

CANINE URINARY BLADDER RECEIVES FUNCTIONAL INNERVATION FROM THE UPPER LUMBAR AND LOWER THORACIC SPINAL CORD AFTER SACRAL ROOT TRANSECTION

Hypothesis / aims of study

It is well established that the bladder receives parasympathetic fibers that directly project motor axons from the sacral spinal cord, as well sympathetic fibers from the lower thoracic and upper lumbar spinal cord. Additionally, we have preliminary evidence of a small number of motor neurons originating in lower thoracic and upper lumbar ventral horns that also directly project to the detrusor. We further explored this innervation pattern and hypothesize that these direct fibers originating outside of the sacral region increase their input to the bladder after sacral root transection.

Study design, materials and methods

This study consisted of two different experiments, an *in vivo* electrophysiological study and a retrograde dye tracing study. For the *in vivo* experiment, female mixed-breed hounds were assigned to either a sham-operated group (n=4) or a sacral root transection group (n=6), which included bilateral transection of all roots caudal to spinal level L7. At terminal surgery 8-12 months following the initial procedure, animals received a laminectomy from spinal level T10 to S4. Roots originating from each level were stimulated and changes in pressure were continuously recorded with external pressure transducers interfaced with the PowerLab® multichannel data acquisition system and LabChart® software (ADInstruments, Colorado Springs, CO). Strength of nerve-evoked bladder contractions after spinal cord or ventral root stimulation were derived from the difference between pressure at resting baseline and peak pressure obtained from continuous stimulation. In a different cohort of sham-operated (n=6) and sacral root transected (n=5) dogs, bladders were cystoscopically injected with Fluoro-gold, a neuronal retrograde label, at four sites around the ureterovesical junction bilaterally three weeks prior to euthanasia. Following euthanasia, the spinal cord was collected, fixed in 4% buffered paraformaldehyde, cryosectioned, and examined for retrogradely labelled neurons in the ventral horns of spinal cord segments T10 through S3.

Results

At one year after sacral root transection, there was a significant decrease in maximum bladder contraction after stimulation of S1-S3 spinal cord/roots, confirming complete transection. Interestingly, sacral root transection also caused an increase, just shy of statistical significance, in maximum bladder contraction following stimulation of T12-L3 spinal cord segments and roots compared to control animals (16.0±3.8 and 8.0±3.3 cmH₂O respectively; p=0.1714). An increase in sample size is needed to better study this effect.

Also at one year after sacral root transection, we observed a significant decrease in the number of retrogradely labelled cells in the S1-S3 ventral horns, confirming completely transected spinal roots caudal to L7 in the sacral root transected animals. However, in both groups, similar numbers of retrogradely labelled neuronal cell bodies were observed in the ventral horns of T10 through L2 spinal cord segments following injection of a retrograde dye into the bladder wall, indicative of direct axonal projections from these segments to the bladder. These neurons were located in lamina VIII and IX of the ventral horns, strongly suggestive of motor function.

