A Ventral Root Avulsion Injury Model for Neurogenic Underactive Bladder Studies

Huiyi H. Chang^{1*}, Leif A. Havton²

¹Institute of Urology, University of Southern California, Los Angeles, CA; ²Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA

*Corresponding author

Huiyi Harriet Chang, Ph.D.

Assistant Professor of Research

Institute of Urology, University of Southern California

2011 Zonal Avenue HMR 602

Los Angeles, CA 90033

Phone: 1-323-442-1447

Email: HuiyiHarriet.Chang@med.usc.edu

A list of nonstandard abbreviations

8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)-tetralin
CGRP	calcitonin gene-related peptide
CM/CE	conus medullaris/cauda equina
DU	detrusor underactivity
EMG	electromyography
EUS	external urethral sphincter
LUT	lower urinary tract
PPN	preganglionic parasympathetic neuron
UAB	underactive bladders
VE	voiding efficiency
VMR	visceromotor reflex
VRA	ventral root avulsion
VRI	ventral root avulsion and re-implantation

ABSTRACT

Detrusor underactivity (DU) is defined as a contraction of reduced strength and/or duration during bladder emptying and results in incomplete and prolonged bladder emptying. The clinical diagnosis of DU is challenging when present alone or in association with other bladder conditions such as detrusor overactivity, urinary retention, detrusor hyperactivity with impaired contractility, aging, and neurological injuries. Several etiologies may be responsible for DU or the development of an underactive bladder (UAB), but the pathobiology of DU or UAB is not well understood. Therefore, new clinically relevant and interpretable models for studies of UAB are much needed in order to make progress towards new treatments and preventative strategies. Here, we review a neuropathic cause of DU in the form of traumatic injuries to the cauda equina (CE) and conus medullaris (CM) portions of the spinal cord. Lumbosacral ventral root avulsion (VRA) injury models in rats mimic the clinical phenotype of CM/CE injuries. Bilateral VRA injuries result in bladder areflexia, whereas a unilateral lesion results in partial impairment of lower urinary tract and visceromotor reflexes. Surgical re-implantation of avulsed ventral roots into the spinal cord and pharmacological strategies can augment micturition reflexes. The translational research need for the development of a large animal model for UAB studies is also presented, and early studies of lumbosacral VRA injuries in rhesus macaques are discussed.

Key words: spinal cord injury, external urethral sphincter, electromyography, serotonin, cauda equine, and conus medullaris

INTRODUCTION

Detrusor underactivity (DU) is a common cause of a variety of lower urinary tract symptoms in both men and women. An underactive bladder (UAB) is typically defined as DU, and its diagnosis is based on urodynamic evaluations (Chapple et al., 2015). According to the International Continence Society, DU is defined as a contraction of insufficient strength and/or duration such that the bladder fails to completely empty or the bladder empties in a prolonged duration (Abrams et al., 2003). The urodynamic diagnosis of DU is based on a pressure-flow study, and it is characterized by a low bladder pressure during voiding and a poorly sustained or wave-like detrusor contraction with an associated poor flow rate (Smith, 2010; Taylor and Kuchel, 2006). The consequences are chronic and debilitating with the potential need for self-catheterization, risk of urinary tract infection, possible need for nursing home care and a general decrease in the quality of life. Several conditions have been associated with the etiology of UAB including bladder partial outlet obstruction, diabetes, pelvic surgery, spinal cord injury (SCI) and neurological disorders such as stroke, Parkinson's disease and multiple sclerosis. These clinical observations underscore that multiple different pathologies may independently result in a UAB syndrome. However, limited attention has been paid to studies of the underactive bladder by the research community to date. Therefore, new clinically relevant and interpretable models for studies of the UAB syndrome are much needed in order to make progress towards new treatments and preventative strategies (Chancellor and Kaufman, 2008; Miyazato et al., 2013; Valente et al., 2014). Here, we will review a neuropathic cause of the UAB syndrome, i.e. traumatic injuries to the conus medullaris and cauda equina portions of the spinal cord, and discuss recent progress on experimental model development and the evaluation of different surgical and pharmacological interventions.

Most injuries to the thoracolumbar spine take place at the thoracolumbar junction and commonly result in a conus medullaris syndrome (Maynard et al., 1997; Holmes et al., 2001). The location of the conus medullaris varies extensively between individuals and the position for the tip of the conus ranges from the lower portion of the T11 vertebra to the upper portion of the L3 vertebra (Saifuddin et al., 1998; Soleiman et al., 2005). As a result, the sacral portion of the spinal cord and associated lumbosacral roots are vulnerable in the setting of trauma to the thoracolumbar vertebral junction. It is therefore not surprising that trauma to the thoracolumbar junction commonly result in CM/CE injuries. Patients with such lesions typically present with a conus medullaris syndrome, which is caused by the combined injury to the sacral segments of the spinal cord (conus medullaris, CM) and lumbosacral nerve roots (cauda equina, CE) (Dobkin and Havton, 2004; Hoang and Havton, 2006; Maynard et al., 1997). The literature indicates that around 50–70% of CM/CE injuries patients have urinary retention (Gardner et al., 2011). Injury may extend distally from the spinal cord along the lumbosacral nerve roots, which may have been avulsed, stretched, crushed, lacerated, and/or torn. The majority of CM/CE injuries includes a traumatic crush of the sacral spinal cord and lumbosacral roots, but a subset of cases may also show an avulsion root injury or other forms of a traumatic separation of lumbosacral nerve roots from the spinal cord (Hans et al., 2004; Monga et al., 2004; Moschilla et al., 2001). CM/CE injuries typically result in a lower motor neuron syndrome with denervation of peripheral targets, including skeletal muscles and autonomic ganglia, resulting in a combination of motor, sensory, and autonomic dysfunction. In these patients, paralysis of the

