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.

Running Title: Neuromodulation of the lower urinary tract

Highlights

- Functional links between the neural control of locomotion and lower urinary tract in awake unanesthetized rats.
- Contrasting effects of central vs. peripheral neuromodulation to activate lower urinary tract.
- Potential impact of chronic engagement of neural networks to enable locomotion and lower urinary tract function during central and/or peripheral neuromodulation.

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) [1]. Micturition is initiated when the neural networks relax the EUS and simultaneously activate bladder contractions [2, 3]. 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 [4]. 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 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) [5, 6]. 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.

Methods

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 [7]. 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 [8-11]. 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 [9, 11]. In terminal experiments electrophysiological responses in non-injured and SCI rats were recorded under anesthesia (urethane administered s.c.) [12, 13].

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 [10, 14]. The tibialis anterior (TA) and soleus muscles were implanted bilaterally with intramuscular EMG recording electrodes [10, 14]. 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 [8, 15].

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 [10, 11, 16-18].

Step training

Five SCI rats were step trained bipedally [14, 19] on a specially designed motor-driven rodent treadmill using a body weight support system [20, 21] under the influence of ES between L2 and S1 (40 Hz) and quipazine a 5-HT_2 agonist [8, 15, 22] (0.3 mg/kg, i.p.) and strychnine a glycinergic antagonist [8, 15] (0.5 mg/ kg, i.p.) at a treadmill speed of 13.5 cm/s [10] (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 [9, 16, 19, 22-25].

Testing procedures

The 5 SCI rats were stepped bipedally at a treadmill speed of 13.5 cm/s while in a body weight support system under the influence of ES (40 Hz bipolar stimulation between L2 and S1). With the animals suspended in the air (without paw contact), motor potentials were evoked via bipolar ES between L2 and S1 (1, 5, and 40 Hz) and recorded from the hindlimb muscles and EUS. An abdominal incision was made along the midline to expose the bladder and the pubic bone. The rostral portion of the pubic bone was partially removed using rongeurs to clearly expose the EUS muscle. Two fine stainless steel wires (Cooner wires AS 631-2) were inserted into the exposed portion of the EUS by passing them through a 30-gauge needle. Electrodes were formed by removing (notching) ~0.5-1.0 mm of Teflon coating, positioning the electrodes in the belly of the muscle, and securing the wires in the muscle with sutures at their entrance into and exit from the muscle. Stimulation was performed at a low intensity and low frequency to ensure local stimulation of the EUS muscle only. No experimental pharmacological agents (quipazine or strychnine) were administered during the testing procedures.

The 3 non-injured rats were anesthetized via urethane (1.2 mg/kg administered s.c.). The soleus and EUS were implanted acutely with Teflon coated stainless steel wires (Cooner wires AS 631-2) as described above. The tibial and pudendal nerves were isolated and placed on silver hook electrodes in a pool of mineral oil [26]. The pudendal nerve was isolated caudal to the L6-S1 trunk, the remaining branch after the pelvic nerve leaves this trunk as described earlier [27]. Evoked potentials were recorded from the EUS and soleus while the animals were lying on a heating pad on their belly during either tibial or pudendal nerve stimulation (0.5 Hz frequency, 0.05 ms pulse width, 1 to 10 V intensity). The bladder was not emptied prior to the experiment, however, no visible voiding was observed during the course of the experiment.

Data analyses

EMG recordings from the TA, soleus, and EUS were band-pass filtered (1 Hz to 5 KHz), amplified using an A-M Systems Model 1700 differential AC amplifier (A-M Systems, Carlsborg, WA), and sampled at a frequency of 10 KHz using a custom data acquisition program written in the LabView development environment (National

