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SPINAL CORD INJURY LEADS TO AFFERENT-MEDIATED TRIGONE-TO-DETRUSOR COUPLING: REVEALED USING OPTICAL MAPPING

Hypothesis / aims of study: The trigone is defined as a triangular region between the ureteric orifices and the bladder outlet and has been studied extensively; however, its functions are still incompletely understood. In these studies we investigated changes in trigone-to-detrusor cellular communication following spinal cord injury at different levels.

Study design, materials and methods: C57Bl10 mice were anesthetized with 1.5-5% isoflurane. Under sterile conditions, a laminectomy was performed and the spinal cord transected between T8-T9 for upper motor neuron (UMN) and L4-L5 for lower motor neuron (LMN) lesions. Absorbable sponge was packed between the cut, muscle and skin sutured and animals allowed to recover with prophylactic antibiotics. The mice had their bladders expressed twice a day by gentle abdominal compression and were used for experiments 2-6 weeks after surgery.

The optical imaging system (Figure 1A) was custom-built and has been previously described [1]. Animals were anesthetized with 5% isoflurane and their bladders with urethra excised. Preparations were placed in a recording chamber with oxygenated Krebs solution, and cut from outlet to dome along the midline-dorsal aspect to form sheets. The dome was then pinned to a fixed platform with the mucosal surface facing up and the outlet end connected to a tension transducer (Figure 1B). Preparations were stretched to optimal resting tension (1-1.5 g), allowed to equilibrate for 30 min and stained with voltage- (di-4-ANEPPS; 5 μM) and/or Ca^{2+}-sensitive dyes (Rhod-2-AM; 5 μM) for 20 min each. Stretch was applied *via* a tension transducer at a rate of 10 μm/sec, to mimic the slow filling of the bladder. Preparations were stimulated using a bipolar electrode, electric field stimulation or chemical agonists.

Results: Electrical stimulation of the trigone in control mouse bladders resulted in activity that did not spread beyond this region (Figure 1C). However, in LMN (Figure 1G and 2B) and UMN (Figure 2B) lesioned bladders, stimulation of the trigone resulted in activity that spread into the detrusor. This was blocked by tetrodotoxin (TTX), but not by hexamethonium (C6). In addition, administration of capsaicin (1μM x 2) (Figures 1D and E) substantially attenuated trigone stimulated activity (Figure 1F), suggesting this activity is in great part afferent mediated.

