Effects of Lateral Funiculus Sparing, Spinal Lesion Level, and Gender on Recovery of Bladder Voiding Reflexes and Hematuria in Rats

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Abstract

Deficits in bladder function are complications following spinal cord injury (SCI), severely affecting quality of life. Normal voiding function requires coordinated contraction of bladder and urethral sphincter muscles dependent upon intact lumbosacral reflex arcs and integration of descending and ascending spinal pathways. We previously reported, in electrophysiological recordings, that segmental reflex circuit neurons in anesthetized male rats were modulated by a bilateral spino-bulbo-spinal pathway in the mid-thoracic lateral funiculus. In the present study, behavioral measures of bladder voiding reflexes and hematuria (hemorrhagic cystitis) were obtained to assess the correlation of plasticity-dependent recovery to the degree of lateral funiculus sparing and mid-thoracic lesion level. Adult rats received mid-thoracic-level lesions at one of the following severities: complete spinal transection; bilateral dorsal column lesion; unilateral hemisection; bilateral dorsal hemisection; a bilateral lesion of the lateral funiculi and dorsal columns; or a severe contusion. Voiding function and hematuria were evaluated by determining whether the bladder was areflexic (requiring manual expression, i.e., “crede maneuver”), reflexive (voiding initiated by perineal stroking), or “automatic” (spontaneous voiding without caretaker assistance). Rats with one or both lateral funiculi spared (i.e., bilateral dorsal column lesion or unilateral hemisection) recovered significantly faster than animals with bilateral lateral funiculus lesions, severe contusion, or complete transection. Bladder reflex recovery time was significantly slower the closer a transection lesion was to T10, suggesting that proximity to the segmental sensory and sympathetic innervation of the upper urinary tract (kidney, ureter) should be avoided in the choice of lesion level for SCI studies of micturition pathways. In addition, hematuria duration was significantly longer in males, compared to females, despite similar bladder reflex onset times. We conclude that the sparing of the mid-thoracic lateral funiculus on one side is required for early recovery of bladder reflex voiding function and resolution of hematuria.

Key words: bilateral pathways; incomplete SCI; micturition; neuroplasticity; urethral sphincter

Introduction

WHEREAS LOSS OF BLADDER FUNCTION is just one of several functional deficits resulting from spinal cord injury (SCI) in humans, this impairment greatly reduces quality of life, and regaining bladder function is of highest priority for the SCI population, second only to sexual function for paraplegics and regaining hand/arm function for quadriplegics.^{1,2} Therefore, elucidating the neural circuitry and effects of SCI on pelvic reflexes, such as micturition and ejaculation, are of great clinical importance. Bladder function is controlled at the spinal and supraspinal levels by both autonomic and somatic neuronal mechanisms.³ At the spinal level, bladder voiding and filling is mediated by the excitatory and inhibitory interaction of

all of these elements. Regarding spinal control by different spinal segments in the rat, 1) activity in L6–S1 parasympathetic preganglionic motor neurons (in the sacral parasympathetic nucleus) leads to contraction of the detrusor smooth muscle for bladder voiding, 2) activity in sympathetic preganglionic neurons at T10–L2 are responsible for contraction of the smooth muscle in the internal urethral sphincter to allow the bladder to fill, and 3) activity of somatic L5–L6 motor neurons lead to contraction of the striated external urethral sphincter muscle to delay voiding until conditions are appropriate. In addition to motor innervation, pelvic visceral afferents communicate information about bladder distension, which, accordingly, leads to appropriate coordination of autonomic/somatic output to cause retention or voiding of urine.

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There are several supraspinal sites involved in the coordination of micturition, some of which send descending projections to the segmental circuitry.⁴⁻⁶ The pontine micturition center (PMC) has been reported to send fibers bilaterally to inhibitory interneurons to likely relax the external urethral sphincter⁷ and the spinal parasympathetic nucleus to contract the detrusor.^{8,9} Several cortical areas are also implicated in the voluntary regulation of bladder voiding,¹⁰ sending descending projections to regulate the external urethral sphincter. In addition to descending pathways, bladder sensory afferents communicate with interneurons, which, in turn, send ascending projections to brain regions, such as the periaqueductal gray, medullary reticular formation, and on to the PMC. Thus, micturition reflex is dependent on a spino-bulbo-spinal loop.¹¹⁻¹⁴