lower extremities is typically accompanied by e.g. loss of voluntary micturition and bowel control as the neural circuits for bladder contractions and control of external sphincters are impaired (Beric and Light, 1992; Carlstedt and Havton, 2012; Pavlakis et al., 1983). Here, we will review progress with regards to the development of experimental models for studies of the UAB syndrome.

RODENT MODELS OF CM/CE INJURIES TO STUDY NEUROGENIC UAB

Lumbosacral ventral root avulsion (VRA) injury models of CM/CE injuries have been developed in rats for performing studies of UAB and its potential treatment interventions. We will present both complete and partial injury models that allow for studies on mechanisms behind the clinical syndrome and testing of innovative strategies to reverse the clinical phenotype of neurogenic UAB.

Bilateral lumbosacral VRA injury and repair models in rats

The neuronal somata for preganglionic parasympathetic projections to pelvic ganglia and somatic motor outflow to pelvic floor sphincters reside primarily within the L6 and S1 spinal cord segments in rats (Hoang et al., 2003; Schröder, 1980). In an attempt to remove all parasympathetic innervation of the major pelvic ganglia and fully denervate the external urethral sphincter (EUS), a bilateral L5-S2 VRA injury model was developed and demonstrated complete absence of any evoked bladder contractions or EUS electromyographic responses to bladder filling (Chang and Havton, 2008a; Hoang et al., 2006a) (**Fig. 1**). This injury model provides the most severe presentation of a UAB syndrome, which includes a flaccid and underactive lower urinary tract. It should be noted that the current definition of a clinical UAB syndrome is restricted to the functional performance of the bladder, whereas the VRA injury model in rats includes underactivity of both the detrusor and the EUS. This aspect of the model is of interest, as both the detrusor and EUS contribute to lower urinary tract (LUT) function (Chang and Havton, 2013; Kakizaki et al., 1997; Maggi et al., 1986). A VRA injury is responsible for not only denervation of peripheral targets but also results in retrograde effects in the spinal cord. Quantitative studies of motor and autonomic neurons have demonstrated that axotomized efferent neurons in the spinal cord undergo degeneration and death after separation of their axons at the spinal cord surface. A unilateral L5-S2 VRA injury in male rats resulted in a progressive and parallel death of axotomized autonomic neurons and motoneurons within the L6 and S1 segments. At 6 weeks after the VRA injury, the vast majority of the axotomized neurons had degenerated and died with only approximately 20% of preganglionic parasympathetic neurons (PPNs) and motoneurons still surviving and showing signs of marked atrophy (Hoang et al., 2003). Nuclear condensation and cleaved caspase-3 detected in axotomized PPNs and motoneurons suggested apoptosis as a contributing mechanism of the neural death (Hoang et al., 2003), whereas the presence of the lytic membrane attack complex in axotomized motoneurons and expression of clusterin in adjacent astrocytes suggested that complement activation and necrosis contributed to the degeneration and loss of axotomized spinal cord neurons (Ohlsson et al., 2006).

A bilateral lumbosacral VRA injury results in Wallerian degeneration of the disconnected segments of motor and autonomic axons with loss of parasympathetic inputs to the major pelvic ganglia and denervation of the EUS with associated functional consequences. Urodynamic studies demonstrated an areflexic bladder and lack of somatic motor innervation of the EUS in rats after a bilateral L5-S2 VRA injury (Hoang et al., 2006a). In addition, end organ effects included increased bladder weight and size with marked thickening of the urothelium, lamina propria, and smooth muscle layers (Chang and Havton, 2008b). Although lumbosacral VRA injuries are relatively rare in the clinical setting, the functional deficits, including the development of a UAB syndrome, are similar to those encountered after the more commonly occurring crush injuries of the CM and CE (Pavlakis et al., 1983; Pesce et al., 1997; Chen et al., 2012).

