

## BLADDER AFFERENT NEURONS SELECTIVELY INTERACT WITH APICAL UROTHELIAL CELLS

### Hypothesis / aims of study

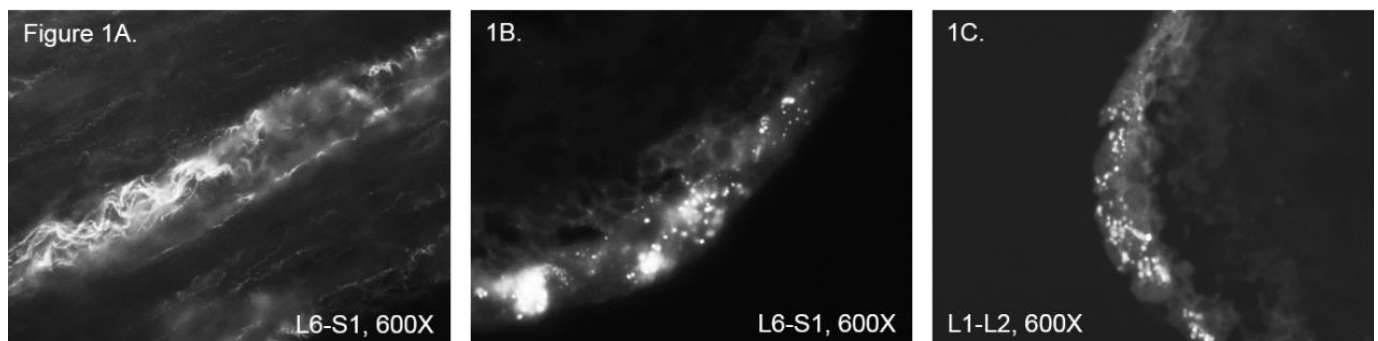
The urothelium is capable of releasing a multitude of signalling factors including ATP, nitric oxide or prostaglandins to modulate the activity of sensory nerves innervating the suburothelium. In turn, sensory nerves innervating the urinary bladder are thought release neuropeptides to regulate urothelial function [1]. It has been demonstrated that neurotrophic viruses injected into the L6-S1 dorsal root ganglia (DRG) can transfect the entire urothelium [2]. However, this study could not determine which urothelial layers communicate directly with afferent nerves. Therefore, the nature of afferent-urothelial communication and its role in bladder function is still not entirely clear. Further information on afferent-urothelial interactions is essential and may be important in uncovering a variety of bladder pathological mechanisms including interstitial cystitis / bladder pain syndrome. Our aim was to investigate afferent to urothelial interactions by injection of fluorescent microbeads into the DRG containing afferent neurons that travel through the pelvic (L6-S1) and hypogastric (L1-L2) nerves.

