in vivo* with BTX-A 48 hours prior to isolation. The preparation was stained with a  $Ca^{2+}$ -sensitive dye and imaged. In this approach, the smooth muscle is used as a sensor to record neuropeptide evoked  $Ca^{2+}$  transients which are inhibited in the BTX-A treated area in 2A. However, BTX-A failed to inhibit intrinsic (spontaneous) contractions in bladder sheets isolated from SCT mice two weeks after surgery (2B).

Figure 3 depicts the sites of action (3A, C and D) and inaction (3B) of BTX-A in human and rodent bladders.

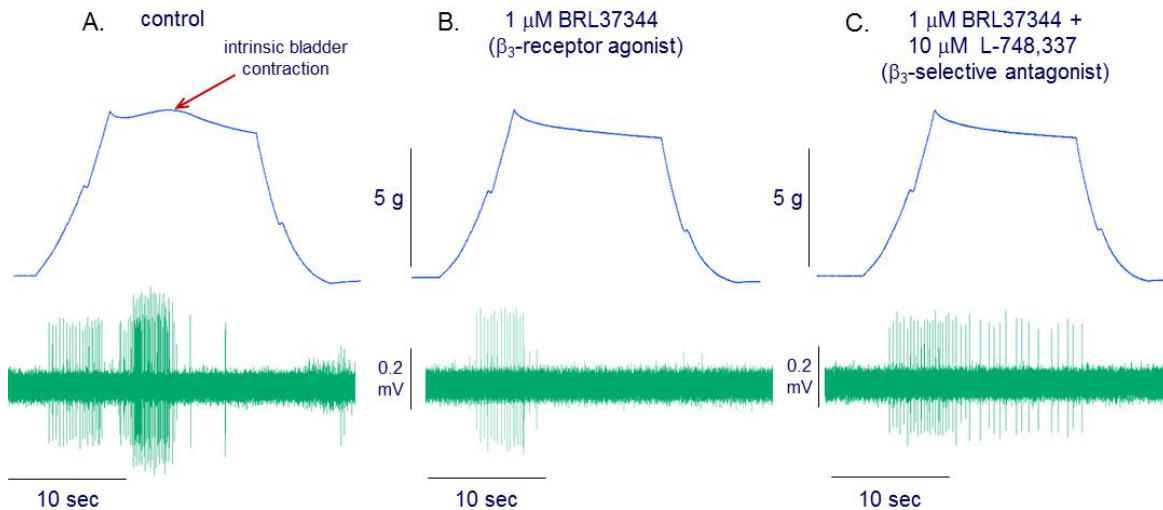


$\beta_3$ -adrenergic receptors are highly expressed on urothelium, afferent nerves, interstitial cells and detrusor smooth muscle where they promote relaxation. Moreover, we have also just shown that they are expressed on mouse DRG neurons that send projections to the bladder (figure 4).