Interestingly, many of the VRA injury effects can be ameliorated or reversed following an acute surgical re-implantation of avulsed ventral roots into the spinal cord (**Fig. 1**). Early studies demonstrated that an acute re-implantation of avulsed ventral roots (VRI) into the lumbosacral spinal cord of rats was neuroprotective and had neurotropic effects, as root re-implantation increased survival of both autonomic and motor neurons and promoted extension of regenerating axons into the re-implanted ventral roots (Hoang et al., 2006a; Hoang et al., 2006b). Of translational research interest is also the finding that even a single re-implanted ventral root may attract regenerative axonal growth from axotomized neurons in multiple spinal cord segments after a lumbosacral VRA injury (Hoang and Havton, 2006). With regards to the functional outcomes of root repair, a bilateral VRA injury followed by re-implantation of bilateral replanted roots resulted in successful re-innervation of the LUT and return of micturition reflexes (Hoang et al., 2006; Chang and Havton, 2008). These studies also highlight the importance of the EUS, as the degree of re-innervation of the EUS was directly related to voiding efficiency (VE) (Hoang et al., 2006b). The return of micturition reflexes in the VRI treatment group represented a significant improvement compared to the complete areflexic LUT after a bilateral VRA injury, but detailed urodynamic studies also showed that the functional recovery was not complete. Specifically, rats with VRI showed shorter periods of urine expulsion and decreased EUS bursting activity during voiding. The bladder contraction was not well synchronized with the EUS indicating the detrusor-sphincter incoordination (Chang and Havton, 2008a). Anatomically, the root re-implantation protected the LUT against VRA-induced increase in bladder size and weight as well as protection against VRA injury-induced thickening of the bladder wall (Chang and Havton, 2008b; Hoang et al., 2006b) (**Fig. 2**).

We conclude that functional re-innervation of the LUT with a return of micturition reflexes is possible. There is also room for further improvement to optimize VE. Future studies will be able to determine whether the combination of surgical root repair strategies in combination with other treatment strategies may result in a further improvement in LUT function. We also recognize that re-implantation of avulsed ventral roots as a potential future treatment of CM/CE injuries would be limited to the subset of patients with an anatomical separation of nerve roots between the spinal cord and periphery.

A unilateral lumbosacral VRA injury as a model for incomplete CM and CE injuries

Many CM/CE injuries are not clinically complete. Partial neurological function may remain in these patients and represents an attractive target for new treatments. For the purpose of evaluating new potential treatment strategies in the experimental setting, a unilateral VRA injury model has been developed in the rat. LUT was selected as a particular outcome measure. In rodent models EUS EMG activity demonstrates low-amplitude and tonic activity during bladder filling. This tonic activity increases gradually as infusion volume approaches the voiding threshold. During bladder contraction, the EUS activity markedly increases, consisting of tonic activity, and bursting pattern of activity that consists of active and silent periods (**Fig. 3A**). The urine flow occurs during the silent periods, not during the active periods in rodents (Streng et al., 2004). This is similar to humans showing quiescent during voiding with little activity.

At 12 weeks after a unilateral L5-S2 injury in rats, urodynamic studies showed decreased bladder intravesical pressure and poor voiding efficiency associated with decreased EUS EMG activation with shortened burst and prolonged silent periods during voiding (**Fig. 3**) (Chang and Havton, 2013). The reduced bladder contractility and increased post-void urinary retention are consistent with DU and establishment of a UAB syndrome. An extended investigation of EUS activity after the unilateral VRA injury showed an increase in the amplitude of EUS activity at the ipsilateral side at 12 weeks post-operatively (Chang and Havton, 2013), indicating that the partial denervation of the EUS skeletal muscle resulted in collateral sprouting and an increase in the motor unit size (Edds, 1953; Luff et al., 1988; Rafuse et al., 1992). Furthermore, the EUS EMG recordings showed a reduced bursting period during voiding, likely contributing to a reduced efficiency of bladder emptying (Chang and Havton, 2013). A similar reduction of EUS bursting in association with a decreased VE have been demonstrated after selective injury to the motor branch of the pudendal nerve (Peng et al., 2008). Studies indicate that 5HT-1A receptors facilitate bladder and EUS relaxation in rats, by prolonging the silent period within EUS bursting during voiding (Cheng and de Groat, 2010). Administration of 8-OH-DPAT, a 5HT_{1A} receptor agonist, promoted voiding function as demonstrated by a longer EUS bursting period during voiding and increased VE at 12 weeks after a unilateral VRA injury (**Fig. 3**) (Chang and Havton, 2013). The increased of EUS bursting period during voiding resulted in improved urethral relaxation and urine expulsion, thereby contributing to an improved VE. The administration of 8-OH-DPAT slightly suppressed bladder intravesical pressure, but the overall effect on LUT function was still a significantly improved VE (Chang and Havton, 2013).

The unilateral lumbosacral VRA injury model may also be useful for testing efficacy of other pharmacological treatments or for innovative cell therapies in attempts to reverse the neurological deficits of CM and CE injuries. For instance, prior studies in rats demonstrated a neuroprotective effect of minocycline on motor but not autonomic neurons after a unilateral L5-S2 VRA injury (Hoang et al., 2008). Preliminary studies on the effects of transplantation of human embryonic stem cell-derived motor neurons into the rat CM after a unilateral lumbosacral VRA injury have recently shown feasibility of approach and ability to determine short and long-term survival of transplanted cells in the host spinal cord (Amin et al., 2013a,b). The addition of functional evaluations, such as urodynamic and sensory threshold studies, will next assist in determining treatment efficacy and safety.