Spinal lesions that disrupt descending pathways from such brainstem areas result in severe dysfunction of the micturition reflex^{12,15,16} and also lead to persistent polyuria.¹⁷⁻²⁰ Micturition controlled purely by spinal segmental reflexes is somewhat inefficient,²¹ and SCI-induced neuroplasticity can exacerbate problems.²² Incomplete bladder emptying can often occur when there is lack of coordination between the contraction of the external urethral sphincter and the detrusor, known as detrusor-sphincter dyssynergia,^{23,24} a common complication that can lead to additional problems, such as urinary tract infection (UTI).

Whereas direct injury to segmental micturition circuit neurons and lower motor neurons often results in permanent loss of bladder function,²⁵ isolated injury to the upper motor neuron axons in the white matter (WM) at more rostral spinal levels does not result in a total loss of bladder function owing to a partial recovery of spinal cord micturition reflexes below the lesion, likely involving a degree of spinal neuroplasticity. Accordingly, previous studies have demonstrated SCI-induced "unmasking" of the segmental bladder voiding reflexes that were present transiently in neonatal animals,^{12,14} in which cutaneous stimulation of the perineal area by the mother triggers voiding contractions in the neonate. Maladaptive neuronal changes after SCI result in detrusor and urethral sphincter dysfunction,^{26,27} which leads to the aforementioned problems of incontinence and detrusor-sphincter dyssynergia. Subsequent to chronic mid-thoracic SCI, some studies describe an increase in the number of lumbosacral neurons contributing synaptic connections to the segmental bladder control circuitry, presumably resulting from formation of new connections in the spinal gray matter (GM)^{28,29} and potentially contributing to detrusor hypertrophy and overactivity. As an example, the observed increase in number of connections could result from unmasking of a C-fiber bladder afferent-triggered micturition reflex.²⁹ Moreover, changes in trophic factors have been implicated in post-SCI plasticity of bladder afferents.^{30,31}

This report utilizes a rat model of SCI to investigate the importance of WM sparing to the integrity of the spino-bulbo-spinal loop and differential effects on behavioral recovery of micturition reflexes after spinal injury. Focus was placed on the lateral funiculus at the mid-thoracic level, given that there is evidence that descending pathways coordinating micturition run through this WM region³²⁻³⁴ and our previous studies³⁵⁻³⁷ have shown that descending pathways regulating a similar segmental reflex (ejaculation) also travel through the lateral funiculus at T7-T8. As with the bulbospinal loop involved in ejaculation, descending pathways from supraspinal sites, such as the PMC, regulating micturition are known to exert a bilateral influence on segmental circuitry⁷⁻⁹; therefore attention was given to behavioral results of 1) unilateral lesions versus bilateral lesions, 2) degree of lateral funiculus

sparing, and 3) proximity of the lesioned mid-thoracic spinal segment to the most rostral neurons of the urinary tract spinal circuitry. We hypothesized that the speed of recovery of bladder voiding reflexes would be correlated to the degree of lateral funiculus sparing and to a more proximal spinal segment of injury.

Another urinary tract complication of SCI is hematuria (hemorrhagic cystitis), which likely results from changes in the integrity of the uroepithelium,³⁸⁻⁴¹ allowing blood to escape into the urine. There are many possible factors involved in SCI-induced hematuria, including hormonal,^{42,43} neurogenic,^{39,40} and immunogenic,⁴⁴ although definitive causes remain unknown. In addition, SCI researchers have historically noted that hematuria may be more pronounced in male rats, compared to female rats, often factoring into the choice of females over males in SCI animal models because of fewer urinary tract complications. Accordingly, this study assessed the effects of SCI on the duration of hematuria in male and female rats.