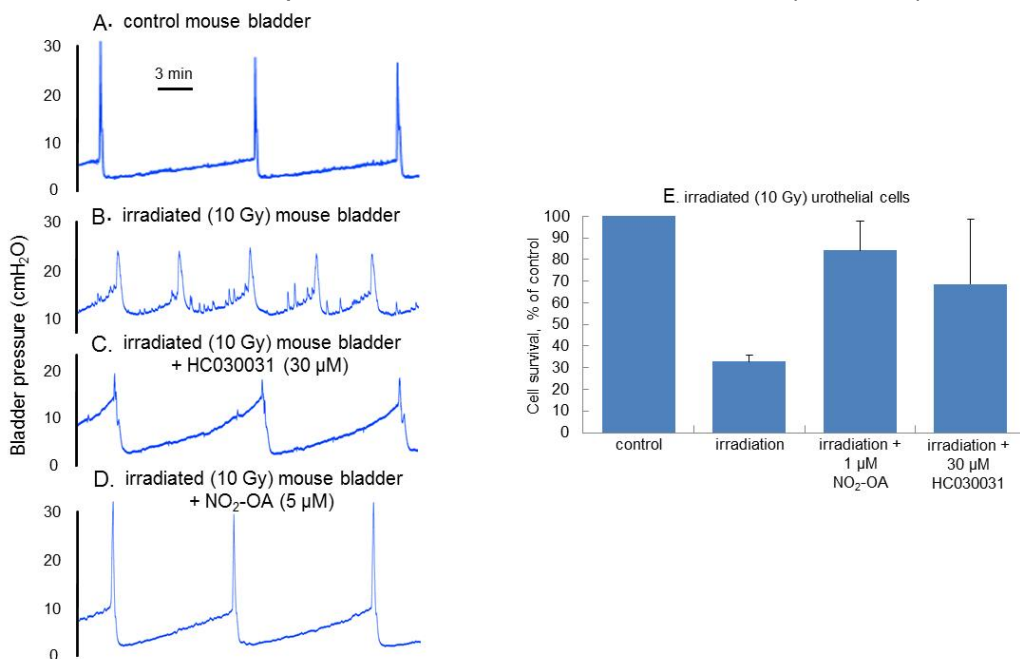
However, to be therapeutically relevant  $\beta_3$ -receptor agonists must be selective enough to decrease bladder overactivity without stimulating  $\beta_1$ -receptors on the heart.  $\beta_3$ -adrenergic receptor agonists are known to decrease afferent firing rates and intrinsic and nerve-evoked smooth muscle contractions. However, whether the afferent and intrinsic effects are direct or secondary to bladder smooth muscle relaxation are key questions.

The data in figure 5 suggests that the  $\beta_3$ -receptor agonist, BRL37344, can reduce afferent firing



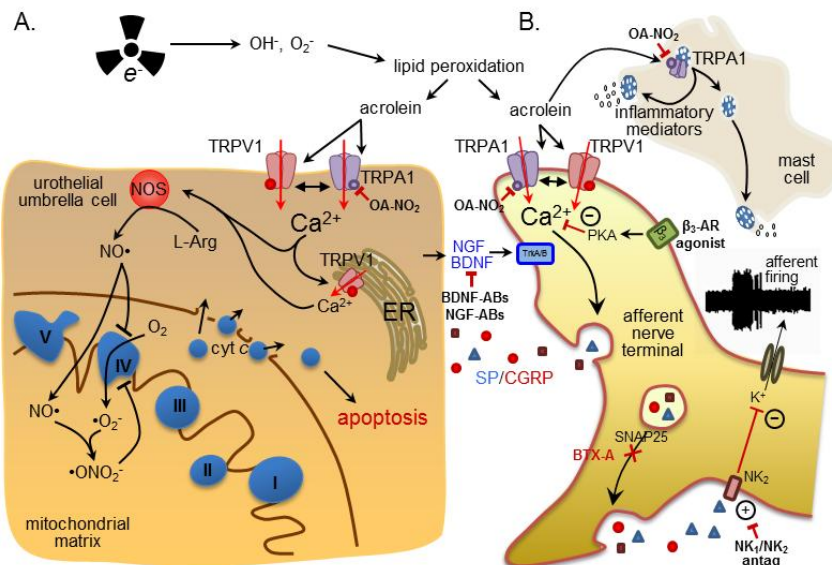
in bladder sheets from SCT mice through a direct action on the nerves. After obtaining single unit activity, in response to stepper motor-controlled stretches, addition of the agonist decreased afferent firing without changing the smooth muscle tension profile (5B). In other words, it could decrease afferent firing without relaxing the bladder. As indicated by the red arrow in 5A, the effects of intrinsic detrusor contractions in enhancing afferent firing can be discerned during these controlled stretches and were blocked by the agonist. The addition of the  $\beta_3$ -receptor antagonist, L-748,377, enhanced afferent firing over controls (5C), unmasking activity that was most likely inhibited by endogenous noradrenalin released from sympathetic nerves.