Somatosensory and visceral pain after CM/CE injury

In addition to the VRA injury effects on the lower urinary tract with the development of a UAB syndrome, neuropathic pain may develop in the form of both somatosensory and visceral pain. Early studies demonstrated that an L5 ventral root transection injury in rats may result in allodynia and mechanical hyperalgesia (Li et al., 2002; Sheth et al., 2002). A unilateral L6 and S1 VRA injury in rats similarly resulted in somatosensory pain in the form of allodynia in the adjacent L5 dermatome, and the VRA injury was also associated with an astrocytic, microglial, and macrophage response within the L5 dorsal horn of the ipsilateral side of the spinal cord (Bigbee et al., 2007). Combined, these studies provide support for the notion that motor lesions may result in sensory consequences, including the development of neuropathic pain. Although the precise mechanism for the development of pain remains unclear, it is possible that Wallerian degeneration of motor axons within mixed motor-sensory nerves in the periphery may release molecules that affects sensory function. Interestingly, surgical re-implantation of the avulsed roots into the spinal cord gradually ameliorated the level of neuropathic pain towards baseline sensory threshold levels (Bigbee et al., 2007). It is possible that the implanted roots provide neurotrophic or other support directly to the lumbosacral spinal cord to affect sensory function, because a peripheral nerve injury results in Schwann cell proliferation in the denervated segment and an increased expression of e.g. nerve growth factor, glial-derived neural growth factor, brain-derived neurotrophic factor, and neurotrophin 4/5 (Funakoshi et al., 1993; Meyer et al., 1992; Salzer and Bunge, 1980). It is also possible that the amelioration of pain may depend on successful regeneration of efferent spinal cord axons and peripheral target re-innervation.

The effects of a lumbosacral VRA injury on visceral sensory function have also been evaluated in rats. For this purpose, the visceral pain-related visceromotor reflex (VMR) was used as a functional outcome measure to evaluate for the possibility of visceral pain development. The VMR is detected as evoked EMG activity from the abdominal external oblique muscle in response to urinary bladder filling and activation of the micturition reflex. Prior studies have shown that an elevated VMR response is a reliable and reproducible sign of visceral pain caused by noxious pelvic organ stimulation from e.g. cystitis and chronic psychological stress (Cruz and Downie, 2006; DeBerry et al., 2007; Ness and Elhefni, 2004; Ness and Follett, 1998).

After a unilateral VRA injury at the L6 and S1 segments, the VMR was increased at 1 and 3 weeks post-operatively, suggestive of visceral pain development, whereas the VMR activity had spontaneously normalized at 8-12 weeks after the injury (Chang and Havton, 2012). The VMR activity was also further modulated when the avulsed ventral roots were re-planted into the spinal cord with earlier normalization of the VMR activity already at 3 weeks after the injury (Chang and Havton, 2012). One possible contributing mechanism for VRA-induced increase in VMR activity is plasticity of neuropeptide expression, and the expression of calcitonin gene-related peptide (CGRP) was increased in the lamina propria portion of the bladder wall after VRA injury, but this CGRP level elevation was ameliorated by acute re-implantation of the avulsed ventral roots into the spinal cord (Chang and Havton, 2008b). CGRP is a sensory and vasodilator neuropeptide increased by neurogenic inflammation (Benarroch, 2011). Furthermore, the combined L6 and S1 VRA injury resulted in a selective decrease of CGRP expression in nociceptive fibers innervating the dorsal horn at the L1 and L2 segmental levels, and this VRA injury-induced effect was partially restored by the VRI (Wu et al., 2012).

This motor lesion-induced reduction of CGRP-immunoreactivity in the dorsal horn of the L1 and L2 dorsal horn was not a diffuse phenomenon affecting multiple spinal levels but instead a highly specific effect that seemed isolated to the spinal segments providing sympathetic innervation the pelvic organs (Wu et al., 2012). We conclude that both somatosensory and visceral pain associated with VRA injury may be ameliorated by acute re-implantation of the avulsed ventral roots into the spinal cord. However, the molecular mechanisms responsible for this sensory plasticity in response to an injury to efferent fibers is complex and only partially understood.

DEVELOPMENT OF A LARGE ANIMAL MODEL FOR CM/CE INJURY STUDIES

In order to translate to the clinic a surgical ventral root re-implantation strategy and pharmacological interventions for the treatment of UAB after CM/CE injuries, a large animal model is needed (Carlstedt and Havton, 2012). Translation of surgical re-implantation of avulsed lumbosacral ventral roots is needed in a large mammal to demonstrate reproducibility of positive urodynamic outcomes documented initially in the rat models, to show feasibility of scaling up surgical procedures involving the CM and CE to a size closer to the human condition, to evaluate functional LUT-related outcome measures longitudinally in long-term studies, and to train spine surgeons in delicate root repair procedures not currently performed in North America.