Methods

Spinal lesions

All procedures used for this study were approved by the institutional animal care and use committee in accord with U.S. Department of Agriculture regulations. Bladder and urinary tract data were collected over many years from spinally injured animals used for our separately published electrophysiological/neural tracing studies of reproductive function. The data reported here are based on a comprehensive analysis of historical postoperative care data from those studies, obtained during the first 2 postoperative weeks. Some preliminary data using a less-rigorous analysis were reported earlier.³⁵ Adult Wistar rats, approximately 90 days of age, were anesthetized for surgery using a mixture of ketamine (80 mg/kg intraperitoneally [i.p.]) and xylazine (10 mg/kg i.p.). Surgeries were done under aseptic conditions. Before surgery, a long-acting antibiotic was administered subcutaneously (s.c.). In male rats, laminectomies of the caudal half of one vertebra were performed to expose one segment of the T6-T9 spinal cord. One of the following types of lesions was made through a longitudinal dural incision using a pair of microdissecting scissors: complete spinal transection; bilateral dorsal columns; lateral hemisection; dorsal hemisection; or a lesion completely destroying the bilateral lateral funiculus in addition to bilateral dorsal columns (lesion micrographs and diagrams shown in Figs. 1 and 2, respectively). After each lesion was performed, a small piece of thrombin-soaked absorbable gelatin sponge (Gelfoam; Pfizer, Inc., New York) was placed into the vertebral defect. The dura and overlying musculature were sutured with 10-0 and 4-0 monofilament, respectively, and the skin was closed with wound clips. In addition to the spinal-injured animals, 10 spinal injury shams were used as controls for effects of general anesthesia and laminectomy. Shams received all the same surgical procedures, but without the dural incision and spinal lesion. Postoperatively, an analgesic (Buprenex; Reckitt Benckiser Pharmaceuticals, Inc., Richmond, VA) was administered to alleviate postoperative discomfort. The antibiotic, gentamicin sulfate (5 mg/kg), was administered s.c. for the first 5 postoperative days⁴⁵ to prevent UTIs.

A separate analysis of historical data was used to determine bladder reflex recovery and hematuria resolution times in male and female rats with a "severe" contusion injury to the T8 spinal cord by rapid compression with a concave probe having the same radius and size of the overlying T7 lamina and attached to a displacement-controlled device driven with a trapezoid waveform generator (2.0-mm displacement for 5 sec⁴⁶). This severe contusion injury spared a minimal number of axons through the epicenter,⁴⁶ but did not completely sever the cord as occurs with spinal transection. All postoperative data on bladder reflex voiding and hematuria in female rats were obtained using the same procedures performed in males.

FIG. 1. Examples of histological sections from each of the animal groups with partial lesions of the T8 spinal cord. Lesion groups shown are the dorsal column (A), lateral hemisection (B), dorsal hemisection, which spared the ventral half of the lateral funiculus (C), and the dorsal column plus lateral funiculus, which spared the ventral funiculus (D). Photomicrographs were taken from paraffin sections near the lesion epicenter. Sections were stained with Luxol fast blue and cresyl violet (Klüver-Barrera stain). The calibration bar applies to all sections. Color image is available online at www.liebertpub.com/neu

Postoperative treatment and data collection

Animals recovered in a temperature-controlled environment while housed singly in plastic cages with wood shavings. Animals were examined three times daily at approximate 8-h intervals, 7 days a week. At these postoperative times, recorded as a one-third fraction of the postoperative day (e.g., 1.3 days, corresponding to a postoperative time of approximately 32 h), animals were observed for evidence of infection or other complications and given an injection of sterile lactated Ringers solution (3 cubic centimeters [cc] injected s.c. every 8 h) until measured water consumption returned to normal levels (approximately 40 cc/day). Bladder voiding function was evaluated during these sessions (at 8-h intervals) by first determining whether the bladder was distended (determined by gentle manual palpation) and, if so, whether bladder status was 1) “areflexic”, that is, distended bladder required a gentle bladder squeeze for urine expression, a “crede maneuver,”²⁰ or 2) “reflexive,” that is, distended bladder required gentle tactile stroking stimulation of the perineum to initiate reflex voiding contractions. If the bladder was not distended, we noted that “automatic” bladder voiding had occurred (i.e., spontaneously triggered by

contact with cage bedding or otherwise without caretaker assistance). Caretakers were meticulously trained in the bladder palpation and expression technique and were blinded to severity of the spinal lesion. In addition to determining status of bladder voiding reflexes, the presence or absence of gross hematuria (i.e., visible blood in the urine) was recorded at each 8-h postoperative interval. However, it should be noted that slight levels of blood products in the urine not visible to the naked eye can only be determined with a dipstick or smear analysis, procedures that were not performed here. In all animals across all lesion groups, bladder voiding function had developed “automatic” status and hematuria had resolved within a 2-week period after spinal lesion surgery.