Figure 6 demonstrates the radioprotective effects of nitro-oleic acid (NO<sub>2</sub>-OA) versus the TRPA1



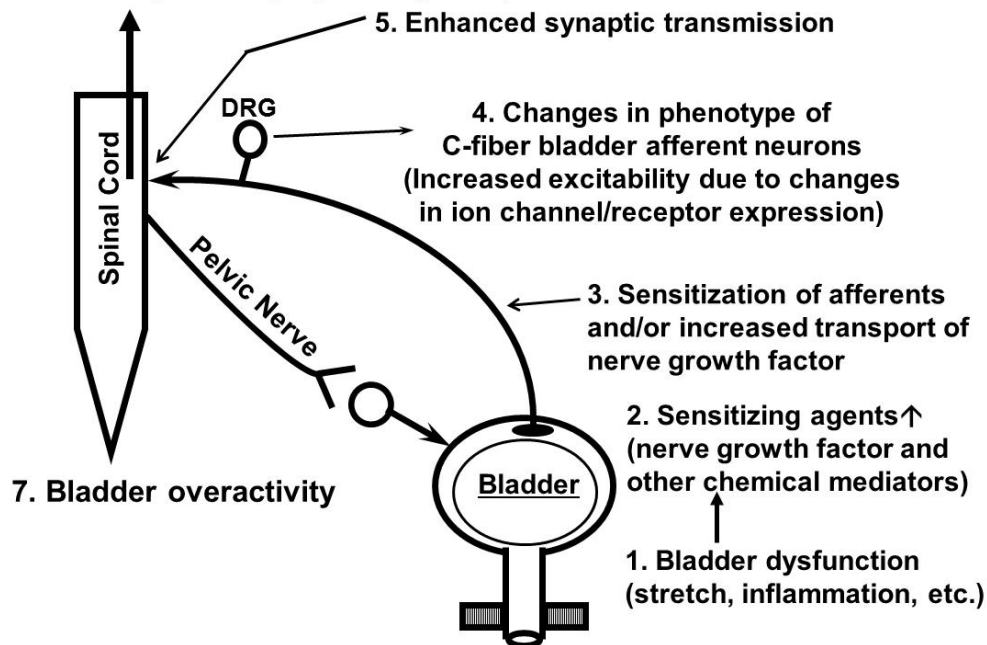
channel blocker, HC030031, when instilled in mouse bladders during irradiation (10 Gy). The results demonstrate that TRPA1 channel blockade (6C) prevents shortening of intercontractile intervals and nonvoiding contractions seen in unprotected bladders (6B). However, NO<sub>2</sub>-OA was even more protective such that cystometrograms from irradiated bladders instilled with this agent (6D) and nonirradiated bladders (6A) look similar. In irradiated primary urothelial cells, exogenously applied NO<sub>2</sub>-OA and HC030031 were also radioprotective. These results support our hypothesis that TRPA1 channels in urothelial cells are responsible for the radiation-induced Ca<sup>2+</sup> influx that activates nitric synthase leading to urothelial cell apoptosis/necrosis, disruption of barrier function and cystitis. Since NO<sub>2</sub>-OA can desensitize both TRPA1 and TRPV1 channels, this suggests the involvement of both and it may be necessary to combine the HC compound with the TRPV1 channel blocker, capsazepine, to obtain comparable radioprotection.

Figure 7 suggests mechanisms of action in the urothelium and afferent nerves for BTX-A, β<sub>3</sub>-adrenergic receptor antagonists, NO<sub>2</sub>-OA and TRPA1/TRPV1 channel blockers.