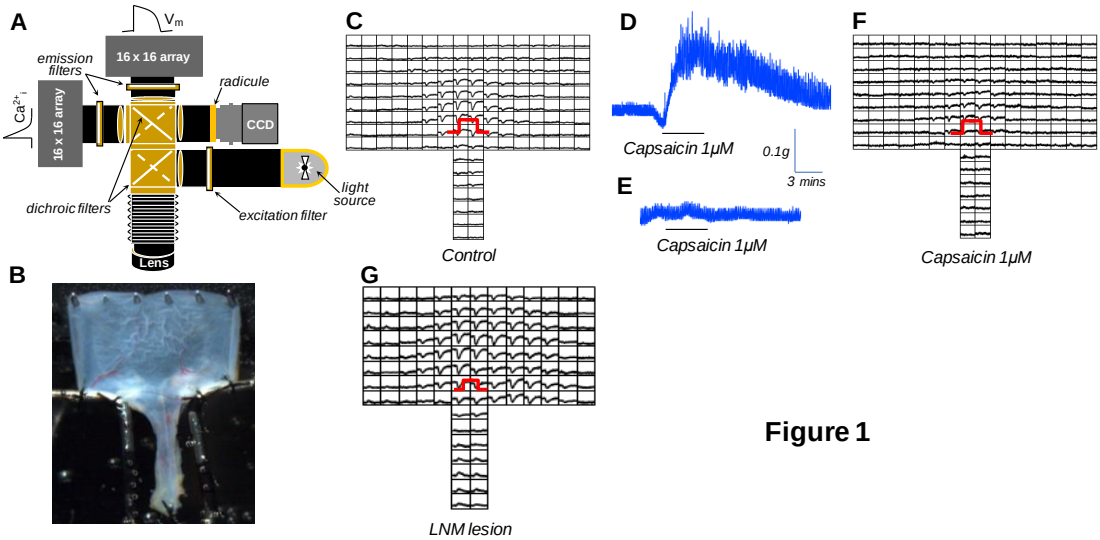

Figure 1

At 6 weeks following LMN lesion, bladder-urethra preparations failed to respond to electrical stimulation suggesting substantial denervation within the tissues (Figure 2A and B). However, the detrusor did contract in response to an exogenously applied muscarinic agonist (arecaidine, 1 μM, not shown).

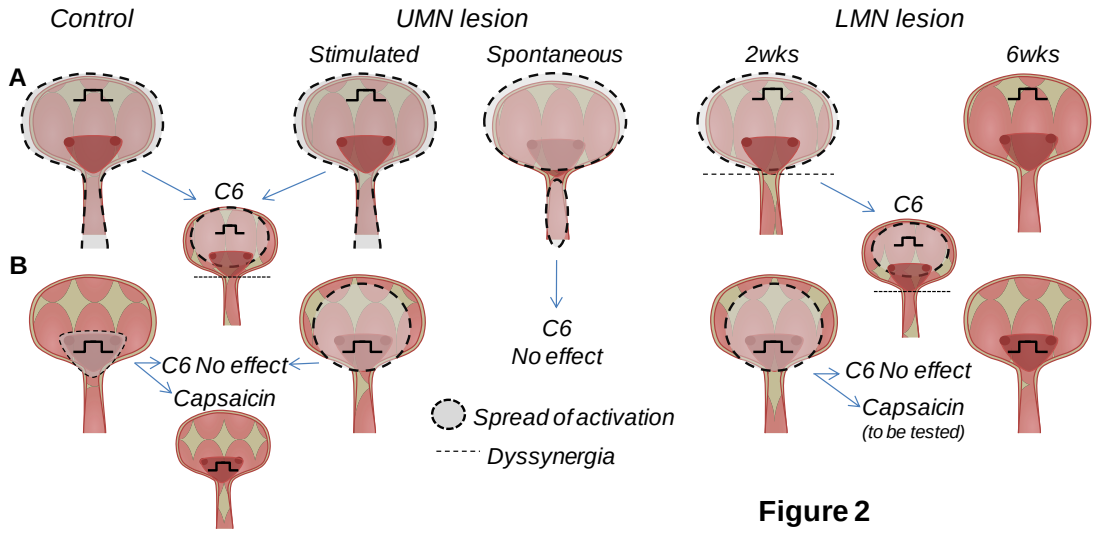

Figure 2

Interpretation of results: The trigone, which we have shown is electrically discrete from the detrusor in normal bladders, becomes coupled to the detrusor following spinal cord injury *via* neural pathways which can be blocked by TTX but not by a ganglionic blocking agent (hexamethonium), indicating a nerve-mediated noncholinergic mechanism. Thus, afferent nerves may become hyperexcitable and undergo morphological expansion from the trigone to the detrusor. Excitatory signals passing from the trigone to the detrusor may be responsible in part for the neurogenic detrusor overactivity occurring after UMN lesions. Our findings demonstrate that capsaicin-induced desensitization of afferent nerves decreased electrically evoked trigonal activity.

Concluding message: Our studies indicate that trigone-to-bladder cellular communication is altered by spinal cord injury and that these changes are likely to contribute to neurogenic urine storage disorders.

References

1. Kanai A, Roppolo J, Ikeda Y et al. Am J Physiol Renal Physiol. 292: F1065-F1072, 2007.

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Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional Animal Care and Use Committee of University of Pittsburgh**