Spinal cord lesion reconstructions

At 1–2 months postinjury, each animal was anesthetized for a terminal experiment for which the obtained electrophysiological or neuroanatomical data have been reported in previously published data from the our labs, including those cited here.^{35–37,46,47} Following those terminal experiments, animals were euthanized with an overdose of urethane and transcardially perfused with 4%

FIG. 2. Effects of complete and partial spinal cord injury on recovery of bladder voiding and duration of hematuria in male rats. Postinjury onset (in days) of both “perineal reflex” voiding and “automatic” voiding behavior in male rats was related to degree of bilateral damage to the lateral funiculus. Compared to total transection lesions, recovery of both voiding measures was significantly faster ($*p < 0.01$) in all groups. Recovery was fastest in animals with dorsal column lesions or lateral hemisections, when compared to the slower ($#p < 0.05$) recovery in animals with increased bilateral damage to the lateral funiculus (dorsal hemisection; dorsal columns/lateral funiculus). Duration of hematuria was significantly shorter than complete transection ($*p < 0.01$) only in animals with dorsal column lesions or lateral hemisections. There were no significant differences between the latter two lesion groups. The y-axis represents the number of days necessary for resolution of hematuria and perineal reflex-based and automatic bladder voiding onset in rats after receiving the following categories of spinal lesions: complete transection; dorsal columns; lateral hemisection; dorsal hemisection; and dorsal columns plus lateral funiculus. Standard error bars are shown.

paraformaldehyde. Specific spinal cord segment blocks from the T6–T9 spinal cord were removed after careful confirmation of spinal segment number with cadaveric dissection of spinal nerves and vertebral segments. These blocks were embedded in paraffin and transversely sectioned at $8\ \mu\text{m}$ with a rotary microtome. Sections were stained with the standard Luxol fast blue (LFB) and cresyl violet procedure (Klüver-Barrera stain⁴⁸), which consisted of deparaffinization in xylene, dehydration in ethanol, overnight immersion in warm 0.1% LFB, two cycles of lithium carbonate with ethanol/distilled water rinse/wash, and 6-min immersion in warm cresyl violet. Precise reconstruction drawings were made of the injured cord to confirm the exact and total extent of the damage along the length of the entire lesion (in addition to the epicenter) by an observer blinded to both the surgical procedure and behavioral results,^{47,49} thereby reducing the possibility of underestimating the size of the lesion while also verifying that specific spared WM regions were continuous through the mid-thoracic spinal cord. Lesions were then grouped into specific categories, eliminating

those from analysis that did not fit the lesion extent criteria. The lesion categories were: complete transection, in which the cord was severed completely; dorsal columns lesion, which destroyed the dorsal columns bilaterally; lateral hemisection, which eliminated the entire left or right side of the cord (see Fig. 1); dorsal hemisection, which ablated the dorsal columns and the more dorsal portions of the lateral funiculus bilaterally; and a lesion including both the dorsal columns and lateral funiculi bilaterally. Severity of the contusion injuries used in the second experiment resembled that of complete transections, although there was sometimes a thin rim of spared axons in some portions of the peripheral WM. All of these contusion injuries were classified as severe (using outcome measures we have described previously).^{20,46}

Statistical analysis

Distribution normality tests were conducted for each set of grouped data, and because normal distributions were not verified in

all cases, the more appropriate (and more conservative) nonparametric Mann-Whitney's rank-sum test was used to determine significant differences between group pairs. Levels of significance were between $p < 0.001$ and $p < 0.05$. Data are presented as mean $\pm$ standard error of the mean.