2) Therapeutic targeting of peripheral afferents and the spinal cord. Figure 8 shows putative mechanisms for inducing lower urinary tract symptoms (LUTS) and bladder overactivity due to afferent sensitization. Bladder dysfunction (1) causes the increased production of nerve

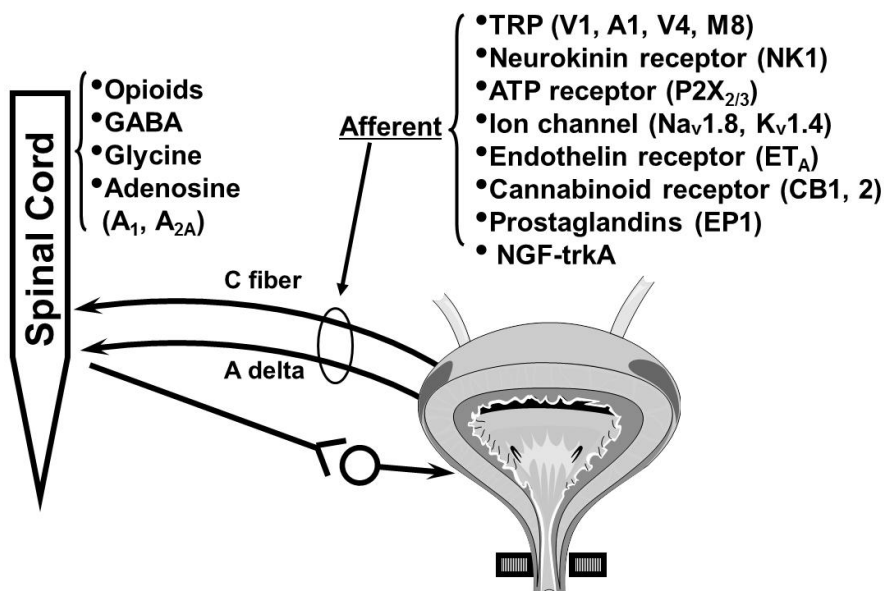
**6. Lower urinary tract symptoms (LUTS)**



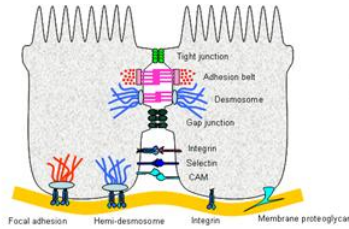
growth factors (NGF) or other chemical mediators in the bladder wall (2). NGF or chemical mediators, in turn, sensitize afferent nerves. NGF is taken up by afferent nerves and transported to the dorsal root ganglion (DRG) cells where it changes gene expression (3). This leads to changes in the functional properties of ion channels and receptors resulting increased neuronal excitability (4). Increased afferent nerve activity results in enhanced synaptic transmission in the spinal cord (5), leading to LUTS (6) and bladder overactivity (7).

Figure 9 show potential therapeutic targets, on receptors or ion channels in bladder afferent pathways and the spinal cord, for the treatment of overactive bladder.

**Potential therapeutic targets**

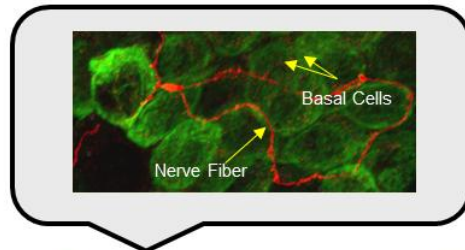


**3) New concepts regarding urothelial involvement in bladder function.** The urothelium receives 'sensory inputs' in a number of different forms and from a variety of sources. These include mechanical stimuli such as the increased stretch associated with bladder filling, soluble mediators such as growth factors found in the urine (e.g., epidermal growth factor, EGF), or neurotransmitters including ATP, adenosine, substance P, acetylcholine, and noradrenalin released from nerve processes or other cell/tissue types including the urothelium (figure 10).



