

## STRETCH DEPENDENT ACTIVATION OF UROTHELIAL MUSCARINIC RECEPTORS PROMOTES DETRUSOR OVERACTIVITY FOLLOWING SPINAL CORD INJURY—REVEALED USING OPTICAL IMAGING

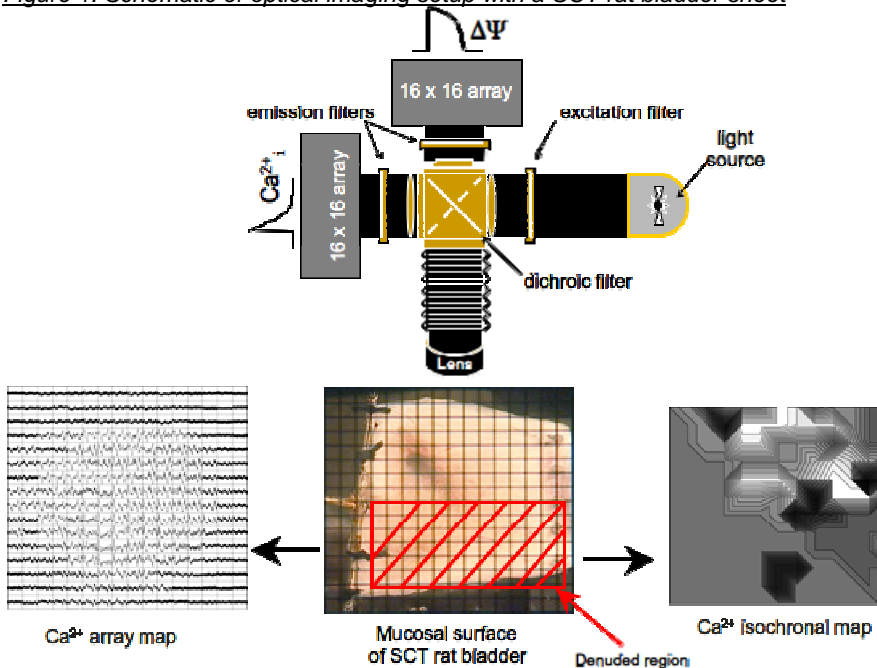
### Hypothesis / aims of study

Antimuscarinics are effective in patients with overactive bladder symptoms during the filling phase, when acetylcholine (ACh) is not thought to be released from efferent bladder nerves supplying the detrusor. Preliminary results, obtained using novel optical techniques, suggest that stretch releases acetylcholine (ACh) from the urothelium that activates urothelial muscarinic receptors in the bladders of both normal and spinal cord transected (SCT) rats. In SCT animals, there was increased stretch-induced spontaneous detrusor contractions which could be mimicked by low-dose carbachol, blocked by atropine or abolished by removal of the urothelium. Accordingly, our aim was to determine if urothelial muscarinic receptors are the site of action of antimuscarinic agents.

### Study design, materials and methods

Bladders from normal adult (3 months old) and spinal cord transected (T<sub>8</sub>-T<sub>10</sub>, 2 weeks postoperative) rats were harvested and cut from outlet to dome along the dorsal aspect to form a sheet. The sheet preparations were either left intact or partially denuded of the mucosal surface. The bladders were then stained using Ca<sup>2+</sup>- (10 μM Rhod-2-AM) and voltage-sensitive (10 μM Di-4-ANEPPS) dyes. After staining the bladders were transferred to a recording chamber, where the base of the bladder was secured to a fixed platform with pins while dome was connected to a tension transducer. The bladder sheets were perfused with Tyrode's solution (95% O<sub>2</sub> and 5% CO<sub>2</sub>, pH 7.35) at 37 °C, stretched to 1 g of tension and imaged from the mucosal surface. Isochronal maps were generated from the local activation time-points for up to 256 optical action potentials and intracellular Ca<sup>2+</sup> transients using cross-correlation analysis. The schematic for the optical imaging set-up is shown in Figure 1. Drugs were added to the bath from stock solutions for final working concentrations. The preparations were stimulated using small increments of stretch (up to 10% of resting length) or by the addition of low-dose carbachol (50 nM).