Some of these goals have been achieved or are in progress in a variety of experimental models. In early translational studies, a VRA injury at the lumbar spinal cord in cats followed by surgical re-implantation of avulsed roots into the spinal cord resulted in motor axon regeneration and reinnervation of skeletal muscles of the hind limb (Cullheim et al., 1989). Subsequently, the VRA injury and VRI repair model was developed at the cervical spinal cord segments in cynomolgus monkeys (*Macaca fascicularis*) as a model for brachial plexus injury and repair, and re-implantation of avulsed ventral roots resulted in reinnervation of upper extremity muscles as demonstrated by retrograde labeling and functional studies (Carlstedt et al., 1993; Hallin et al., 1999). Surgical re-implantation of avulsed ventral roots has since been performed after traumatic brachial plexus injuries in select patients with demonstration of primarily proximal muscle reinnervation in the upper limb (Carlstedt et al., 2000; Carlstedt et al., 1995). However, there are to the best of our knowledge no reported clinical cases of surgical re-implantation of avulsed or separated lumbosacral ventral roots for the repair of CM/CE injuries.

Recently, a unilateral CM/CE injury model has been developed in the rhesus macaque for the purpose of studying new treatments for UAB and related dysfunctions of the pelvic floor. In this model, a unilateral lumbosacral VRA injury in the adult rhesus macaque results in a significant retrograde loss of motor neurons and an associated glial activation in the affected ventral horn (Ohlsson et al., 2013). Functional outcome measures have been developed, including cystometrogram recordings and EMG recordings from both the EUS and external anal sphincter, to allow for comprehensive functional assessments in rhesus macaques (Christe et al., 2013; Nielson et al., 2015). Preliminary studies are in progress to determine the effects of a unilateral lumbosacral VRA injury on LUT and pelvic floor function (Lee et al., 2012a, 2012b; **Fig. 4**). When the bladder was infused with the same volume of saline, the voiding contraction was evoked. The control subjects presented a continuous urine flow and shorter voiding

duration. The VRA subjects showed an intermittent voiding and longer voiding duration. However, the VRA subjects voided less volume with lower bladder intravesical pressure compared to the controls. These preliminary results from the VRA subjects represented the symptoms of UAB including lower amplitude of bladder intravesical pressure, less voided volume, and longer voiding duration.

PERSPECTIVE

CM/CE injuries commonly result in severe neurological impairments, including paralysis, sensory disturbance, pain, as well as bladder, bowel, and sexual dysfunctions. Rodent models of lumbosacral VRA injury provide useful tools for experimental studies and mimic key features of clinical CM/CE injuries, including, retrograde neuronal loss, LUT dysfunction, and a combination of neuropathic and visceral pain. However, the VRA injury model is probably less informative as a model for UAB syndromes that develop in neurological conditions that primarily affect the brain and brain stem, including stroke and Parkinson's disease. Interestingly, early re-implantation of avulsed ventral roots into the spinal cord may be neuro-protective and improve many functional impairments, including DU-associated symptoms, in rats after a lumbosacral VRA injury. Ongoing studies will determine the corresponding clinical phenotype in rhesus macaques after lumbosacral VRA injuries and assess the utility of emerging treatment interventions, including surgical root repair procedures, in long-term studies.

ACKNOWLEDGEMENTS

Supported by California Institute for Regenerative Medicine (CIRM, TR-01785), Department of Defense Spinal Cord Injury Research Program (SC090273), and Dr. Miriam and Sheldon G. Adelson Medical Research Foundation to LA Havton, and NIH/NIDDK (DK106181) to HH Chang.

Figure legends

Fig. 1: Anatomical schematics showing the avulsion injury of L5-S2 ventral roots from the surface of the spinal cord (A), and re-implantation of avulsed L6-S1 ventral roots into the lateral funiculus of the spinal cord (B).

Fig. 2: The bladder wall thickness is reduced in rats after a lumbosacral VRA injury and is partially restored after an acute implantation of avulsed ventral roots into the CM. The whole bladder wall thickness (A) was measured as was the thickness of the epithelium (B), lamina propria (C) and smooth muscle (D) layers in rats of the sham, VRA and re-implanted series; * indicates $p < 0.05$. Reprinted from (Chang and Havton, 2008b) with permission from Elsevier.

Fig. 3: Urodynamic recordings obtained from the sham rat (A-C) and a rat with unilateral L5-S2 VRA injury (D-F). Top tracings demonstrate the intravesical bladder pressure and continuous voiding contractions evoked by saline infusion. Middle and bottom tracings show the EUS EMG activity from ipsilateral and contralateral sides, respectively, associated with voiding contractions. Drug administrations of 8-OH-DPAT were intravenously given at the dosages of 0.3 and 1.0 mg/kg. The generation of this new figure was based on unpublished traces collected from data sets generated for our previous publication (Chang and Havton, 2013). The upper right corners at sham (A) and VRA (D) rats showed the faster time of EUS bursting (1-second period) at ipsilateral side, where the asterisks indicated the voiding contractions, before administration of 8-OH-DPAT.