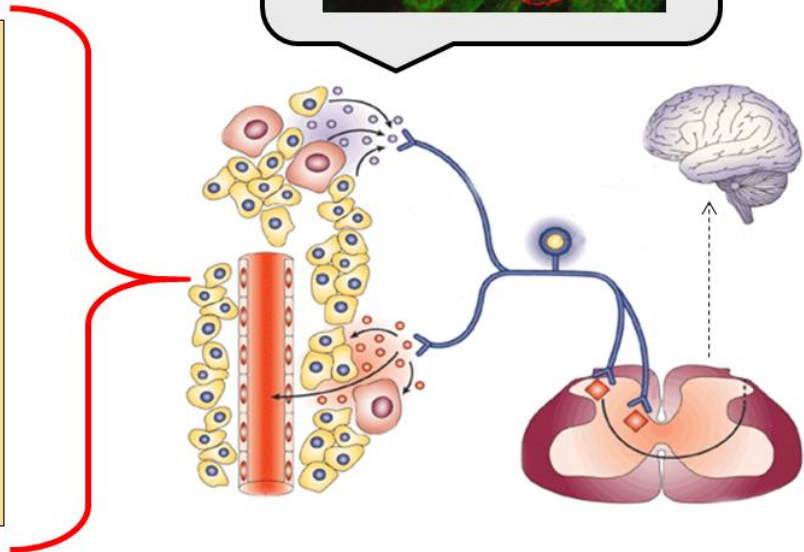
**More than just a barrier: The Urothelium Functions as a Primary Transducer of Physical and Chemical Stimuli**

*(Bladder afferents and efferents are located in close proximity to the uro-epithelium)*



Urothelial Cells Can Synthesize/Release a Number of Mediators:

- ATP
- Prostaglandins
- Nitric Oxide
- Acetylcholine
- Nerve Growth Factor

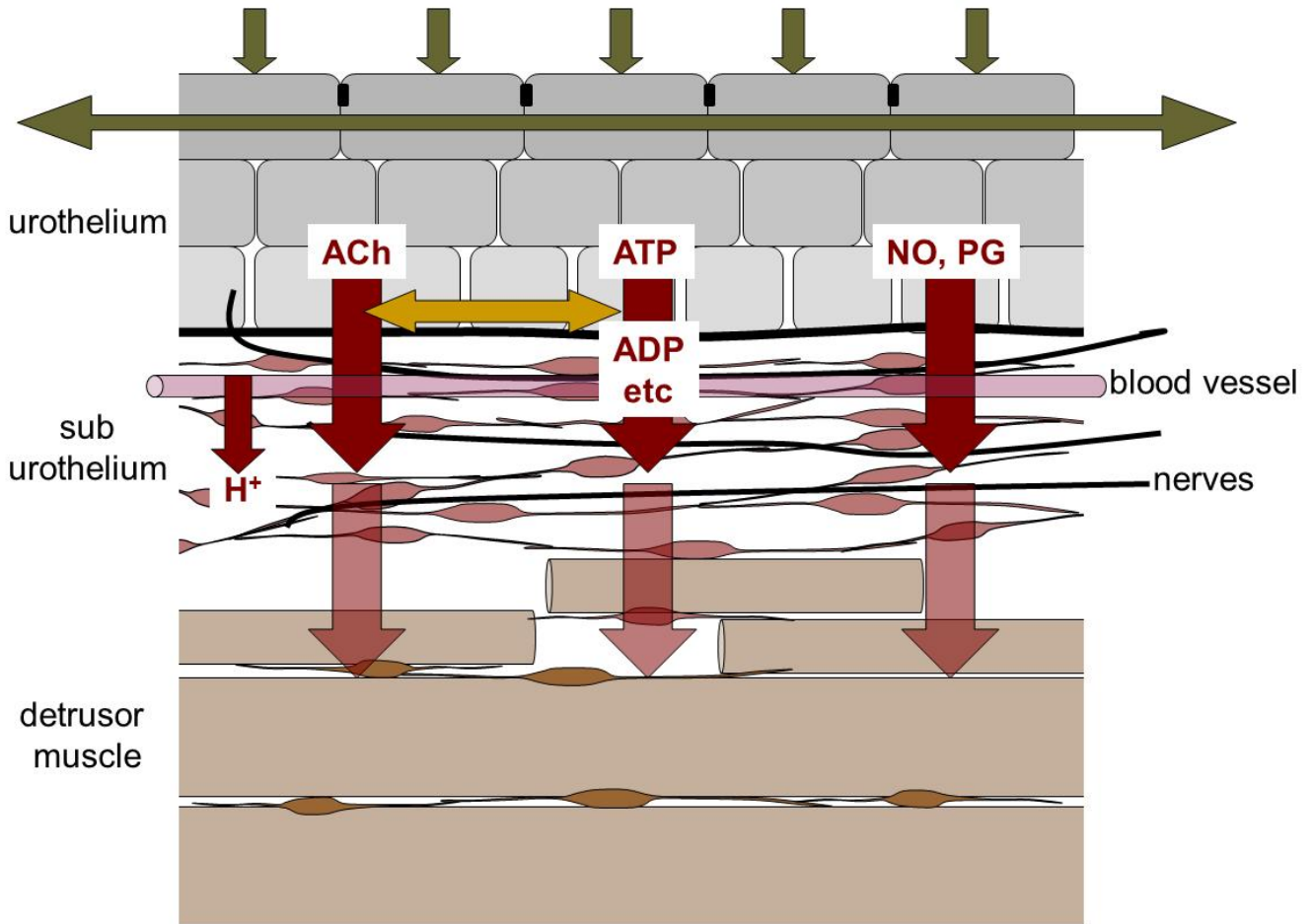


In response to the myriad of signaling factors, the urothelium can, in turn, release ATP and/or acetylcholine. Afferent nerves form close contact with the urothelium and it is likely that these sensory terminals are targets for urothelial ATP. However, in pathological conditions there is increased urothelial ATP release and this has been demonstrated in chemically irritated and spinal cord injured rodent bladders, as well as patients diagnosed with painful bladder syndrome/interstitial cystitis. ATP can also act in an autocrine manner to enhance its own release from the urothelium of patients with chronic bladder disease. Therefore, higher levels of urothelial ATP seen in pathology are likely to contribute to progression of bladder diseases and to afferent sensitization. As such, urothelial-associated receptor and mediator release pathways may serve as important targets for the pharmacological management of bladder disorders.

**4) Urothelial-interstitial cell communication as a therapeutic target.** Figure 11 depicts signaling between the urothelium, suburothelium and detrusor smooth muscle in the bladder wall during stretch or imposition of pressure. The urothelium releases several chemical



transmitters when subjected to stresses, including ATP, acetylcholine (ACh), nitric oxide (NO) and prostaglandins (PG). Furthermore,  $H^+$  may be released due to the relative ischaemia. These transmitters may also be degraded, in particular ATP to ADP and even adenosine. There are several potential targets for these molecules that may initiate sensory or motor responses on suburothelial nerves and interstitial cells and detrusor smooth muscle. In addition, these agents may feedback on the urothelium to further modulate transmitter release. The evidence for these different pathways will be discussed during normal and pathological bladder function.

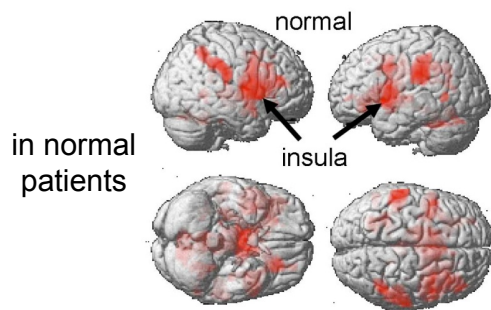


**5) Central regulation of micturition studied using brain imaging.** Over the past 10 to 15 years much has been learned about the control of the urinary bladder, especially as a result of new methods of imaging brain function. Foremost among them is functional magnetic resonance imaging (fMRI), which has been adapted for use in bladder studies mainly by our group in Pittsburgh. fMRI has the advantage of being relatively inexpensive and noninvasive, but requires a complicated apparatus, an unnatural position (at least for voiding), and repeated measurements (to enable averaging of the noisy fMRI signals). For these reasons, fMRI studies have been limited to the storage or filling phase. Voiding has been investigated in only a few studies, mainly using PET imaging. Conveniently however, overactive bladder (OAB) syndrome is a failure of storage function and so fMRI has proved ideally suited to investigating the differences between normal and OAB patients, in particular, those with urgency incontinence of unknown etiology. During bladder filling, the detrusor has to be kept under control so that it