### Study design, materials and methods

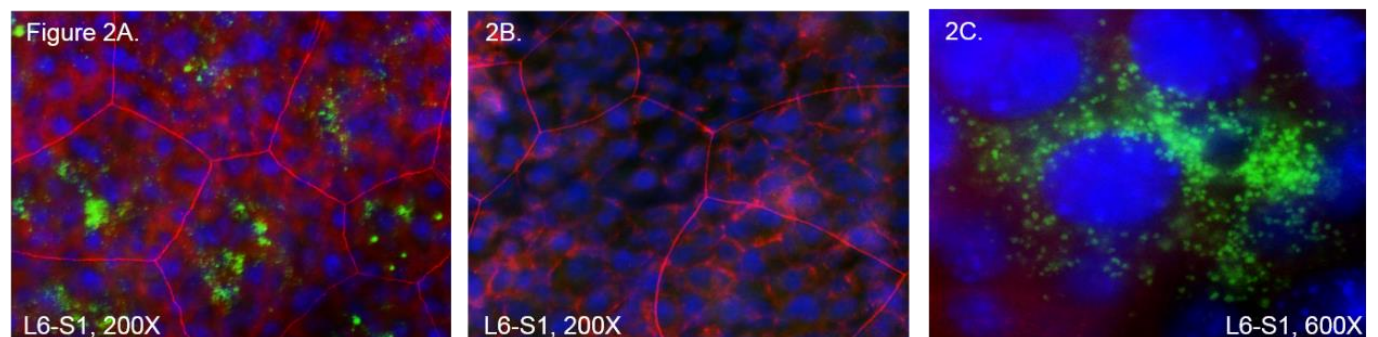
Adult (6-8 weeks) female C57Bl/6 mice were used for this study. Mice were anesthetized with isoflurane (5% induction, 2% maintenance in 100% oxygen) and L6-S1 or L1-L2 DRG were exposed. Ganglia were injected with red-fluorescent or streptavidin-coated microbeads (1  $\mu$ l/DRG, 0.02-0.04  $\mu$ m diameter, Life Technologies) using 1 mm quartz micropipettes bevelled to 45°. Sham controls were injected with sterile saline. The surgical site was packed with haemostatic sponge, the wound sutured and animals allowed to recover for 7-10 days. For tissue isolation, mice were given 200 units of heparin, anesthetized with Avertin (2,2,2-tribromoethanol, 2 mg/kg) and transcardially perfused with oxygenated Krebs solution. Urinary bladders were fixed with 4% paraformaldehyde and examined using fluorescence microscopy as whole-mounts or frozen tissue sections (8  $\mu$ m thick). Zonula occluden-1 (ZO-1) expression was detected by immunohistochemical staining and streptavidin-microbeads were visualized in the same preparation by incubating tissues with biotin conjugated FITC. Images were acquired using Hamamatsu cameras and HClmage software. Images were prepared in Photoshop CS5 and only brightness/contrast were adjusted.

### Results

Red fluorescent bead injections into L6-S1 or L1-L2 DRG demonstrated labelling of nerve fibres that run through the muscle layer (Fig 1A) and accumulation of beads in the urothelium (Fig 1B and C). There was high level of bead accumulation within the urothelial layer with both pelvic and hypogastric DRG injections, with notably more intense labelling with L6-S1 injections.



Whole-mount bladder mucosa preparations demonstrated that in bead injected mouse bladders, clusters of FITC fluorescence were present in the apical layer (Fig 2A; red – ZO-1, green – FITC, blue – DAPI nuclear stain). ZO-1 was labelled in order to visualize the characteristic tight junction structure of the apical urothelium. FITC fluorescence was not detected in sham injected bladders (Fig 2B). Higher magnification showed punctate fluorescence, potentially indicating there was vesicular uptake of the beads (Fig 2C).



### Interpretation of results

These data demonstrate the direct interaction between afferent neurons and apical urothelial cells. However, not all umbrella cells exhibited fluorescent labelling, suggesting that only a subpopulation of apical cells communicate with sensory neurons.

### Concluding message

Communication of urothelial cells through transmitter release with afferent nerves has been shown to be an integral part of normal lower urinary tract function. This study has shown that bladder sensory nerves directly interact with apical umbrella cells in a bidirectional manner. This suggests a mechanism by which afferent nerves can sense luminal changes including urine composition, pH, intravesical pressure, *etc.*, to modulate voiding function. Accordingly, the physiological role of afferent to urothelial signalling and its involvement in bladder pathologies (*e.g.* interstitial cystitis) warrants further investigation.

### References

1. Birder and Andersson, Urothelial signalling, *Physiol Rev.*;93(2):653-80, 2013
2. Ikeda Y et al., Bidirectional communication between afferent neurons and urothelial cells in the mouse urinary bladder, *Journal of Urology*, Vol.191(4), e142, 2014.

### Disclosures

**Funding:** NIH/NIDDK grants (DK093424, DK071085 and DK098361) **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Mouse, C57Bl/6 strain **Ethics Committee:** University of Pittsburgh Institutional Animal Care and Use Committee (IACUC)