*Figure 1. Schematic of optical imaging setup with a SCT rat bladder sheet*



### Results

SCT bladder preparations showed high amplitude, low frequency spontaneous contractions, in contrast to the low amplitude, high frequency contractions observed in normal adults. Stretch and 50 nM carbachol caused a significant increase in Ca<sup>2+</sup> and voltage activity across the mucosal surface of the SCT bladders. This corresponded with an increase in the amplitude and frequency spontaneous contractile activity (Carbachol: 124±56% increase in amplitude of control, n=4; Stretch: 79±18% of control, n=3). Normal adult bladders did not show the same degree of increase in activity (Figure 2). The isochronal maps showed that Ca<sup>2+</sup>/voltage transients occur randomly across the bladder surface in normal adults, while in SCT bladders they arise from a single initiation site in the dome (Figure 3). White regions represent initiation sites, while increasing darker regions represent progressively longer conduction delays. The optical and contractile responses to carbachol and stretch were abolished when the urothelial/lamina propria was removed or when 1 μM atropine was added.

*Figure 2. Effects of low-dose carbachol and stretch on spontaneous contractions in normal and SCT rat bladders*

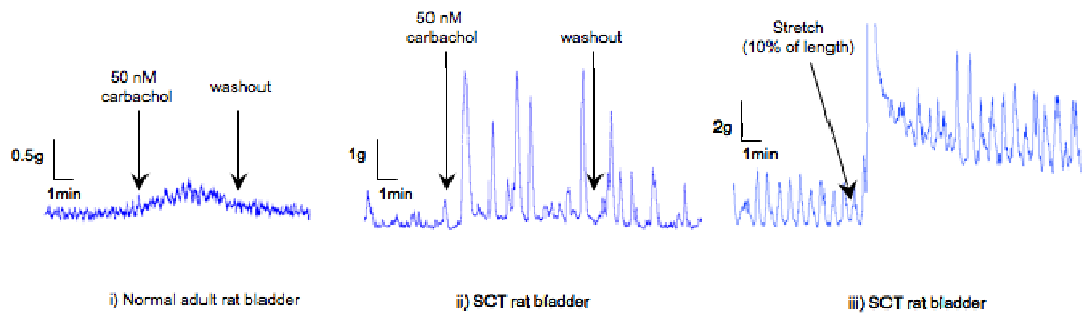
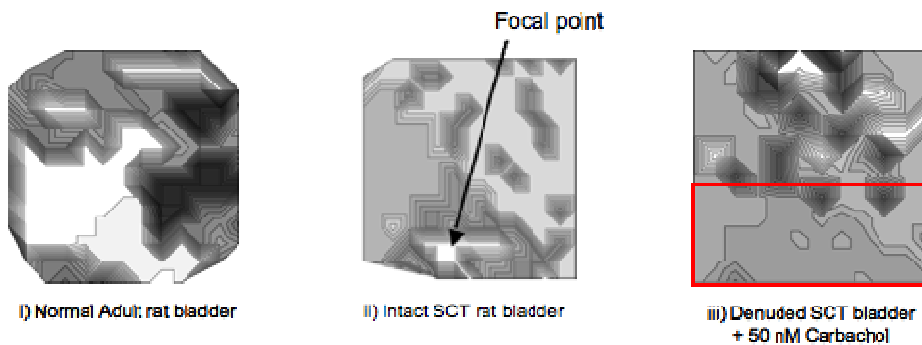


Figure 3.  $Ca^{2+}$  isochronal maps from a normal adult and intact and denuded SCT rat bladders. (Red box indicates mucosa denuded region)



#### Interpretation of results

In SCT rat bladders, optical mapping revealed a change from un-coordinated to single-site initiated contractile activity. This coordination may account for the high amplitude low frequency contractions. The presence of an intact urothelium was essential for the stretch and carbachol-mediated enhancement of spontaneous contractions, suggesting that muscarinic receptors on the urothelium have a role in modulating detrusor contractile activity.

#### Concluding message

The results suggest that the urothelium is required for enhancing spontaneous detrusor contractions *via* the activation of urothelial muscarinic receptors. Furthermore, that ACh is released from the urothelium in response to stretch and activates downstream signaling pathways to enhance intrinsic detrusor contractions. The response to stretch and low-dose carbachol was significantly increased in SCT rats compared to control bladders. This increased sensitivity may play a significant role in detrusor overactivity.

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**ANIMAL SUBJECTS:** This study followed the guidelines for care and use of laboratory animals and was approved by University of Pittsburgh Institutional Animal Care and Use Committee