Instruments, Austin, TX) [8, 19, 28]. Custom scripts written in Matlab were used to measure the evoked potentials from the hindlimb and EUS muscles [17, 18]. Step cycle durations and EMG burst amplitudes and durations were determined using a custom program written in the LabView development environment [15]. Integrated EMG (iEMG) values were calculated as EMG amplitude x duration. The evoked responses were divided into early (ER, latency 1-3 ms), middle (MR, latency 4-7 ms) and late responses (LR, latency 7-10 ms) based on latency post-stimulation. The characteristics of the ER are consistent with the direct activation of motor fibers or motoneurons. This response has a short latency (1–3 ms) and shows a maximum amplitude with supramaximal stimulation. Theoretically, the short latency of the ER includes only the neuromuscular synaptic delay and the conduction time of the stimulus to the muscle and involves no synaptic delay within the spinal cord. The MR reflects the inclusion of one or more synaptic delays. The MR has a latency of about 4-6 ms. The LR has the longest latency of the three responses (7-10 ms) and represents a polysynaptic event. The latency difference between the ER and LR was about 6 to 8 ms and this difference may correspond to as many as three synaptic delays. The latencies used were based on previously published data [18, 29]. The phase difference between the left and right hindlimbs was calculated based on the difference in the onset time of the TA between the limbs divided by the step cycle duration.

Statistical analyses

All data are reported as mean±SEM. One-way repeated measures analysis of variance (ANOVA) was used to determine overall differences and paired t-tests to determine individual differences. The criterion level for the determination of a statistical difference was set at $P < 0.05$ for all comparisons.

Results

Interaction between stepping and voiding

The EMG patterns for the hindlimb muscles of a spinal rat while stepping bipedally on a treadmill under the influence of ES at 7 weeks post-SCI are shown in Figure 1A. Note that the EMG burst patterns and frequencies observed during normal stepping (blue highlights) change several seconds prior to voiding (yellow highlights) as well as during voiding (green highlight). In addition, the specific evoked responses (the MRs and LRs) vary during these time periods (Fig. 1B). MRs appear just prior to voiding and remain during the entire voiding period in both the TA and soleus. In contrast, the LRs are more prominent prior to voiding (yellow highlight) than the voiding period in both muscles. During the voiding period, the EMG bursts consist primarily of MRs with amplitudes lower than those observed pre-voiding. The iEMG burst amplitude for each step when transitioning to a different pattern in the progression from one period to the next is different for each period, with a slight increase during pre-voiding but similar during voiding compared to normal stepping for the soleus muscle (Fig. 1C). The TA, however, shows an increase in iEMG for each step during pre-voiding compared to normal stepping or during voiding (Fig. 1C). The step cycle duration decreases as the animal transitions from normal stepping to the prior to voiding period and then plateaus throughout the voiding period (Fig. 1D). Marked differences in the phase between the left and right hindlimbs are observed during the three periods shown in Figure 1A (Fig. 1E). During normal stepping (blue highlight) the phase difference is $<50^0$, whereas as the animal transitions to the prior to voiding period (yellow highlight) the phase difference begins to increase and reaches $\sim150^0$ during the voiding period (green highlight).

Neuromodulation of the EUS via ES

Stimulation of the lumbosacral spinal cord generated evoked potentials in the EUS similar to those in the hindlimb muscles in the unanesthetized SCI rats. The ERs appear first (Fig. 2B) with the MRs and LRs appearing at higher intensities (Fig. 2A & B). The amplitudes of ERs, MRs, and LRs increase with increasing intensity of stimulation (Fig. 2B). In contrast, the latencies of ERs, MRs, and LRs remain relatively constant with increasing stimulation voltage (Fig. 2C). As the frequency of stimulation increases, the ER amplitudes decrease and the MRs and LRs are abolished at 5 and 40 Hz (Fig. 2D). This lowered activation is reflected in the mean integral of the evoked potentials for all rats (Fig. 2E**).** The frequency of ES under <1 Hz, i.e., a frequency that produces a bilateral flexion motion as described in one of our earlier publications [9].

Alternating air stepping-like motion is observed only during higher frequency of ES (e.g., 40 Hz) or when the bladder is being filled with saline as shown in Gad et al. [9].