Fig. 4: Bladder intravesical and urethral pressures were recorded from a neurologically intact female rhesus macaque (A) and after a unilateral lumbosacral VRA injury (B). The saline was infused at the rate of 15-30 mL per minute. A total volume of 180 mL was infused in each subject to partially fill the bladder and facilitate the voiding responses. Urethral pressure was used to indicate the duration of urine expulsion. In the control subject (A), the bladder intravesical pressure was higher during voiding and generated 68 mL of urine by a continuous flow. The subject after a unilateral lumbosacral VRA injury (B) presented the intermittent voiding and voided 41 mL of urine.

References

- Abrams, P., Cardozo, L., Fall, M., Griffiths, D., Rosier, P., Ulmsten, U., Van Kerrebroeck, P., Victor, A., Wein, A., Standardisation Sub-Committee of the International Continence, S., 2003. The standardisation of terminology in lower urinary tract function: report from the standardisation sub-committee of the International Continence Society. *Urology* 61, 37-49.
- Benarroch, E.E., 2011. CGRP: sensory neuropeptide with multiple neurologic implications. *Neurology* 77, 281-287.
- Beric, A., Light, J.K., 1992. Function of the conus medullaris and cauda equina in the early period following spinal cord injury and the relationship to recovery of detrusor function. *J Urol* 148, 1845-1848.
- Bigbee, A.J., Hoang, T.X., Havton, L.A., 2007. At-level neuropathic pain is induced by lumbosacral ventral root avulsion injury and ameliorated by root reimplantation into the spinal cord. *Exp Neurol* 204, 273-282.
- Carlstedt, T., Anand, P., Hallin, R., Misra, P.V., Noren, G., Seferlis, T., 2000. Spinal nerve root repair and reimplantation of avulsed ventral roots into the spinal cord after brachial plexus injury. *J Neurosurg* 93, 237-247.
- Carlstedt, T., Grane, P., Hallin, R.G., Noren, G., 1995. Return of function after spinal cord implantation of avulsed spinal nerve roots. *Lancet* 346, 1323-1325.
- Carlstedt, T., Havton, L., 2012. The longitudinal spinal cord injury: lessons from intraspinal plexus, cauda equina and medullary conus lesions. *Handb Clin Neurol* 109, 337-354.
- Carlstedt, T.P., Hallin, R.G., Hedstrom, K.G., Nilsson-Remahl, I.A., 1993. Functional recovery in primates with brachial plexus injury after spinal cord implantation of avulsed ventral roots. *J Neurol Neurosurg Psychiatry* 56, 649-654.
- Chancellor, M.B., Kaufman, J., 2008. Case for pharmacotherapy development for underactive bladder. *Urology* 72, 966-967.
- Chang, H.H., Havton, L.A., 2012. Modulation of the visceromotor reflex by a lumbosacral ventral root avulsion injury and repair in rats. *Am J Physiol Renal Physiol* 303, F641-647.
- Chang, H.H., Havton, L.A., 2013. Serotonergic 5-HT(1A) receptor agonist (8-OH-DPAT) ameliorates impaired micturition reflexes in a chronic ventral root avulsion model of incomplete cauda equina/conus medullaris injury. *Exp Neurol* 239, 210-217.
- Chang, H.Y., Havton, L.A., 2008a. Re-established micturition reflexes show differential activation patterns after lumbosacral ventral root avulsion injury and repair in rats. *Exp Neurol* 212, 291-297.
- Chang, H.Y., Havton, L.A., 2008b. Surgical implantation of avulsed lumbosacral ventral roots promotes restoration of bladder morphology in rats. *Exp Neurol* 214, 117-124.
- Chapple, C.R., Osman, N.I., Birder, L., van Koeveeringe, G.A., Oelke, M., Nitti, V.W., Drake, M.J., Yamaguchi, O., Abrams, P., Smith, P.P., 2015. The underactive bladder: a new clinical concept? *Eur Urol* 68, 351-353.
- Chen, S.L., Huang, Y.H., Wei, T.Y., Huang, K.M., Ho, S.H., Bih, L.I., 2012. Motor and bladder dysfunctions in patients with vertebral fractures at the thoracolumbar junction. *Eur Spine J* 21, 844-849.
- Cheng, C.L., de Groat, W.C., 2010. Role of 5-HT1A receptors in control of lower urinary tract function in anesthetized rats. *Am J Physiol Renal Physiol* 298, F771-778.
- Christe, K.L., Lee, U.J., Lemoy, M.J., Havton, L.A., 2013. Generalized seizure activity in an adult rhesus macaque (*Macaca mulatta*) during ketamine anesthesia and urodynamic studies. *Comp Med* 63, 445-447.

