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Research Paper

# Neuromodulation of the neural circuits controlling the lower urinary tract

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ABSTRACT

The inability to control timely bladder emptying is one of the most serious challenges among the many functional deficits that occur after a spinal cord injury. We previously demonstrated that electrodes placed epidurally on the dorsum of the spinal cord can be used in animals and humans to recover postural and locomotor function after complete paralysis and can be used to enable voiding in spinal rats. In the present study, we examined the neuromodulation of lower urinary tract function associated with acute epidural spinal cord stimulation, locomotion, and peripheral nerve stimulation in adult rats. Herein we demonstrate that electrically evoked potentials in the hindlimb muscles and external urethral sphincter are modulated uniquely when the rat is stepping bipedally and not voiding, immediately pre-voiding, or when voiding. We also show that spinal cord stimulation can effectively neuromodulate the lower urinary tract via frequency-dependent stimulation patterns and that neural peripheral nerve stimulation can activate the external urethral sphincter both directly and via relays in the spinal cord. The data demonstrate that the sensorimotor networks controlling bladder and locomotion are highly integrated neurophysiologically and behaviorally and demonstrate how these two functions are modulated by sensory input from the tibial and pudental nerves. A more detailed understanding of the high level of interaction between these networks could lead to the integration of multiple neurophysiological strategies to improve bladder function. These data suggest that the development of strategies to improve bladder function should simultaneously engage these highly integrated networks in an activity-dependent manner.



## 1. Introduction

The principal function of the lower urinary tract (LUT) is to collect and store urine and periodically expel it from the body in a socially acceptable time and place. The LUT consists of two mechanically simple components: the urinary bladder that stores urine at low pressures and the urethra that provides the conduit for voiding the stored urine. The urethra consists of two subcomponents each working as a release-valve: the internal urethral sphincter (IUS) and the external urethral sphincter (EUS) (de Groat, 1997). Micturition is initiated when the neural networks relax the EUS and simultaneously activate bladder contractions (Griffiths et al., 1986; Mallory et al., 1989). Afferent sensory feedback from the urethra conveys information to the lumbosacral spinal cord related to the urine flow further facilitating bladder contraction.

After a severe spinal cord injury (SCI), the sensitivity and responsiveness to sensory input from the bladder and pelvic nerves is drastically altered (Yoshimura, 1999). This results in abnormal coordination of bladder-sphincter contractions, resulting in inefficient or no bladder voiding. Current treatments for neurogenic LUT dysfunction including detrusor sphincter dyssynergia consists of a combination of pharmaceutical, mechanical, and surgical interventions, focusing primarily on the suppression of overactive detrusor contractions to improve bladder capacity, reduction in leakage and incontinence episodes, and reduction in the risk of autonomic dysreflexia.

More recent findings in rats and humans with severe spinal cord injury demonstrate a more complex and highly integrated neural networks playing an important role in the control of LUT function. More specifically there is a clear functional link between the postural and

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locomotor networks and LUT networks. Four human subjects with complete paralysis were implanted with a spinal cord electrode array. These subjects showed the ability to stand with full weight bearing and recovered some voluntary control of movement in the lower extremities in the presence, but not the absence, of epidural stimulation (ES) (Angeli et al., 2014; Harkema et al., 2011). All four subjects showed anecdotal evidence of improved bladder function, including the ability to sense a filled bladder as well as the ability to volitionally, partially void the bladder even in the absence of ES. This improvement was attributed to the adaptations resulting from repeated treatment sessions consisting of a combination of weight-bearing standing and ES, although the mechanisms involved are unknown. Along with an improvement in bladder function, the subjects reported an improvement in cardiovascular function, thermoregulation, and sexual function. The overlap in the neural control of somatic and bladder function suggest that there is extensive sharing of neurons that control of the two functional neural networks. It seems likely that the two functional control systems can be differentiated via varying frequencies of activation and proprioceptive inputs. Alternatively there could be two completely independent networks albeit both modulated by the same afferent inputs. The main purpose of the present study, therefore, was to assess the acute effects of varying frequencies and amplitudes of ES in the lumbosacral region of the spinal cord and/or peripheral nerve stimulation (tibial and pudendal nerve stimulation) on the neuromodulation of the LUT.

## 2. Methods

### 2.1. Experimental design

Data were obtained from 8 (3 non-injured and 5 SCI) adult female Sprague Dawley rats (270–300 g body weight). Pre- and post-surgical animal care procedures have been described in detail previously (Roy et al., 1992). The rats were housed individually with food and water provided ad libitum. All survival surgical procedures were conducted under aseptic conditions with the rats deeply anesthetized with isoflurane gas administered via facemask as needed. All procedures were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Animal Research Committee at UCLA. SCI rats were allowed to recover for 7 days after which step training under the influence of spinal cord ES was initiated. Step training was performed 5 days/week for 20 min/day for 6 weeks. At 7 weeks post-injury, the rats were tested to step bipedally on a treadmill with ES (40 Hz) while the rat was supported by a body weight support (Gad et al., 2013, 2014; Courtine et al., 2009; Lavrov et al., 2008a). Motor evoked potentials were generated by ES (1, 5, and 40 Hz) and recorded in selected hindlimb muscles and the EUS while the rat was suspended in a harness (Gad et al., 2014; Lavrov et al., 2008a). In terminal experiments electrophysiological responses in non-injured and SCI rats were recorded under anesthesia (1.2 mg/kg urethane administered s.c.) (Chang and Havton, 2008a,b).