Neuromodulation of the EUS via peripheral nerve stimulation

Supra-threshold intensities of stimulation (2 V and above) of either the pudendal or tibial nerve result in activation of both the soleus and EUS (Fig. 3A-D). The activation patterns and thresholds, however, are very different between the two muscles and the two sites of stimulation (reflected by the slope of Fig. 3G). LRs (latency 10 ms and above) in the EUS occur at ~4 V and above during pudendal nerve stimulation and at ~6 V and above during tibial nerve stimulation (green highlight; Fig. 3C & D). The short latency responses during stimulation of either the tibial nerve or pudendal nerve occur at ~1-3 ms latency (Fig. 3E). A high frequency (~100 Hz, Fig. 3H) bursting pattern is observed in the EUS muscle at long latencies (50 ms and above) during pudendal nerve stimulation (at all intensities) and during tibial nerve stimulation (6V and above, Fig. 3F). This high-frequency bursting phenomenon was observed consistently in all non-injured rats. Further evidence of the contrasting nature of tibial vs. pudendal nerve stimulation on the EUS can be seen in the recruitment curves (Fig. 3G). The EUS responses to pudendal nerve stimulation remain constant post-threshold whereas the tibial nerve responses gradually increase with an increase in stimulation intensity. Occasionally, pudendal nerve stimulation at low intensities results in very long latency bursts (100-300 ms) in the EUS (Fig. 3I). These bursts are not time linked with every pulse and are seen only at moderate intensities of stimulation (3-4 V).

Discussion

Data presented here suggest a functional links between the neural control (biomechanical and electrophysiological) of locomotion and lower urinary tract in awake unanesthetized rats and the contrasting effects of central vs. peripheral neuromodulation on the lower urinary tract. Chronic engagement of neural networks to enable locomotor and lower urinary tract function during central, peripheral neuromodulation and locomotor training demonstrate the potential clinical impact these strategies offer.

ES facilitates functional overlap of locomotor and LUT function in human and animal subjects after a SCI

Significant progress has been made using activity-based training paradigms and the introduction of ES in combination with locomotor training has enabled improved motor and autonomic function in both animal [11, 14, 19, 23, 30] and human [6, 31, 32] subjects. In four human subjects with complete paralysis implanted with a spinal cord electrode array, anecdotal evidence reported improved bladder function, along with cardiovascular, thermoregulation, and sexual function. These results are consistent with there being an overlap of the neural circuits controlling locomotor and lower urinary tract function. Animal studies have since begun to address the potential interactions between motor and autonomic functions in response to locomotor training. Interestingly, it has been shown recently that LUT function can be improved in rats undergoing locomotor training [33, 34] that is enabled by the use of ES in combination with the administration of quipazine and strychnine. Specifically, enabling of locomotor-related spinal neuronal circuits by ES can influence neural networks controlling voiding with the onset of bladder emptying within seconds of the onset of the stimulation [9].

Spinal neural networks: In what order do they decide control

Neuromodulation in the form of ES, pharmacological agents, or chronic locomotor training can be used to activate afferents projecting to the spinal networks as well as directly affecting the neural networks intrinsic to the spinal cord and to the ganglia associated with the LUT. The overlap in the neural networks receiving these afferent projections with the networks responsible for controlling LUT function, including bladder and EUS activation, results in both acute as well as chronic changes when these perturbations are used repetitively. Results observed in both animal and human experiments demonstrate the degree to which the neural networks controlling LUT are affected when subjected to locomotor rehabilitation [9, 33, 34]. The physiological responses generated, however, are dependent on the *'physiological state of all systems'*. Considering the great flexibility of spinal networks [35] and their common localization in the lumbar segments, ES of the spinal cord is not only affecting the locomotor circuits but all systems with overlapping networks in the lumbosacral spinal cord.