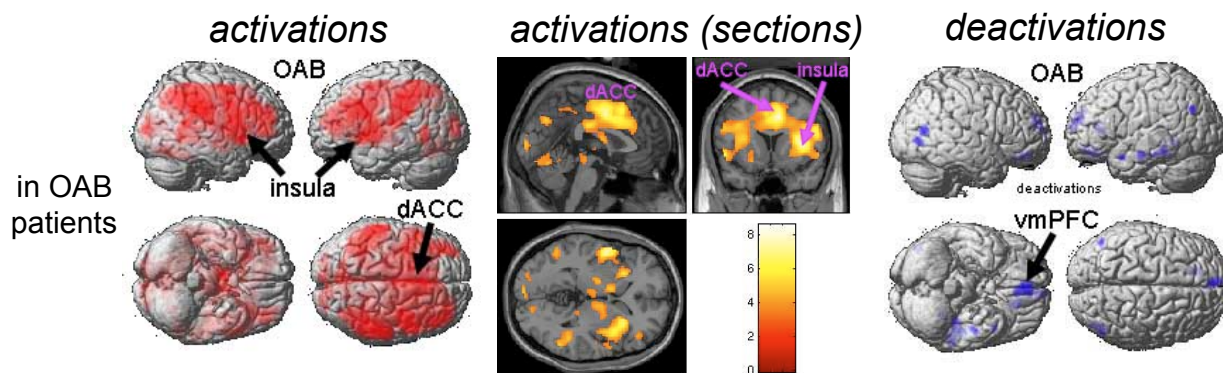
does not react by contracting involuntarily. Normally, contraction of the detrusor should occur only if voiding is consciously and voluntarily desired. This voluntary aspect of control implies that cortical regions of the brain must be involved in the control mechanism, and indeed the measurements to be described in this presentation suggest that control of the bladder and urethra depends on an extensive supraspinal neuronal network that provides sensations of bladder filling together with motor output to affect control.

Early on, our group in Pittsburgh developed a repetitive infusion/withdrawal paradigm (which is still employed) to mimic natural filling in the scanner and enable us to gauge the brain responses to bladder filling. The early measurements quickly established that regions near the insula, in the dorsal part of the anterior cingulate cortex (dACC), and in the ventral part of the medial prefrontal cortex (vmPFC) responded to filling (figure 12). The insula is believed to be

## Activations and deactivations with full bladder



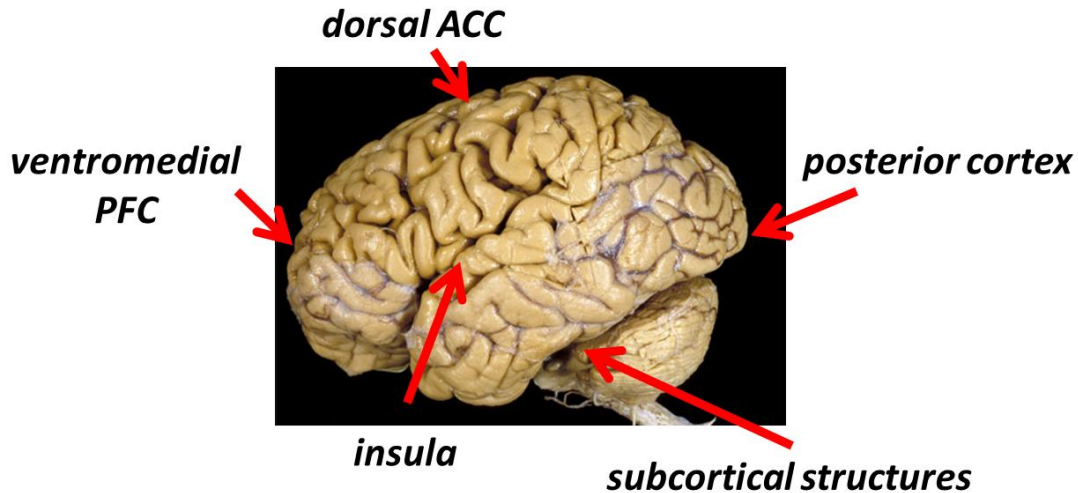
dACC = dorsal anterior cingulate cortex  
vmPFC = ventromedial prefrontal cortex



