

Joint Annual Meeting of the International Continence Society
(ICS) and International Urogynecological Association (IUGA)
23-27 August, 2010, Toronto, Canada



Workshop 47, Tuesday, 24 August, 14:00-18:00

Pathophysiological Mechanisms of Detrusor Overactivity and New
Therapeutic Targets Including Afferents Nerves, Interstitial Cells,
Trigone and Urothelium

Chair: Anthony Kanai

Speaker 1: William de Groat, PhD, United States

Expert in autonomic regulation and pharmacology of the lower urinary tract. Yu Y and de Groat W. Sensitization of pelvic afferent nerves in the in vivo rat urinary bladder-pelvic nerve preparation by purinergic agonists and cyclophosphamide pretreatment. Am J Physiol Renal Physiol 294:F1146-F1156, 2008 (PMID: 18322018).

Speaker 2: Lori Birder, PhD, United States

Expert in urothelial sensory mechanisms in the urinary bladder. Kullmann F, Artim D, Beckel J, de Groat W and Birder L. Heterogeneity of muscarinic receptor-mediated Ca²⁺ responses in cultured urothelial cells from rat. Am J Physiol Renal Physiol 294:F971-F981, 2008 (PMID: 18272602).

Speaker 3: Anthony Kanai, PhD, United States

Expert in new imaging approaches for studying intracellular communication in the bladder and urethra. McCarthy C, Zabbarova I, Brumovsky P, Roppolo J, Gebhart G and Kanai A. Spontaneous contractions evoke afferent nerve firing in mouse bladders with detrusor overactivity. J Urol 181:1459-1466, 2009 (PMID: 19157431).

Speaker 4: Christopher Fry, PhD, United Kingdom

Expert in muscle and interstitial cell physiology of the bladder. Sui G, Wu C, Roosen A, Ikeda Y Kanai A and Fry C. Modulation of bladder myofibroblast activity: implications for bladder function. Am J Physiol Renal Physiol 295:F688-F697, 2008 (PMID: 18632799).

Speaker 5: Karl-Erik Andersson, MD/PhD, Sweden

Expert in the physiology and pharmacology of the lower urinary tract. Werkstrom V, Hedlund P, Lee T and Andersson K-E. Vardenafil-induced relaxation and cyclic nucleotide levels in normal and obstructed rat urinary bladder. BJU Int, 104:1740-1745, 2009 (PMID: 19694713).

Agenda & Itinerary

Time	Time	Topic	Speaker
14:00	14:10	Introduction	Dr Anthony Kanai
14:10	14:50	Neurogenic and Myogenic Bladder Overactivity	Dr William de Groat
14:50	15:30	Role of the Urothelium in Sensation	Dr Lori Birder
15:30	16:00	Break	
16:00	16:40	Afferent Sensitization in the Bladder and Trigone	Dr Anthony Kanai
16:40	17:20	Smooth Muscle and Interstitial Cell Interactions	Dr Christopher Fry
17:20	18:00	New Agents for Overactivity and Sensation	Dr Karl-Erik Andersson
18:00		Final Discussion/End of Session	All Speakers

Aims of the Workshop

To summarize the latest information regarding new mechanisms for the development of detrusor overactivity and new therapeutic targets in afferent nerves, interstitial cells, trigone and urothelium.

Summary

The opening talk will cover in-depth mechanisms for the development of neurogenic and myogenic bladder overactivity. Relevant animal models for studying each pathology will be discussed. The second presentation will present new information on the sensory role of the urothelium in promoting overactivity and sensation. The third discussion will present new findings on afferent sensitization in the detrusor, trigone and urethra following upper and lower motor neuron lesions. The fourth presentation will focus on detrusor smooth muscle and its interaction with submucosal interstitial cells and those within the muscle layer. The closing talk will cover new therapeutic approaches for treating detrusor overactivity. These will include antimuscarinic agents acting on the urothelium, β_3 -adrenergic agonists promoting detrusor relaxation and botulinum toxin-A acting on afferent nerves, trigone and the urothelium.

1) Neurogenic and Myogenic Bladder Overactivity: Myogenic overactivity results from enhanced intrinsic bladder smooth muscle contractions that may stimulate primary bladder afferents leading to urgency, reflex bladder contractions and incontinence. The cause of this form of overactivity is controversial but is believed to involve altered signalling by the urothelium and interstitial cells (IC). Neurogenic overactivity, on the other hand, involves sensitization of peripheral or central sensory pathways and/or sensitization of efferent outflow resulting in reflex bladder contractions leading to incontinence. Of course, bladder overactivity can involve a combination of these mechanisms. Animal models for neurogenic and/or myogenic overactive are shown in figure 1.