The EMG patterns of a spinal rat stepping on a treadmill under the influence of ES change several seconds prior to and during voiding. Perhaps, the continuously adapting and different state-dependent reconfiguration of neural networks shared between the two circuits can serve to select the postural and locomotor outputs that can accommodate autonomic functions such as bladder voiding. To quantify the functional overlap of the different circuits we characterized the EMG patterns and the corresponding evoked potentials during the two tasks. The signature of the physiological state of the spinal cord is embedded in the EMG patterns and the corresponding evoked potentials. As the spinal rat transitions from smooth, robust bipedal stepping to uncoordinated stepping with shorter steps a few seconds prior to voiding to more disrupted patterns during voiding, the corresponding evoked responses in the form of MRs and LRs reflect these transitions in stepping kinematics. When initially stepping (blue highlight, Fig. 1A)**,** the patterns of the evoked potentials were stochastic with little to no time-linked responses observed. Note that the animals were tested at 6 weeks post-injury and had completely recovered their ability for bipedal stepping, thus we did not expect any MRs in either the flexor or extensor muscles [15]. As the state of the bladder transitioned to a pre-voiding state, however, time-linked MRs appeared in both the flexor and extensor hindlimb muscles and several LRs appeared at the start of the burst. These patterns of evoked responses are similar to those observed in untrained spinal animals several weeks post-injury [11]. Furthermore, as the animals began to void, the patterns of the evoked potentials were further modified with the bursting patterns being primarily dependent on MRs with little to no LRs. Previously we have shown that the pattern of the MRs and LRs reflect both the functional state of the spinal cord and the physiological state of the *in vivo* preparation [8].

In the present study, as the physiological state of the animal is transitioning from pre-voiding to voiding, the spinal circuitry is adapting to the change and generating responses in the hindlimb flexor and extensor muscles. The MRs in the pre-voiding and voiding periods suggest a higher reliance of the ES pulses to generate an EMG bursting pattern in both the flexors and extensors. During the normal stepping period (Fig. 1A, blue highlight), the spinal circuitry is dedicated to posture and locomotion. As the rat transitions to the pre-voiding period and then to the voiding period, the lumbosacral spinal circuitry was generating both stepping and voiding. Since some portion of the spinal circuitry seems to be shared in enabling stepping, the effect of the ES pulses on MR responses were (Fig. 1) clear time-linked MRs. Furthermore, as the animals began to void a much greater component of the spinal circuitry seemed to be dedicated to control the more immediate task (voiding), resulting in fewer LRs in both the flexors and extensors. It is interesting to note that the patterns of evoked responses in the MRs and LRs during normal stepping resemble those in rats that have completely recovered their stepping abilities [18], whereas the evoked potentials during voiding resemble those in animals being stepped prior to any step training. Similar observations have been made when spinal animals are being step trained with the pattern of stepping varying markedly and often leading to dragging of the hindlimbs while they are either voiding or defecating (unpublished observations).

Central vs. peripheral neuromodulation of the LUT

Historically, multiple techniques have been proposed to induce micturition after a SCI, including stimulation of the bladder wall and pelvic plexus [36, 37], the pelvic nerve [38, 39], and/or the sacral nerve [40] resulting in contractions of the bladder with or without relaxation of the EUS. Some of these strategies involve severing of nerves, resulting in permanent loss of some motor and sensory function. These strategies also completely *bypass the automaticity* that is intrinsic to the sensorimotor neural circuitries present in the spinal cord [30, 41-43]. In contrast with the more invasive techniques described above, we are activating the sensorimotor networks via spinal cord stimulation that underlie the automaticity of micturition as occurs with the recovery of stepping and standing after a SCI. We predict that improved bladder function can be largely re-established by re-enabling the inherent automaticity present within the spinal cord [9]. Interestingly, clinical studies have demonstrated that percutaneous tibial nerve stimulation in patients with an overactive bladder can result in an improvement of overactive bladder symptoms [44]. Both pudendal and tibial nerve stimulation lead to activation of the hindlimb and EUS muscles (Fig. 3). The pattern and recruitment of activation, however, are very different. Based on the latency and threshold of the responses to tibial or pudendal nerve stimulation in the EUS, we speculate that the origin of the response is either direct activation or indirectly via secondary afferents synapsing in the lumbosacral spinal cord. In addition, stimulation of either nerve results in a slow bursting EMG pattern in the EUS during the long latency phase (10-100 ms) at a frequency of ~100 Hz. This bursting pattern was observed at both low and high intensities (range of 1-9 V) for pudendal nerve stimulation, but only at high intensities (6V and above) for tibial nerve stimulation, suggesting the presence of a low level of continuous activity in the muscle is needed to maintain a state of continence. These responses also were only present in the long latency timeframes. These observations suggest a potential for a strong influence of the spinal networks controlling the ensembles of multiple sources of afferent input rather than direct nerve-to-muscle activation as indicated by the early response. The similarity of responses from EUS during peripheral and central stimulation (especially at higher intensities) could be due to recruitment of similar afferent fibers, including Group Ia, Ib, and II afferents, the activation of interneurons, and/or direct activation of motoneurons. Further experiments to isolate the source of each response during peripheral vs. central stimulation are important and need to be performed.