- Cruz, Y., Downie, J.W., 2006. Abdominal muscle activity during voiding in female rats with normal or irritated bladder. *Am J Physiol Regul Integr Comp Physiol* 290, R1436-1445.
- Cullheim, S., Carlstedt, T., Linda, H., Risling, M., Ulfhake, B., 1989. Motoneurons reinnervate skeletal muscle after ventral root implantation into the spinal cord of the cat. *Neuroscience* 29, 725-733.
- DeBerry, J., Ness, T.J., Robbins, M.T., Randich, A., 2007. Inflammation-induced enhancement of the visceromotor reflex to urinary bladder distention: modulation by endogenous opioids and the effects of early-in-life experience with bladder inflammation. *J Pain* 8, 914-923.
- Dobkin, B.H., Havton, L.A., 2004. Basic advances and new avenues in therapy of spinal cord injury. *Annu Rev Med* 55, 255-282.
- Edds, M.V., Jr., 1953. Collateral nerve regeneration. *Q Rev Biol* 28, 260-276.
- Funakoshi, H., Frisen, J., Barbany, G., Timmusk, T., Zachrisson, O., Verge, V.M., Persson, H., 1993. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J Cell Biol* 123, 455-465.
- Gardner, A., Gardner, E., Morley, T., 2011. Cauda equina syndrome: a review of the current clinical and medico-legal position. *Eur Spine J* 20, 690-697.
- Hallin, R.G., Carlstedt, T., Nilsson-Remahl, I., Risling, M., 1999. Spinal cord implantation of avulsed ventral roots in primates; correlation between restored motor function and morphology. *Exp Brain Res* 124, 304-310.
- Hans, F.J., Reinges, M.H., Krings, T., 2004. Lumbar nerve root avulsion following trauma: balanced fast field-echo MRI. *Neuroradiology* 46, 144-147.
- Hoang, T.X., Akhavan, M., Wu, J., Havton, L.A., 2008. Minocycline protects motor but not autonomic neurons after cauda equina injury. *Exp Brain Res* 189, 71-77.
- Hoang, T.X., Havton, L.A., 2006. Novel repair strategies to restore bladder function following cauda equina/conus medullaris injuries. *Prog Brain Res* 152, 195-204.
- Hoang, T.X., Nieto, J.H., Dobkin, B.H., Tillakaratne, N.J., Havton, L.A., 2006a. Acute implantation of an avulsed lumbosacral ventral root into the rat conus medullaris promotes neuroprotection and graft reinnervation by autonomic and motor neurons. *Neuroscience* 138, 1149-1160.
- Hoang, T.X., Nieto, J.H., Tillakaratne, N.J., Havton, L.A., 2003. Autonomic and motor neuron death is progressive and parallel in a lumbosacral ventral root avulsion model of cauda equina injury. *J Comp Neurol* 467, 477-486.
- Hoang, T.X., Pikov, V., Havton, L.A., 2006b. Functional reinnervation of the rat lower urinary tract after cauda equina injury and repair. *J Neurosci* 26, 8672-8679.
- Holmes, J.F., Miller, P.Q., Panacek, E.A., Lin, S., Horne, N.S., Mower, W.R., 2001. Epidemiology of thoracolumbar spine injury in blunt trauma. *Acad Emerg Med* 8, 866-872.
- Kakizaki, H., Fraser, M.O., De Groat, W.C., 1997. Reflex pathways controlling urethral striated and smooth muscle function in the male rat. *Am J Physiol* 272, R1647-1656.
- Lee U, Chang HH, Christe K, Havton LA (2012a) Analysis of evoked voiding contractions and corresponding urethral sphincter electromyography in non-human primates. *Female Pelvic Medicine & Reconstructive Surgery*. 18: s159-160.
- Lee U, Chang HH, Christe K, Havton LA (2012b) Evoked electromyographic activity in the external anal sphincter muscle of non-human primates differs from corresponding patterns in humans. *J Urol* 187:e16-e17

Li, L., Xian, C.J., Zhong, J.H., Zhou, X.F., 2002. Effect of lumbar 5 ventral root transection on pain behaviors: a novel rat model for neuropathic pain without axotomy of primary sensory neurons. *Exp Neurol* 175, 23-34.

Luff, A.R., Hatcher, D.D., Torkko, K., 1988. Enlarged motor units resulting from partial denervation of cat hindlimb muscles. *J Neurophysiol* 59, 1377-1394.

Maggi, C.A., Santicioli, P., Meli, A., 1986. The nonstop transvesical cystometrogram in urethane-anesthetized rats: a simple procedure for quantitative studies on the various phases of urinary bladder voiding cycle. *J Pharmacol Methods* 15, 157-167.

Maynard, F.M., Jr., Bracken, M.B., Creasey, G., Ditunno, J.F., Jr., Donovan, W.H., Ducker, T.B., Garber, S.L., Marino, R.J., Stover, S.L., Tator, C.H., Waters, R.L., Wilberger, J.E., Young, W., 1997. International Standards for Neurological and Functional Classification of Spinal Cord Injury. American Spinal Injury Association. *Spinal Cord* 35, 266-274.

Meyer, M., Matsuoka, I., Wetmore, C., Olson, L., Thoenen, H., 1992. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J Cell Biol* 119, 45-54.

Miyazato, M., Yoshimura, N., Chancellor, M.B., 2013. The other bladder syndrome: underactive bladder. *Rev Urol* 15, 11-22.

Monga, P., Ahmed, A., Gupta, G.R., Rao, K.S., 2004. Traumatic lumbar nerve root avulsion: evaluation using electrodiagnostic studies and magnetic resonance myelography. *J Trauma* 56, 182-184.