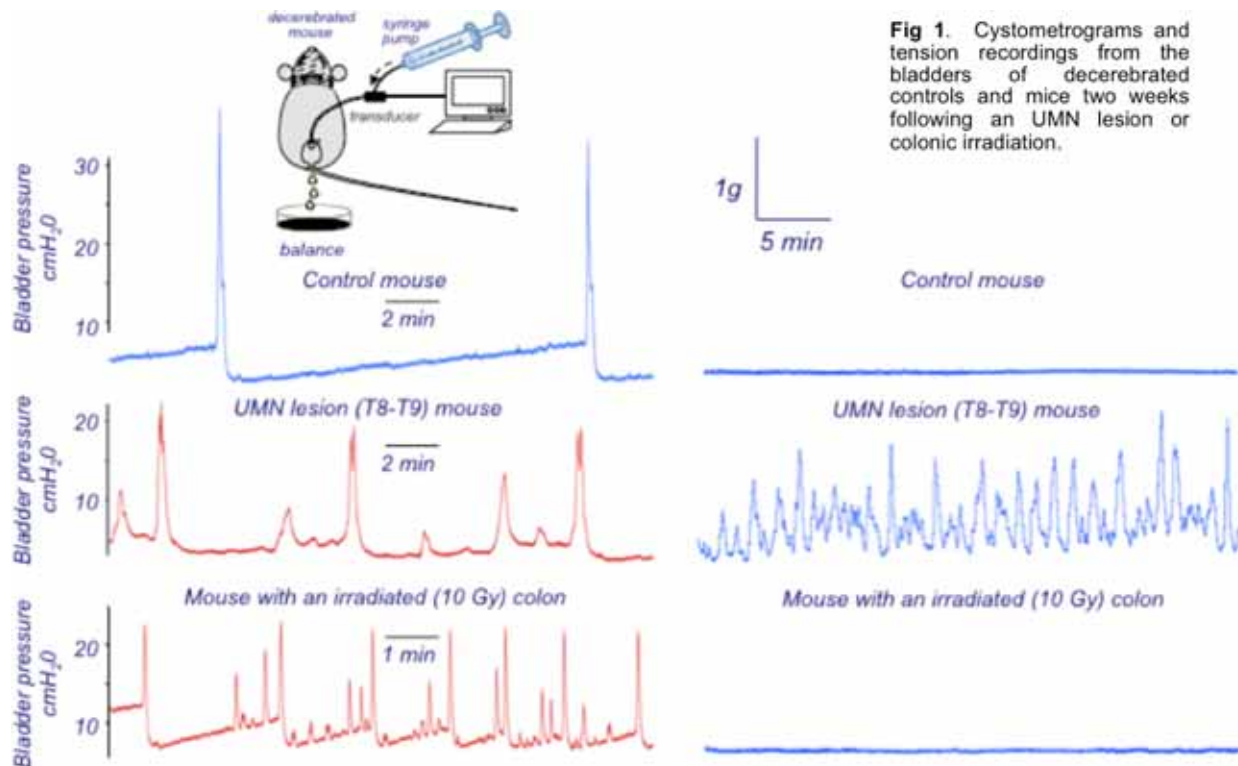
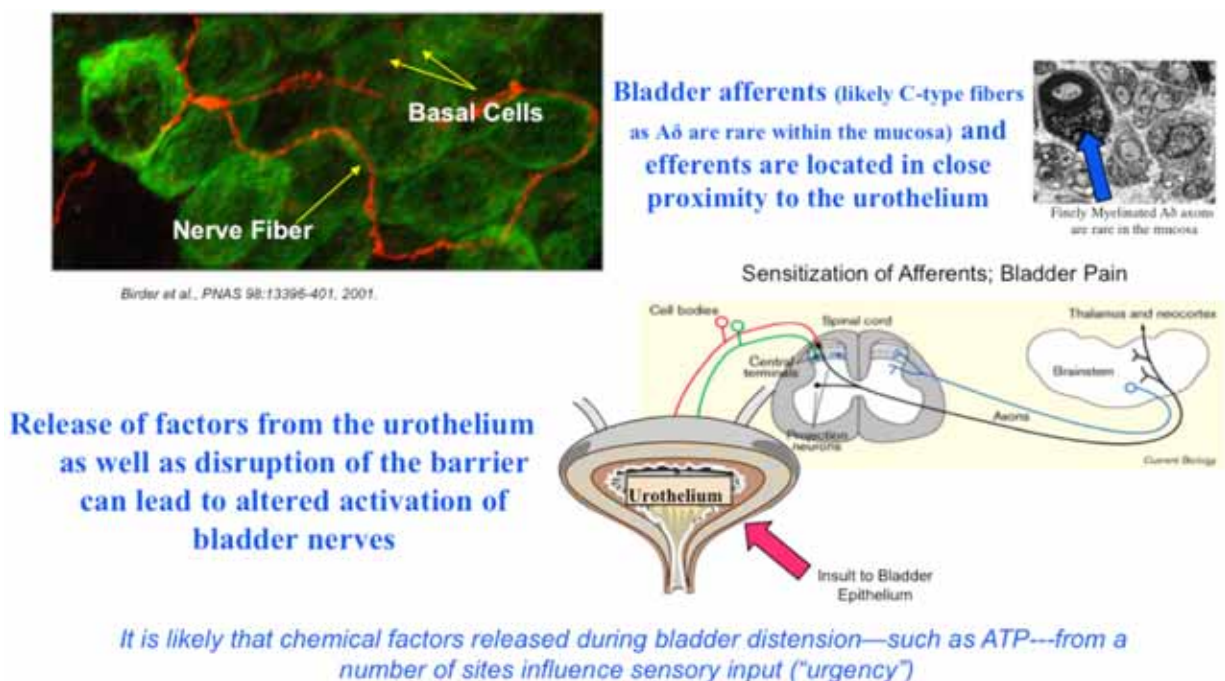