### 2.2. Head connector and chronic intramuscular EMG electrode implantation

A small incision was made at the midline of the skull. The muscles and fascia were retracted laterally, small grooves were made in the skull with a scalpel, and the skull was dried thoroughly. Two amphenol head connectors with Teflon-coated stainless steel wires (AS632, Cooner Wire, Chatsworth CA) were attached securely to the skull with screws and dental cement (Courtine et al., 2009; Ichiyama et al., 2008a). The tibialis anterior (TA) and soleus muscles were implanted bilaterally with intramuscular EMG recording electrodes (Courtine et al., 2009; Ichiyama et al., 2008a). Skin and fascial incisions were made to expose the belly of each muscle. Two wires extending from the skull-mounted connector were routed subcutaneously to each muscle. The wires were inserted into the muscle belly using a 23-gauge needle and a small notch (0.5–1.0 mm) was removed from the insulation of each wire to expose the conductor and form the electrodes. The wires were secured in the belly of the muscle via a suture on the wire at its entrance into and exit from the muscle belly. The proper placement of the electrodes was verified during the surgery by stimulating through the head connector and post-mortem via dissection (Gad et al., 2013, 2015a).

### 2.3. Spinal cord transection, epidural electrode implantation, and post-surgical animal care procedures

A partial laminectomy was performed at the T8–T9 vertebral level to expose the spinal cord. A complete spinal cord transection to include the dura was performed at approximately the T8 spinal level using microscissors. Two surgeons verified the completeness of the transection by lifting the cut ends of the spinal cord and passing a glass probe through the lesion site. Gel foam was inserted into the gap created by the transection as a coagulant and to separate the cut ends of the spinal cord. For epidural electrode implantation, partial laminectomies were performed to expose the spinal cord at spinal levels L2 and S1. Two Teflon-coated stainless steel wires from the head connector were passed under the spinous processes and above the dura mater of the remaining vertebrae between the partial laminectomy sites. After removing a small portion (~1 mm notch) of the Teflon coating and exposing the conductor on the surface facing the spinal cord, the electrodes were sutured to the dura mater at the midline of the spinal cord above and below the electrode sites using 8.0 Ethilon suture (Ethicon, New Brunswick, NJ). Two common ground (indifferent EMG and stimulation grounds) wires (~1 cm of the Teflon removed distally) were inserted subcutaneously in the mid-back region. All wires (for both EMG and ES) were coiled in the back region to provide stress relief. All incision areas were irrigated liberally with warm, sterile saline. All surgical sites were closed in layers using 5.0 Vicryl (Ethicon, New Brunswick, NJ) for all muscle and connective tissue layers and for the skin incisions in the hindlimbs and 5.0 Ethilon for the back skin incision. All closed incision sites were cleansed thoroughly with saline solution.

Analgesia was provided by buprenex (0.5–1.0 mg/kg, s.c. 3 times/day). The analgesics were initiated before completion of the surgery and continued for a minimum of 2 days. The rats were allowed to fully recover from anesthesia in an incubator. The rats were housed individually in cages that had ample CareFresh bedding and the bladders of the spinal rats were expressed manually 3 times daily for the first 2 weeks after surgery and 2 times daily thereafter. The hindlimbs of the spinal rats were moved passively through a full range of motion once per day to maintain joint mobility. These procedures have been described in detail previously (Courtine et al., 2009; Lavrov et al., 2008a,b, 2006; Gad et al., 2012).

### 2.4. Step training

Five SCI rats were step trained bipedally (Ichiyama et al., 2008a; Shah et al., 2012) on a specially designed motor-driven rodent treadmill using a body weight support system (de Leon et al., 2002a,b) under the influence of ES between L2 and S1 (40 Hz) and quipazine a 5-$HT_2$ agonist (Gad et al., 2013, 2015a; Ichiyama et al., 2008b) (0.3 mg/kg, i.p.) and strychnine a glycinergic antagonist (Gad et al., 2013, 2015a) (0.5 mg/kg, i.p.) at a treadmill speed of 13.5 cm/s (Courtine et al., 2009) (pharmacological agents were administered 10 min prior to training). The rats were trained for a period of 6 weeks starting one week after the spinal cord transection surgery. Step training in spinal rats under the influence of pharmacological cocktails and/or spinal cord stimulation interventions are routine procedures with established protocols that have been performed in our laboratory for several years (Gad et al., 2012, 2014; Shah et al., 2012, 2013; Ichiyama et al., 2008b; Johnson et al., 2011; Gerasimenko et al., 2007).