Moschilla, G., Song, S., Chakera, T., 2001. Post-traumatic lumbar nerve root avulsion. *Australas Radiol* 45, 281-284.

Ness, T.J., Elhefni, H., 2004. Reliable visceromotor responses are evoked by noxious bladder distention in mice. *J Urol* 171, 1704-1708.

Ness, T.J., Follett, K.A., 1998. The development of tolerance to intrathecal morphine in rat models of visceral and cutaneous pain. *Neurosci Lett* 248, 33-36.

Nielson, J.L., Haefeli, J., Salegio, E.A., Liu, A.W., Guandique, C.F., Stuck, E.D., Hawbecker, S., Moseanko, R., Strand, S.C., Zdunowski, S., Brock, J.H., Roy, R.R., Rosenzweig, E.S., Nout-Lomas, Y.S., Courtine, G., Havton, L.A., Steward, O., Reggie Edgerton, V., Tuszynski, M.H., Beattie, M.S., Bresnahan, J.C., Ferguson, A.R., 2015. Leveraging biomedical informatics for assessing plasticity and repair in primate spinal cord injury. *Brain Res* 1619, 124-138.

Ohlsson, M., Hoang, T.X., Wu, J., Havton, L.A., 2006. Glial reactions in a rodent cauda equina injury and repair model. *Exp Brain Res* 170, 52-60.

Ohlsson, M., Nieto, J.H., Christe, K.L., Havton, L.A., 2013. Long-term effects of a lumbosacral ventral root avulsion injury on axotomized motor neurons and avulsed ventral roots in a non-human primate model of cauda equina injury. *Neuroscience* 250, 129-139.

Pavlakakis, A.J., Siroky, M.B., Goldstein, I., Krane, R.J., 1983. Neurourologic findings in conus medullaris and cauda equina injury. *Arch Neurol* 40, 570-573.

Peng, C.W., Chen, J.J., Cheng, C.L., Grill, W.M., 2008. Role of pudendal afferents in voiding efficiency in the rat. *Am J Physiol Regul Integr Comp Physiol* 294, R660-672.

Pesce, F., Castellano, V., Finazzi Agrò, E., Giannantoni, A., Tamburro, F., Vespasiani, G., 1997. Voiding dysfunction in patients with spinal cord lesions at the thoraco-lumbar vertebral junction. *Spinal Cord* 35, 37-39.

Rafuse, V.F., Gordon, T., Orozco, R., 1992. Proportional enlargement of motor units after partial denervation of cat triceps surae muscles. *J Neurophysiol* 68, 1261-1276.

Saifuddin, A., Burnett, S.J., White, J., 1998. The variation of position of the conus medullaris in an adult population. A magnetic resonance imaging study. *Spine* 23, 1452-1456.

Salzer, J.L., Bunge, R.P., 1980. Studies of Schwann cell proliferation. I. An analysis in tissue culture of proliferation during development, Wallerian degeneration, and direct injury. *J Cell Biol* 84, 739-752.

Schrøder, H.D., 1980. Organization of the motoneurons innervating the pelvic muscles of the male rat. *J Comp Neurol* 192, 567-587.

Sheth, R.N., Dorsi, M.J., Li, Y., Murinson, B.B., Belzberg, A.J., Griffin, J.W., Meyer, R.A., 2002. Mechanical hyperalgesia after an L5 ventral rhizotomy or an L5 ganglionectomy in the rat. *Pain* 96, 63-72.

Smith, P.P., 2010. Aging and the underactive detrusor: a failure of activity or activation? *Neurourol Urodyn* 29, 408-412.

Soleiman, J., Demaerel, P., Rocher, S., Maes, F., Marchal, G., 2005. Magnetic resonance imaging study of the level of termination of the conus medullaris and the thecal sac: influence of age and gender. *Spine* 30, 1875-1880.

Streng, T., Santti, R., Andersson, K.E., Talo, A., 2004. The role of the rhabdosphincter in female rat voiding. *BJU Int* 94, 138-142.

Taylor, J.A., 3rd, Kuchel, G.A., 2006. Detrusor underactivity: Clinical features and pathogenesis of an underdiagnosed geriatric condition. *J Am Geriatr Soc* 54, 1920-1932.

Valente, S., DuBeau, C., Chancellor, D., Okonski, J., Vereecke, A., Doo, F., Lajiness, M., Diokno, A., Chancellor, M., 2014. Epidemiology and demographics of the underactive bladder: a cross-sectional survey. *Int Urol Nephrol* 46 Suppl 1, S7-10.

Wu, L., Wu, J., Chang, H.H., Havton, L.A., 2012. Selective plasticity of primary afferent innervation to the dorsal horn and autonomic nuclei following lumbosacral ventral root avulsion and reimplantation in long term studies. *Exp Neurol* 233, 758-766.

A. Ventral root avulsion

B. Ventral root reimplantation

The spinal levels of L6-S1 primarily innervate the lower urinary tract.

Figure 2

Figure 3

Figure 4

A. Control

Bladder intravesical pressure

Urethral pressure

B. 1 month after VRA injury

Bladder intravesical pressure

Urethral pressure