Fig 1. Cystometrograms and tension recordings from the bladders of decerebrated controls and mice two weeks following an UMN lesion or colonic irradiation.

2) Role of the Urothelium in Sensation: The urothelium receives ‘sensory inputs’ in a number of different forms and from a variety of sources. These inputs 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 such as ATP, adenosine, substance P, acetylcholine, or norepinephrine released from nerve processes or other cell/tissue types including the urothelium itself.

In response to the myriad of signaling factors, the urothelium can in turn release ATP and/or acetylcholine (ACh). Afferent nerves form close contact with the urothelium and it is likely that these sensory terminals are targets for the urothelial ATP (fig 2).

Fig 2. The location of bladder nerves in close proximity to the epithelium suggests potential for a chemical dialogue.



However, in pathological conditions there is increased urothelial ATP release and this has been demonstrated in chemically irritated and spinal cord injured rat 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, the 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.

3) Afferent Sensitization in the Bladder and Trigone: It has long been held that spinal cord injury results solely in bladder overactivity dependent upon reflex mechanisms in the spinal cord. However, recent studies have demonstrated a

myogenic component. Voiding cystometry performed on mice with upper motor neuron (UMN) lesions (T₈-T₉) demonstrated a decreased intercontraction interval and non-voiding contractions not present in control mice. When these bladder were excised and tension recorded from whole sheets this overactivity remained demonstrating a myogenic detrusor overactivity as shown in figure 1 above. When animals underwent urinary diversion (to the uterus) and then spinal cord transected, overactivity did not develop. This suggests that this overactivity is a consequence of detrusor-sphincter discoordination, bladder distension and damage. On the other hand, animals with lower motor neuron (LMN) lesions (L₄-L₅) developed atonic bladders (not shown).