In summary, the main findings include the demonstration of the 1) functional links between the neural control (biomechanical and electrophysiological) of locomotion and lower urinary tract in awake unanesthetized rats, 2) contrasting effects of central vs. peripheral neuromodulation, and 3) potential impact of chronic engagement of locomotor networks to enable locomotor and lower urinary tract function during central and/or peripheral neuromodulation.

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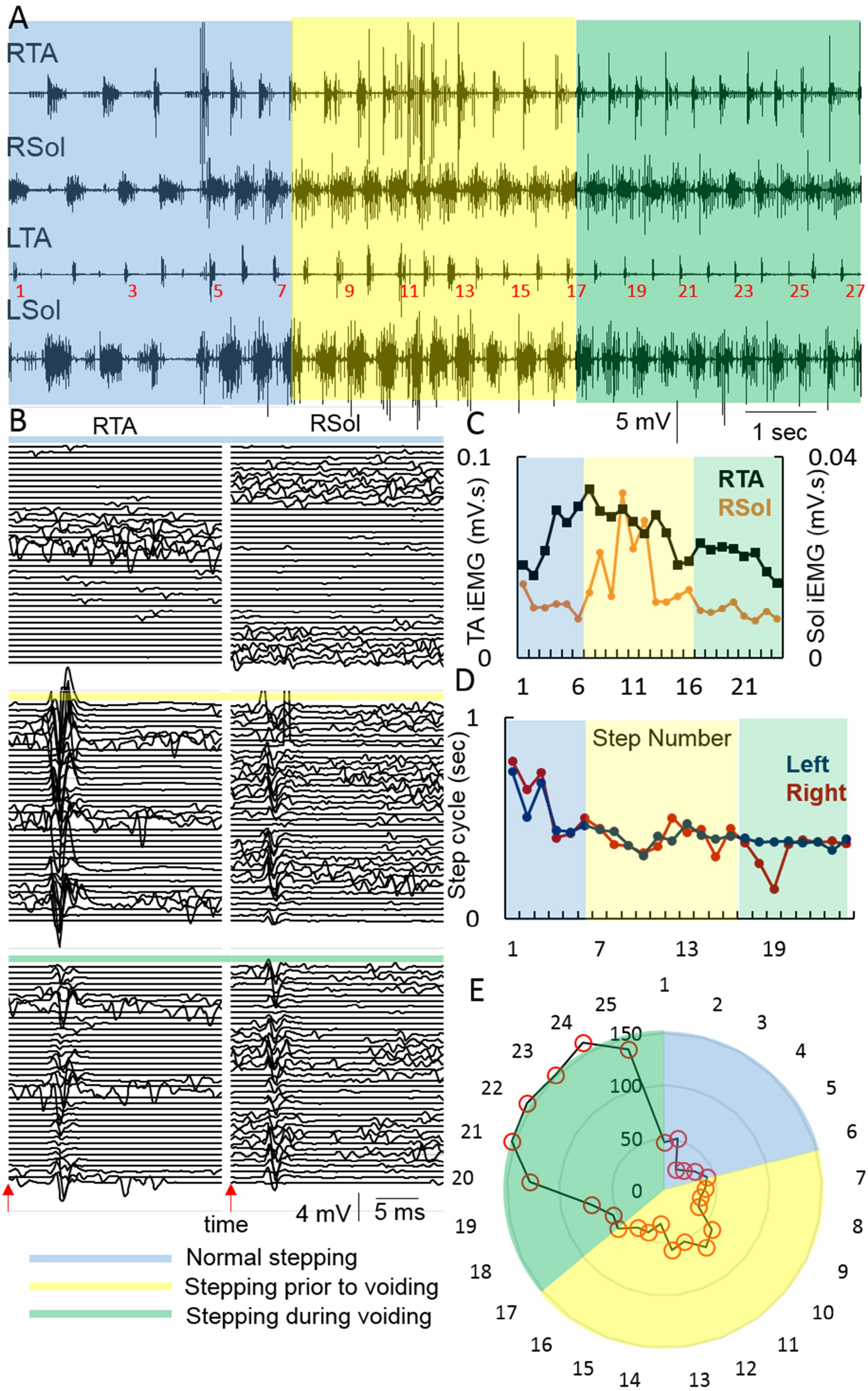
Author Disclosure Statement