Results

Effects of lateral funiculus sparing

Following chronic mid-thoracic SCI in male rats, functional recovery of bladder voiding (for both reflexive and automatic voiding measures) was fastest in animals with a significant degree of lateral funiculus sparing, when compared to animals with either a complete spinal cord transection or a bilateral lesion of the lateral funiculus (Fig. 2). In other words, those with bilateral dorsal column lesions ($n = 7$) recovered bladder function quickly (1.2 ± 0.48 days for reflexive voiding and 1.7 ± 0.67 days for automatic voiding), as did those with lateral hemisections ($n = 11$) in which one side of the lateral funiculus was left intact (1.9 ± 0.41 days reflexive and 2.9 ± 0.63 days automatic). Rats with lesions that completely ablated the lateral funiculus bilaterally ($n = 8$), classified as "dorsal columns plus lateral funiculus" lesions, recovered reflex voiding (4.3 ± 0.36 days) and automatic voiding (6.3 ± 0.45 days) significantly more slowly than those with either lateral hemisections or dorsal column lesions ($p < 0.001$), but significantly faster than

animals with complete transections ($n = 47$, 5.9 ± 0.30 days reflexive; 9.5 ± 0.43 days automatic), possibly because of the sparing of the most ventral portions of the lateral and ventral funiculus. Similar results were obtained with lesions classified as dorsal hemisections ($n = 7$), which involved bilateral ablation of both dorsal columns and the dorsal parts of the lateral funiculus bilaterally, and the times for this group to recover reflexive (3.5 ± 0.48 days) and automatic (4.8 ± 0.54 days) bladder voiding were not significantly shorter than the recovery times for the dorsal columns plus lateral funiculus group. The possible effects of general anesthesia and laminectomy on bladder reflex function and hematuria were tested in 10 surgical sham control animals. There was no apparent effect on bladder function, that is, at the first postoperative assessment, the bladders were not distended and there was no evidence of hematuria.

Duration of hematuria roughly paralleled the bladder function data (Fig. 2); hematuria resolved more quickly for animals with a greater degree of lateral funiculus sparing. Specifically, animals with at least one side of the lateral funiculus left intact (lateral hemisection) exhibited faster resolution of hematuria than those with bilateral ablation of the dorsal half of the lateral funiculus (dorsal hemisection) and those with dorsal hemisections, which incompletely destroyed the lateral funiculus bilaterally. However, unlike the voiding onset results, the time to resolution of hematuria was similar (not significantly different) in those animals with dorsal

FIG. 3. Transection of the spinal cord closer to T10 resulted in a slower recovery of bladder voiding behavior in male rats. Compared to lesions at T6–T7 ($n = 26$), lesions at T8 ($n = 13$) and T9 ($n = 8$) showed a significantly slower onset (in days) of automatic voiding ($*p < 0.001$) and for perineal reflex voiding at T9 only ($*p < 0.01$). Duration of hematuria, however, was not affected by lesion level. Standard error bars are shown.

columns plus lateral funiculus lesions and those with complete transections. The amount of water consumed did not correlate with the presence of hematuria, given that animals drank much less water in the first few days after surgery regardless of the type of lesion or the presence or absence of hematuria.

Effects of spinal lesion level

Given that it has been shown that spinal contusion lesions at different segmental levels may have differential effects on disruption of micturition,¹⁶ we then analyzed the results from male rats based on the segmental lesion level (as confirmed with postmortem lesion reconstructions). Lesions ranged from T6 to T9, and levels did not substantially vary between groups of different lesion types. To determine whether differences in lesion level were correlated with a difference in bladder functional recovery or duration of hematuria, we compared the data between three lesion level subgroups within the complete transection lesion group; those with transection lesions at three levels (T6/T7, T8, and T9). Results revealed significant differences in certain bladder voiding onset measures between the three subgroups (Fig. 3). Specifically, those with T9 epicenter lesions had significantly slower reflex voiding onset (8.0 ± 0.84 days), compared to those with T6/T7 (5.4 ± 1.02 days) and T8 lesions (5.3 ± 0.33 days). Also, those with T9 and T8

lesions had significantly slower automatic bladder voiding onset than those with T6/T7 lesions. The slower recovery times noted with more-caudal lesions may be owing to proximity, in the immediately adjacent T10–L2 spinal segments, to spinal neuronal circuits that control 1) sensory input from the upper urinary tract (kidneys and ureters) and 2) sympathetic preganglionic neurons to the bladder and lower urinary tract.

In contrast to the effects on bladder voiding measures, hematuria duration was not significantly different between the three transection lesion-level subgroups. Whereas the first set of results suggests that severity of injury or degree of WM sparing influences duration of hematuria post-SCI, proximity of the lesion to T10 had no correlation with duration of hematuria.