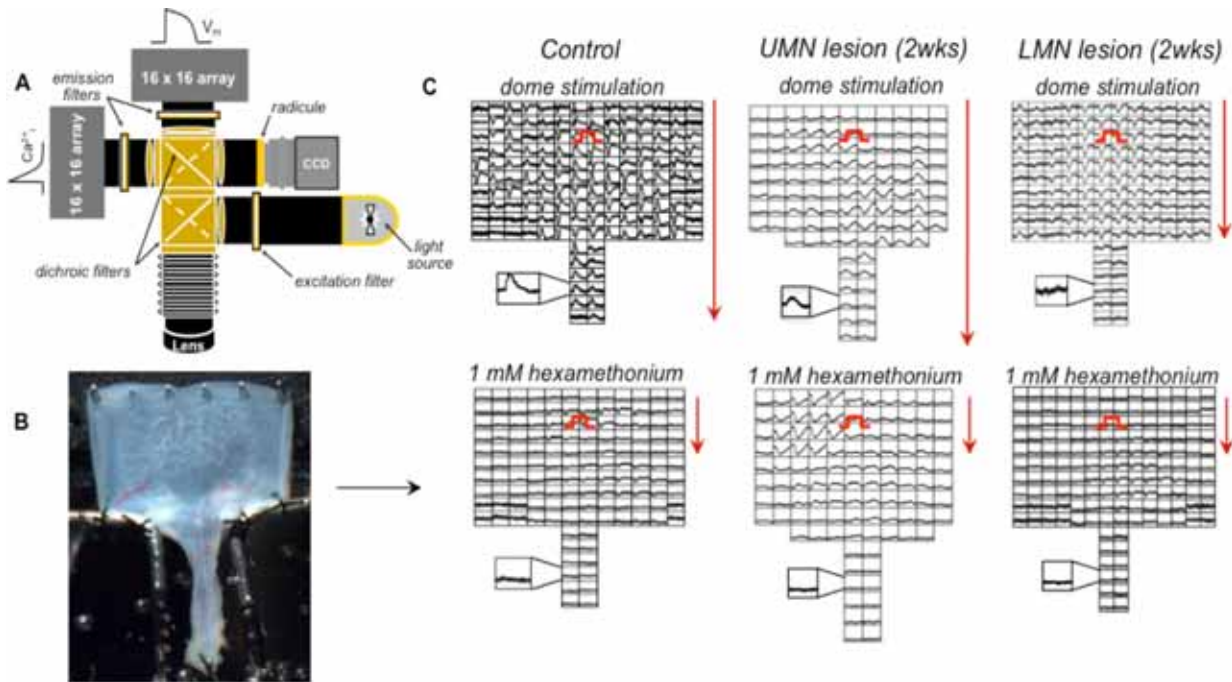
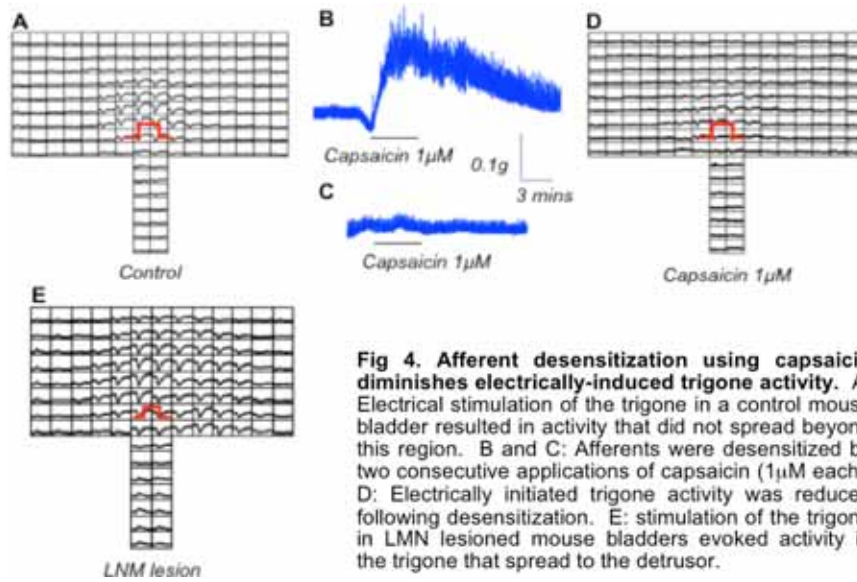


Fig 3. Optical mapping setup to record bladder-urethra communication. A: Optical system showing the light source, filters and photodiode array cameras. B: Bladder-urethra preparation. C: Action potentials recorded from bladder preparations of control, upper motor and lower motor neuron lesioned mice.