VRE, YG, RRR, and PG researchers on the study team hold shareholder interest in NeuroRecovery Technologies. VRE is president and chair of company's board of directors. VRE, YG, RRR, and PG hold certain inventorship rights on intellectual property licensed by the regents of the University of California to NeuroRecovery Technologies and its subsidiaries.


A
RTA
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1 3 5 7 9 11 13 15 17 19 21 23 25 27
LSol
5 mV
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4 mV
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Normal stepping
Stepping prior to voiding
Stepping during voiding
C
0.1
TA iEMG (mV.s)
0
RTA
RSol
0.04
Sol iEMG (mV.s)
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1 6 11 16 21
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1
Step cycle (sec)
0
Step Number
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1 7 13 19
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150
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1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

Figure 1: (A) Representative EMG recordings from the right (R) and left (L) soleus (Sol) and tibialis anterior (TA) muscles from a spinal rat supported in a harness and stepping bipedally on a treadmill at 13.5 cm/s under the influence of ES (40 Hz between L2 and S1). The rat begins to void at the beginning of the green shaded area, however, the pattern of stepping changes several seconds (yellow shaded area) prior to voiding. Blue shading, normal stepping. Note the step numbers are marked in red. (B) Evoked potentials from the RTA and RSol muscles for the first 2 seconds for the three periods shown in (A) The red arrows mark the stimulation pulses with successive sweeps from bottom to top. Note the presence of time-linked MRs only during the pre-voiding and voiding periods. C) iEMG for the RTA and RSol muscles for each step shown in (A). (D) Left and right step lengths for each step shown in (A). (E) Phase difference between the left and the right hindlimbs for each step shown in (A).