Gender differences in hematuria recovery

To investigate other factors that may have played a role in the hematuria results, we compared male versus female animals, given that hormonal^{42,43} and other sex-related differences, such as length of the urogenital tract, may differentially affect hematuria duration. As can be seen in Figure 4, following severe contusion injuries to the T8 spinal level, hematuria took a significantly shorter time to resolve for females than for males (1.3 ± 0.19 vs. 3.7 ± 0.30 days, respectively). However, there was no significant difference in

FIG. 4. Effects of gender on bladder voiding reflexes and hematuria. Onset of bladder voiding (in days) following severe contusion injury of T8 was not significantly different between male ($n=48$) and female rats ($n=19$), but duration of hematuria was significantly shorter ($*p < 0.001$) in female rats. Note that duration of manual bladder expression (i.e., onset of perineal reflex voiding) was the same in both groups, suggesting that manual bladder expression by the caretaker was an unlikely cause of hematuria. Standard error bars are shown.

bladder voiding onset between these two groups, for either the perineal reflex-based voiding or the automatic voiding measures, suggesting that given that the duration (in days) of manual bladder expression (crede maneuver) was the same in both groups, it was unlikely to be the cause of hematuria as stated above.

Discussion

The results of the present study suggest that following incomplete mid-thoracic SCI, sparing of pathways in the lateral funiculus on one side of the cord leads to a relatively quick onset of bladder voiding reflexes and resolution of hematuria. In addition, the data showing that animals with lesions to only one side of the lateral funiculus recovered function faster than those with bilateral lesions of the lateral funiculus gives further support to the bilateral and, possibly, redundant nature of the ascending and descending pathways to pelvic organ circuitry, a conclusion reported in earlier electrophysiological^{35–37} and pseudorabies virus tracing⁴⁷ studies of bilateral (crossed) connections in the lumbosacral GM from a spared lateral funiculus. In the present report, selective bilateral lesions of only the dorsal columns did not produce a significant effect on bladder voiding function, suggesting that whereas the dorsal column may transmit some degree of bladder sensation to the brain, the bilateral loss of those ascending pathways does not cause a significant loss of bladder voiding function or trigger the development of prolonged periods of lumbosacral reflex micturition.³⁴ The somewhat slower recovery for animals with complete transections, compared to those with bilateral ablation of the lateral funiculus and dorsal columns, is consistent with reports of descending micturition pathways traveling in the ventral columns to some extent.⁵⁰ It is important to note that the classification of “automatic” bladder voiding, in animals with incomplete injuries, does not differentiate between voiding resulting from primarily segmental reflex circuits (triggered by pressure of the cage bedding) or supraspinal pathways (recovery of normal voiding patterns), given that we did not measure the residual volume of the urine, which would presumably be low if normal spino-bulbo-spinal pathways were re-established. The “perineal reflex” measure, however, is a strong assay of segmental voiding recovery given that it is not functional in normal adult rats, only in neonates, or as an unmasked circuit in spinalized adult animals.^{12,14}

Both behavioral measures of bladder voiding recovery (reflexive and automatic) were significantly delayed in animals with transections closer to the T10 level. This is in partial agreement with those of another study that compared the effect of moderate contusion injuries at T9 with more cranial levels (T1 and T4) by measuring amount of retained urine to indicate decreased bladder function.¹⁶ Although they found significantly greater bladder impairment for T9 contusion, compared to T1, they found no difference between T9 and T4 lesions. However, their study was different from our lesion level data in the following ways: Only females were tested (vs. only males); “moderate” contusion injuries were given (vs. complete transections); bladders were expressed every 12 h (vs. every 8 h); and bladder functional measure was urine volume (vs. voiding trigger) and was obtained under anesthesia (vs. awake). Preliminary data from our lab have shown that a T9 epicenter transection causes secondary neuronal loss extending caudally into T10 and T11. Therefore, the more caudal lesion epicenters in the present report may have had a greater negative effect on the known low thoracic circuitry governing urinary tract function and/or localized spinal centers that regulate micturition.^{16,21}