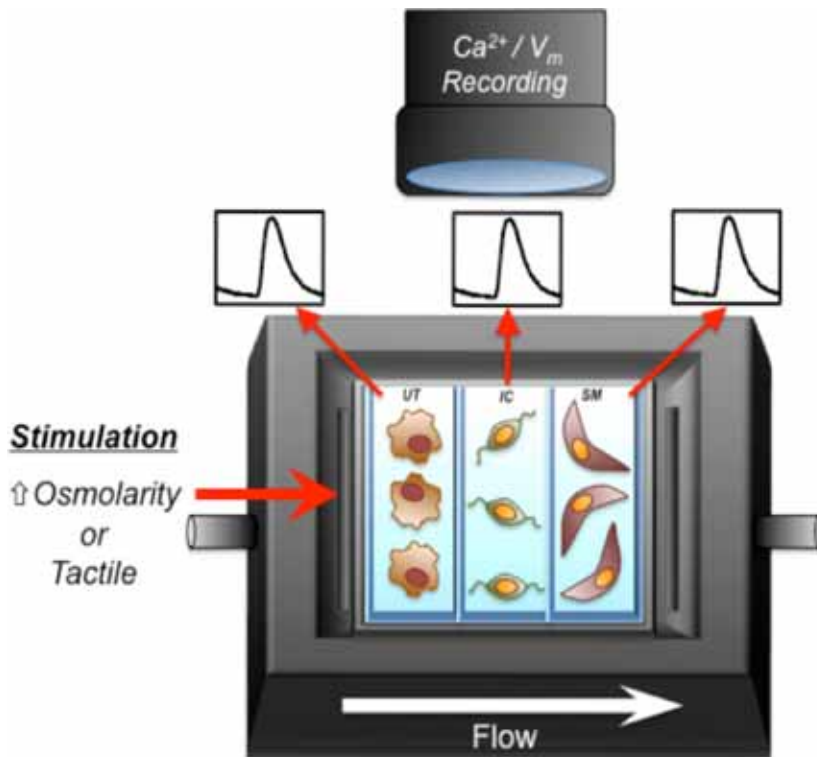
Optical imaging (fig 3A) allows us to map the spread of spontaneous and electrically-evoked action potentials and intracellular Ca²⁺ transients throughout the bladder and urethra (fig 3B). Focal electrical stimulation at the bladder dome evoked activity that spread into the urethra in the bladders of control and UMN lesioned mice. This spread was abolished by the ganglionic blocker, hexamethonium (C6). However, with LMN lesions, activity initiated in the bladder did not spread into the urethra suggesting the involvement of intramural ganglia in bladder urethral coordination (fig 3C).

Electrical stimulation of the trigone in control mouse bladders resulted in activity that did not spread beyond this region (fig 4A). However, with LMN (fig 4E) and UMN (not shown) lesioned animals, stimulation of the trigone resulted in activity that spread into the detrusor. This was blocked by TTX, but not by hexamethonium, indicating a nerve mediated non-cholinergic mechanism. In addition, administration of capsaicin (1μM x 2) (figs 4B and C) substantially attenuated trigone stimulated activity (fig 4D), suggesting

that this activity is afferent mediated.



4) Smooth Muscle and Interstitial Cell Interactions: There are numerous studies that have demonstrated the ability of the urothelium to act as a sensory structure by releasing transmitters, a function generally associated with neurons. The target for these signaling molecules has not been fully elucidated, however structures located in the suburothelium or lamina propria are thought to be likely sites of action.



There are several cell types in close contact with the urothelial layer, the two of particular interest with respect to normal bladder sensory function are the interstitial cells (IC) and afferent nerve terminals. Sensory nerves densely innervate the suburothelial layer and can even extend in to the urothelium itself. IC are also in close contact with the basal layer of the urothelium. The closeness of these structures to the urothelium would allow them to respond to any diffusible factors that may be released during bladder filling. It can be hypothesized that the urothelium may communicate with the underlying IC and afferent terminals, which in turn may affect smooth muscle function and sensation. However, the functional characteristics of bladder IC have not been fully elucidated. In addition, experimental evidence showing direct communication between urothelium, IC and detrusor smooth muscle has yet to be demonstrated. One novel approach is to use cultured urothelial, interstitial and smooth muscle cells and optically map the transmission of activity from one cell type to another (fig 5). In this paradigm, it is possible to selectively activate urothelial cells and determine if signals can propagate to smooth muscle *via* interstitial cells. If so, this can be verified by removing the IC culture and determine if the urothelium is still able to communicate with the smooth muscle. Further understanding of urothelial-IC-smooth muscle communication mechanism may help to uncover new signaling pathways in the bladder and potentially lead to new therapeutic targets.

5) New Agents for Overactivity and Sensation. As a normal bladder fills there is little increase in the intravesical pressure until micturition pressure is reached. In contrast, the wall tension will increase linearly as the bladder expands. Micturition contractions are triggered when mechanosensitive A δ -fiber firing reaches the threshold rate, during this time C-fibers are thought to remain silent. Bladder overactivity may be driven by a number of underlying causes, these include central/peripheral afferent sensitization, bladder wall compliance changes and enhanced spontaneous detrusor contractions. Furthermore, intrinsic bladder activity mediated by the urothelium/suburothelium may contribute to overactivity. Figure 6A shows a flow diagram of the potential mechanism by which factors from the urothelium can drive detrusor overactivity by enhancing spontaneous smooth muscle contractions. However, it should be noted that bladder overactivity is unlikely a result of a single cause but rather a combination of the abovementioned factors. Therefore, there are numerous targets within the bladder that have been considered for pharmacological interventions. Some of the newer targets are shown in the schematic in figure 6B. Including receptors on the urothelium (e.g., TRPV1), interstitial cells (e.g. P2Y₆), smooth muscle (e.g., β_3) and afferent nerve terminals (e.g., P2X₃).