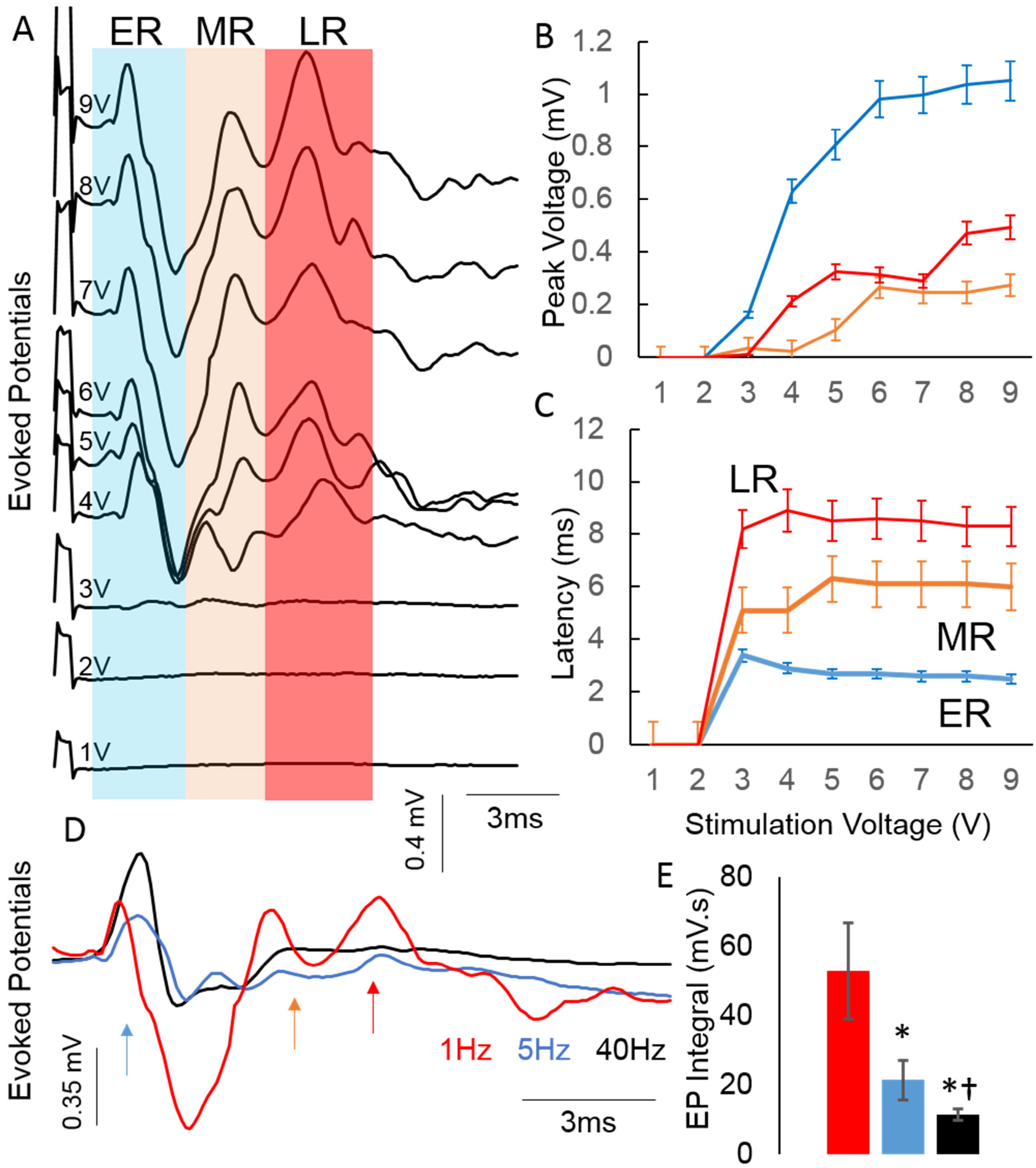
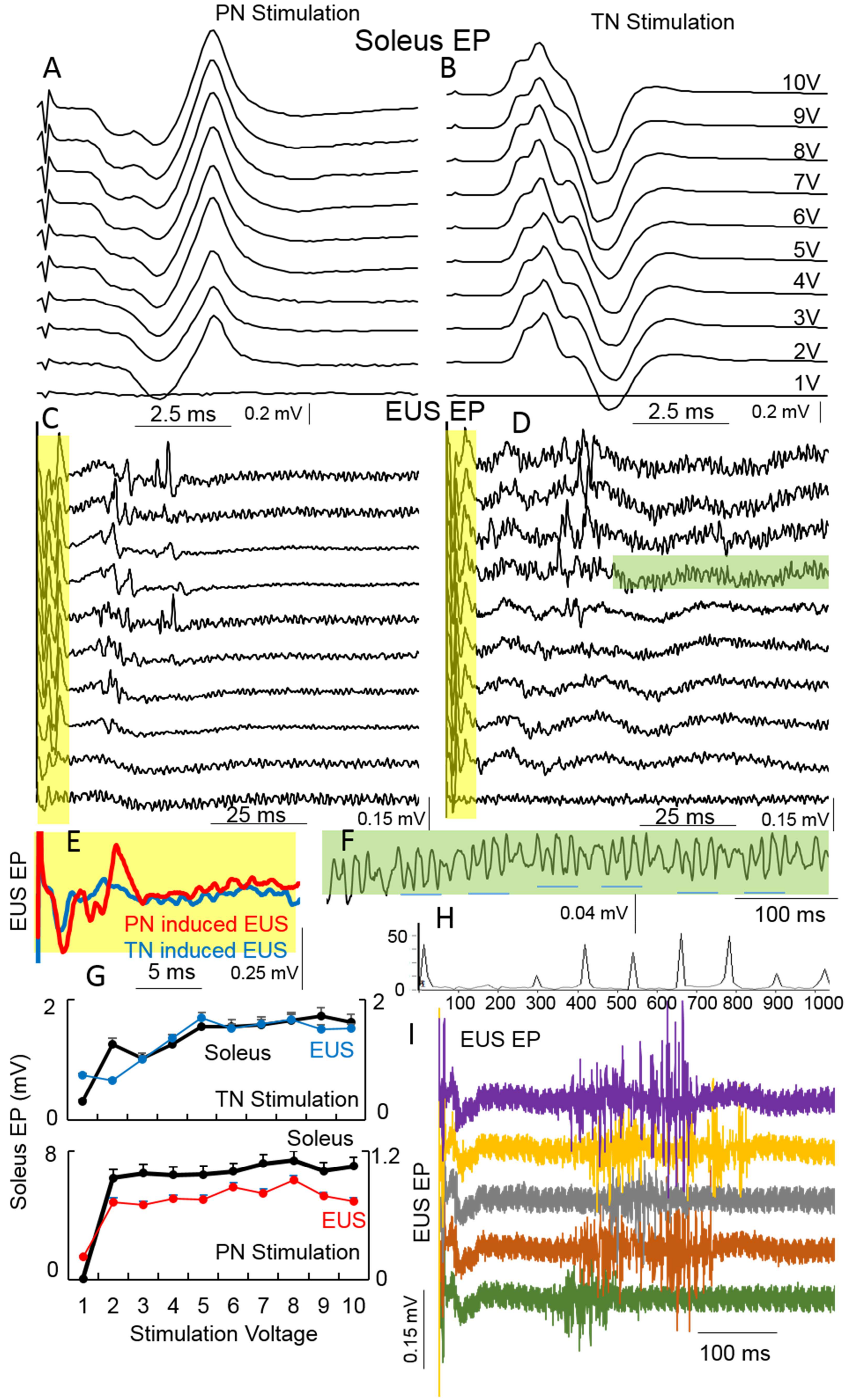