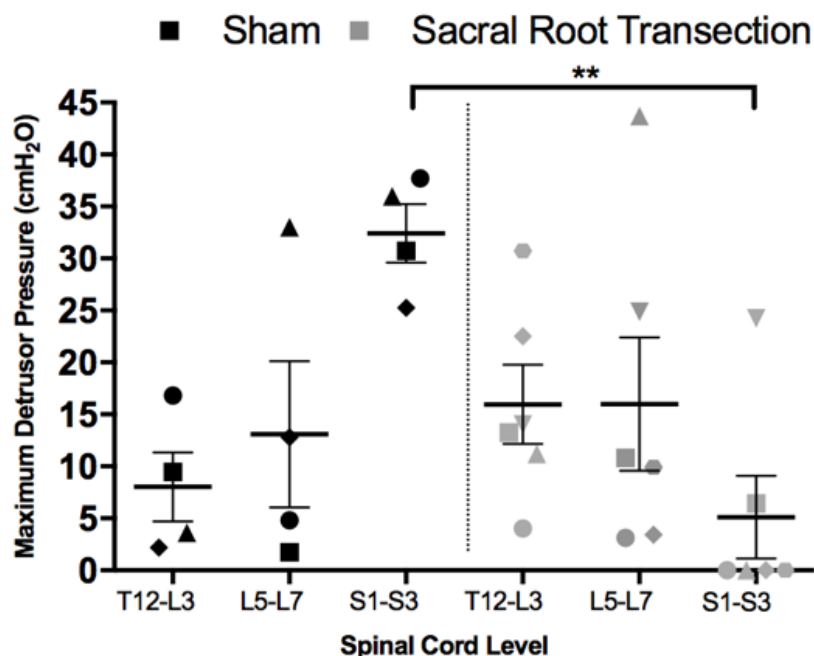


Figure 1. Spinal cord or root stimulation-induced bladder contraction *in vivo*. Spinal cord and spinal roots between T10 and S3 were stimulated with 3-5 mAmp current. Each animal is represented by a unique shape. Maximum contractions in each region are represented by a single point. Sacral root transection significantly decreased the maximum detrusor contraction induced by roots in the sacral spinal cord (S1-S3) compared to controls (p<0.01).

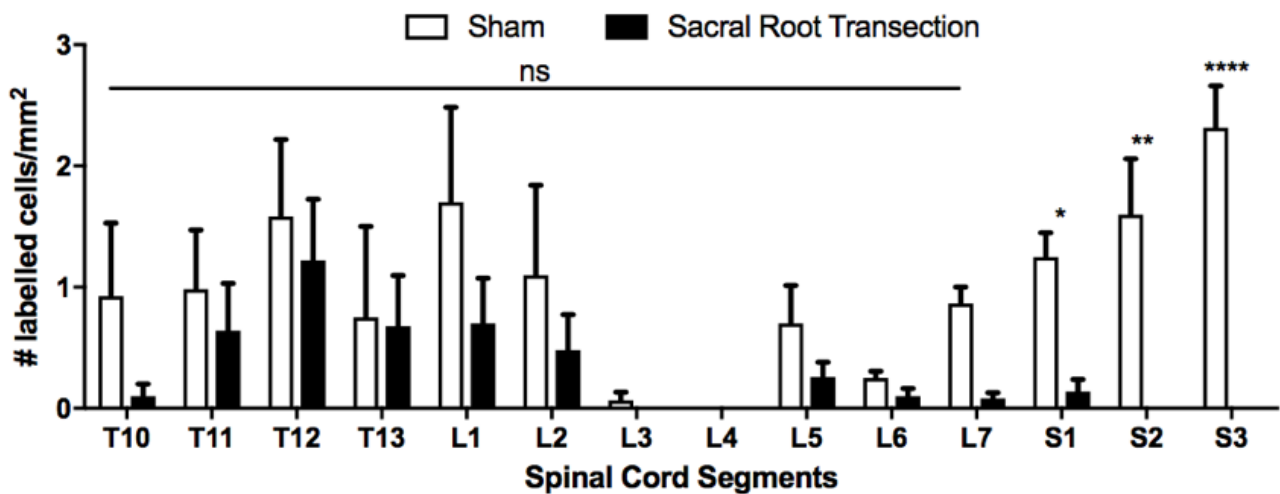


Figure 2. Retrograde labelling of neuronal tracts from bladder wall to spinal cord. Bladder was injected with Fluoro-gold, a neuronal retrograde label. Mean number of retrogradely labelled cells per mm² were counted in ventral horn of different levels of spinal cord. There was no difference in number of positively labelled cells seen between T10 and L7 of the spinal cord. Sacral root transection significantly decreased the number of labelled cells seen at S1 ($p < 0.05$), S2 ($p < 0.01$), and S3 ($p < 0.001$).

Interpretation of results

Little is known about the motor axons originating from the upper lumbar or lower thoracic spinal cord that innervate the bladder. The presence of retrogradely labelled neuronal cell bodies in ventral horn regions of T10-L2 cord segments is the first evidence of direct motor projections from these segments to the bladder. Only low numbers of labelled cells were observed, indicating this is likely not the main innervation of the bladder. This result, combined with the increase in upper lumbar/lower thoracic stimulation-induced bladder contractility (although just short of statistical significance) after long-term sacral root transection, is suggestive of increased sensitivity to stimulation rather than an injury-induced increase in neuronal cell numbers. Beyond mediating bladder contractility, sacral nerves may stimulate interneurons that inactivate tracts originating from the upper lumbar region. Without sacral input, latent tracts may become uninhibited and partially compensate for the contractile deficit.

Concluding message

Future acute experiments will include stimulation of all spinal roots caudal to T9 prior to sacral root transection and immediately after transection to better determine whether intact sacral roots inhibit upper lumbar/lower thoracic input on the bladder. A clearer picture of normal and adaptive bladder innervation will inform our future surgical bladder reinnervation studies.

Disclosures

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