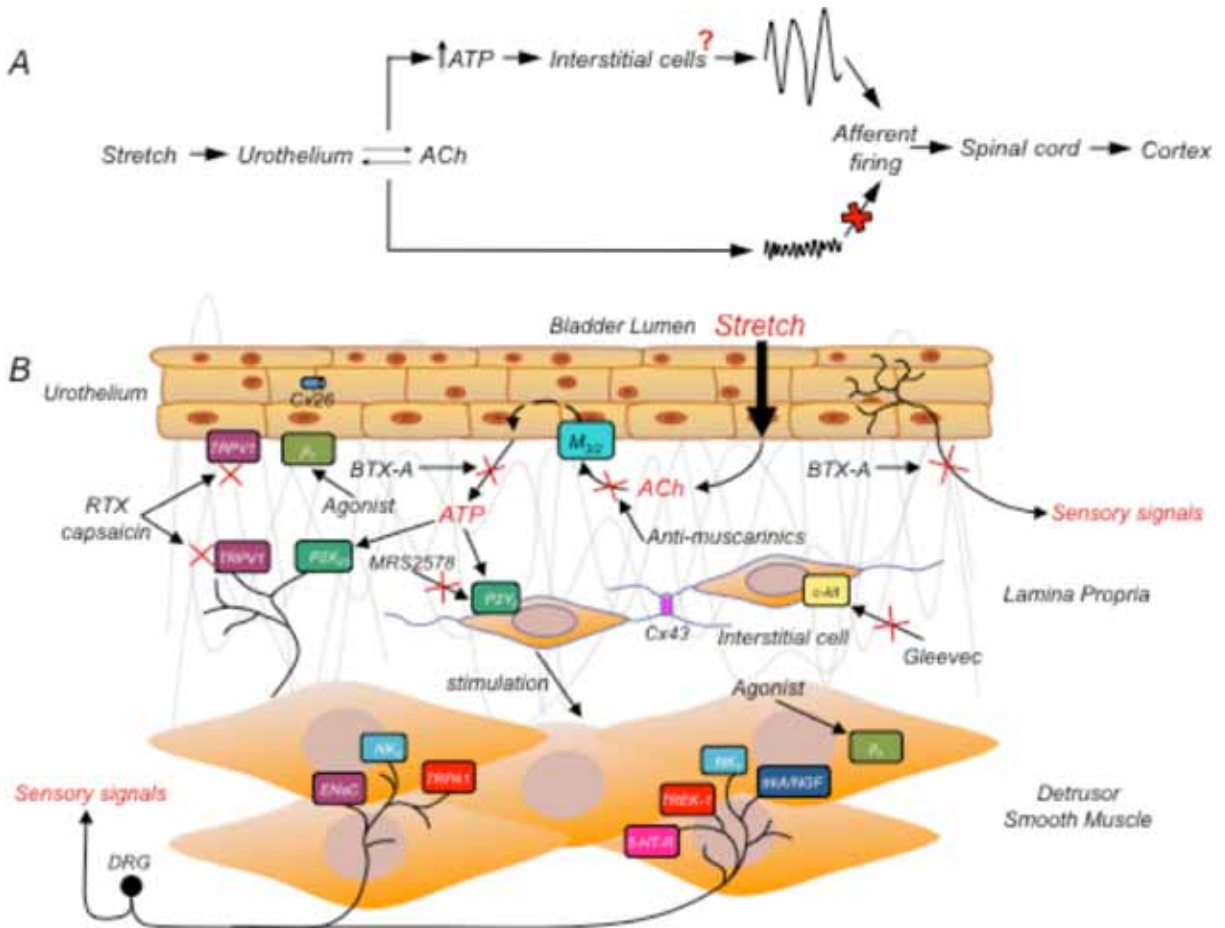


Fig 6. (A) Flow diagram describing the effects on spontaneous bladder contractions on afferent nerve firing. (B) Receptor and other potential drug targets that may be involved in overactivity and sensation.