Figure 2: (A) Evoked responses from the EUS at various stimulation intensities in an unanesthetized spinal rat while suspended in a body weight support system during stimulation of spinal cord between L2 and S1. (B) Mean (±SEM; n = 5 rats) peak-to-peak amplitudes and latencies (C) post-stimulation for the ERs, MRs, and LRs during increasing intensities of spinal cord stimulation are shown. (D) Average evoked

potentials (20 sweeps) with increasing frequencies of spinal cord stimulation at the same intensity (4 V). Blue, orange, and red arrows mark the ERs, MRs, and LRs, respectively. (E) Mean (±SEM; n = 4 rats) area under the evoked potential curves for the first 10 ms post-stimulation at 1, 5, and 40 Hz spinal cord stimulation. *, †: significantly different from 1 and 5 Hz, respectively, at $P < 0.05$.



PN Stimulation
Soleus EP
TN Stimulation
A
B
10V
9V
8V
7V
6V
5V
4V
3V
2V
1V
2.5 ms
0.2 mV
EUS EP
C
D
25 ms
0.15 mV
E
EUS EP
PN induced EUS
TN induced EUS
5 ms
0.25 mV
F
0.04 mV
100 ms
H
50
0
100 200 300 400 500 600 700 800 900 1000
G
Soleus EP (mV)
Soleus
EUS
TN Stimulation
PN Stimulation
Stimulation Voltage
1 2 3 4 5 6 7 8 9 10
I
EUS EP
0.15 mV
100 ms

Figure 3: Evoked potentials (EP) from the soleus muscle during increasing intensities of pudendal nerve (PN) (A) or tibial nerve (TN) (B) stimulation in anesthetized non-injured rats. Evoked potentials from the EUS during increasing intensities of PN (C) or TN (D) stimulation. The yellow and green highlights in the EUS traces highlight the ERs and LRs, respectively. (E) Zoomed in view of the evoked potentials in the EUS at 10 V during stimulation of the PN (red) and the TN (blue). (F) Zoomed in view of the long latency response during PN (7 V in the EUS). (G) Mean (±SEM for n=3 rats) peak-to-peak amplitude from the soleus and EUS during increasing stimulation intensities of the TN and PN. Note the difference in scale between PN and TN, (H)Frequency spectrum for the data shown in (F). (I) Five examples of single EUS responses to PN stimulation at 4 V: note the presence of a long latency (100-300 ms) bursts.

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