Hematuria (also known as hemorrhagic cystitis) was equally prolonged in groups of male rats receiving total transection, severe

contusion, or total/partial bilateral damage to the lateral funiculus. However, hematuria resolved significantly more quickly in animals with at least one side of the cord spared. One possible explanation is that hematuria resolved faster because of a higher fluid intake leading to a higher urine output, possibly because of increased mobility in animals with less-severe injuries. However, we saw no differences in amount of water consumed immediately after surgery and there was no correlation between amount of water consumption and time to resolve hematuria, that is, animals drank much less water in the first few days after surgery regardless of type of lesion or presence or absence of hematuria. It is important to note that although it has been suggested that the manual pressure used by the caretaker to empty the bladder may cause or contribute to hematuria in rats, we do not believe it was an important variable in our results because 1) hematuria was observed during the first crede maneuver and 2) the same minimal manual expression pressure was used across all groups and across all time points. Moreover, our data on hematuria duration after contusion injury in males and females (see below) supports this conclusion.

Another possible explanation is that a more severe lesion (total volume of spinal tissue damaged) results in significantly prolonged hematuria. The possibility of hematuria being a function of lesion severity (volume) is supported by studies demonstrating neurogenic contributions to hematuria.^{39,40} In those studies, it was suggested that transecting the spinal cord could stimulate neural release of norepinephrine and other stress hormones. It was not proposed exactly how, or at what spinal level, such hormones may be released. However, if they are released into damaged spinal cord tissue and are able to directly reach the bladder through the disrupted spinal circulatory system, they may affect the bladder uroepithelium directly. Intravesicular application of norepinephrine indeed significantly reduces epithelial viability.³⁹ In addition, norepinephrine results in a desquamation of the bladder epithelium, possibly owing to alternations in the tight junction from nitric oxide release.^{39,51} A recent study proposed that an SCI severity-correlated infiltration of lymphocytes may cause hematuria in the rat based on the therapeutic benefit of a T-lymphocyte sequestering agent.⁴⁴ Thus, a neurogenic or immunogenic component to hematuria could be a factor in the differences observed with different degrees of spinal cord destruction in the present study.

Although the exact cause or causes of SCI-induced hematuria is not known, the longer duration we have demonstrated in males, compared to females, may be a result of the male urethra being significantly longer and more dependent on burst contractions, compared to females, making it more difficult to excrete the blood.⁵² Alternatively, the observed gender difference may also be owing to a potential protective effect of circulating ovarian steroid hormones given that estradiol has been shown to have a neuroprotective effect.⁴³ The lack of correlation of hematuria duration with lesion level within the T6–T9 range suggests a lesser importance on proximity of the lesion to urinary thoracic segmental circuitry/spinal micturition centers, compared to the disruption of descending pathways, as factors in the persistence of hematuria. Our data also demonstrate that manual expression of the bladder by the caretaker during the initial areflexic bladder phase is likely not the cause of hematuria. In addition, because the animals all received the same amounts of the postoperative antibiotic, gentamicin, it is unlikely that possible effects resulting from a subclinical UTI contributed to the differences in hematuria resolution.

In conclusion, the results of these lesion studies support our hypothesis that the lateral funiculus is the WM spinal location at the thoracic level for descending pathways from supraspinal regions to

the segmental micturition circuitry. In addition, these studies support the bilateral nature of the pathway below the thoracic level; sparing of at least one side of this bilateral pathway is sufficient for quick recovery of voiding reflexes. Behavioral data gathered after administration of lesions at different levels within the mid-thoracic region also demonstrate the potential importance of sparing the spinal circuitry immediately caudal to T9 for rapid recovery of bladder voiding reflexes, possibly owing to the preservation of low thoracic (T10–T13) neural circuits important in the upper urinary tract control of micturition. For this reason, it would be important that investigations of the ascending and descending WM pathways involved in bladder function in the rat model use accurately delivered and verified spinal lesions at the T6–T7 level or above, as opposed to more-caudal thoracic segments. The prolonged duration of hematuria for animals with a greater extent of overall damage is consistent with suggestions made in other studies concerning the possible response of the sympathetic nervous system to such damage and subsequent uroepithelium breakdown, and the quicker resolution of hematuria for females versus males may be owing to protective hormonal or anatomical factors.

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Author Disclosure Statement

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