the seat of visceral sensation. Insular activation in response to bladder filling occurs in normal subjects and most likely implies that there is a desire to void. The dACC is activated strongly only in OAB patients, especially when they report urgency. Thus, dACC activation is likely to be a sign of urgency. The vmPFC appears to be deactivated by bladder filling, although this finding is less certain, partly because it is technically difficult to image this part of the brain. Subcortical structures, e.g., periaqueductal gray (PAG) and thalamus, are believed to be involved in control but are also difficult to image. The posterior parts of the cortex are also clearly important. In the next presentation, the feasibility of drug therapies that may target these areas will be discussed in detail.

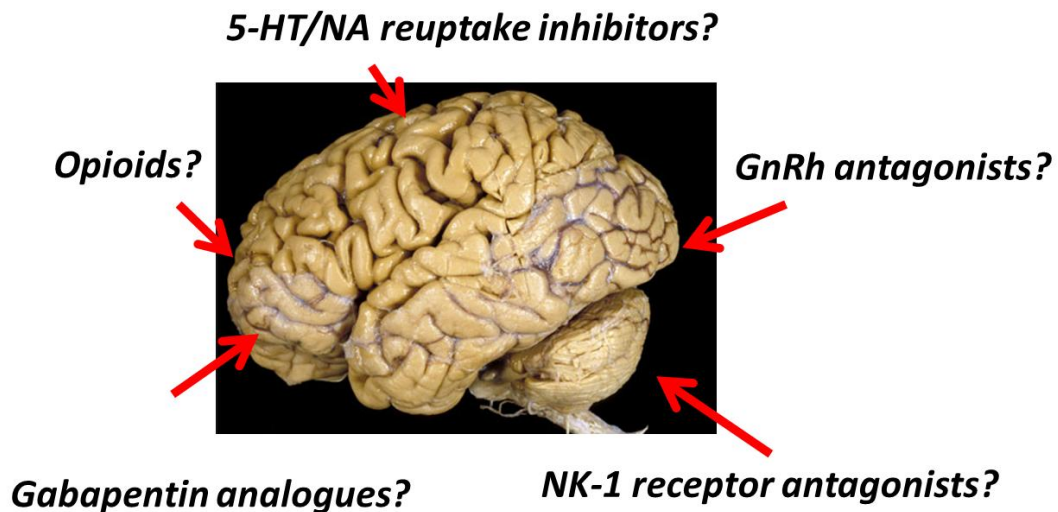
6) **Central targets for treating overactive bladders.** Many regions of the CNS have been suggested to be involved bladder control by different investigations. However, the most likely regions involved are those discussed above and summarized in figure 13. In this presentation

### **Activated and deactivated regions: possible targets for OAB Drugs**



therapeutic agents that may target these areas will be discussed as suggested in figure 14.

### **OAB Drugs – Central Mode of Action**



An important aspect to be discussed is whether the effectiveness of putative therapies can be evaluated using fMRI, given the difficulty in defining urgency. This subjective sensation is hypothesized to be represented by the activation of the dACC. Therefore, if treatment reduces urgency, it may reduce dACC activation which would provide a numerical marker of urgency.