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ORIGINAL ARTICLE

Lower urinary tract function in spinal cord-injured rats: midthoracic contusion versus transection

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Objectives: To compare changes in lower urinary tract (LUT) function with modifications in pathways that regulate LUT function using two different animal models (incomplete and complete) of spinal cord injury (SCI).

Methods: Female Sprague–Dawley rats were used. SCI was made at Th8/9 by a contusion injury (contusion, $n=9$) or a complete transection (transection, $n=9$). Unoperated rats were used as normal controls (normal, $n=6$). LUT function was evaluated by micturition behavior in metabolic cages for 24 h and cystometry in awake animals. Immunocytochemical staining at the L6 spinal cord, spinal areas associated with LUT, was performed to identify descending modulatory fibers and dorsal root afferents that project to the L6 spinal cord.

Results: Volume/micturition in metabolic cages gradually increased in both contusion and transection groups compared with normals, and operated groups did not differ from each other. Urodynamic parameters from cystometry were significantly different in contusion and transection groups compared with normals, but again there was no significant difference between contusion and transection groups. Immunocytochemical analyses at the L6 spinal cord showed no serotonergic or noradrenergic fibers in transection group, but some descending fibers remained in contusion group, indicating sparing. Small dorsal root afferents were denser in both contusion and transection groups than in normals, indicating sprouting.

Conclusions: Although differences were not found in LUT function in operated animals, supraspinal and dorsal root projections to the L6 spinal cord responded differently to contusion and transection. This suggests that the benefits of pharmacologic treatments may be different in two lesion models.

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INTRODUCTION

Spinal cord injury (SCI) is classified clinically into complete injuries, where function below the level of a injury is lost, and incomplete injuries where some sensory and/or motor function is retained. Nevertheless, it is known that some descending pathways are spared in many cases of clinically complete injuries.[1]

Lower urinary tract (LUT) dysfunction in SCI results from damage to descending modulatory pathways and increased sensory input through sprouting of primary afferent pathways. A contusion injury that we use destroys the dorsal spinal cord in the thoracic region, including the dorsal columns, the corticospinal tract and the dorsolateral (DL) funiculus, damages the dorsal horn (DH) and impinges on the intermediolateral column.[2–4] Thus, the ventral funiculi and the ventral portions of the lateral funiculi, which contain descending modulatory pathways, were partially spared in contusion injuries, but not in transection injuries.

Serotonergic axons project to the DL nucleus in the spinal cord, which has been implicated in the central control of the bladder and the urethra and recovery of bladder-external urethral sphincter coordination in SCI rats.[5,6] Brainstem-spinal noradrenergic axons project to the lumbosacral spinal cord including the sacral parasympathetic nucleus (SPN) whose axons innervate the LUT.[5,7] In SCI, disruption of these modulatory descending pathways induces sprouting of the small-diameter dorsal root afferents, for example, calcitonin gene-related peptide (CGRP)-positive fibers[3] in the DH. If the descending fibers are partially preserved even in a clinically compete injury of the spinal cord, they could offer targets for LUT treatment even after severe SCI. However, because most animal studies of LUT function have used a transection model, which is both anatomically and clinically complete spinalized animals, they may not model the clinically complete cases of LUT dysfunction in which some projections may be spared.

In our previous studies,[2–4] we have shown that a cellular graft placed into a contusion injury site, such as we use in this study, improved LUT function and was associated with changes in pathways, presumably due to the neuroprotective effects of the grafts. We also showed that intrathecal administration of adrenergic antagonists further improved function,[3] likely by acting on spared and/or sprouting noradrenergic pathways. Here, we compared LUT function with changes in descending modulatory and primary afferent projections after a complete transection with a clinically relevant model, a contusion injury, in the absence of invasive procedures such as grafting or pharmacological treatment.

MATERIALS AND METHODS

Animal groups

Twenty-four 10-week-old Sprague–Dawley rats (225–250 g; Taconic, Germantown, MA, USA) were used. Nine rats received a contusion injury of the spinal

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cord (contusion). Nine rats received a complete transection of the spinal cord (transection, $n=9$). Unoperated rats were used as normal controls (normal, $n=6$).

Rats had free access to food and water and were kept under a 12-h light/dark cycle. All procedures were approved by the Drexel University College of Medicine's Institutional Animal Care and Use Committee and conformed to the National Institute of Health guidelines for the care and use of laboratory animals.

SCI procedures

Animals were anesthetized with intraperitoneal injection of XAK cocktail containing xylazine (10 mg kg^{-1}), acepromazine maleate (0.7 mg kg^{-1}) and ketamine (95 mg kg^{-1}), and a laminectomy was performed at T8/9. In a contusion model, a modified moderate contusion injury was created using the impact rod of the MASCIS device dropped from a height of 25 mm and allowed to rest on the spinal cord for 5 s.[2,3] In a complete transection model, the spinal cord was cut with microscissors at T8/9 as previously described.[8] Muscle and skin were closed in layers after SCI.

Rats were placed on heating pads, and closely observed until awake before returning them to their home cage. Bladders were manually expressed twice daily until killing, except during testing in metabolic cages.

Micturition pattern

Rats with SCI were placed in metabolic cages (Nalgene Metabolic Cage, Nalge Co., Rochester, NY, USA) for 24 h to measure micturition behavior preoperatively and at weekly intervals from week 2 to week 9 after SCI. The bladders were expressed manually before the animals were placed in the metabolic cage. The urine voided during the next 24 h was collected on an electronic scale (FORT250, World Precision Instruments, Sarasota, FL, USA), connected to a microcomputer, for recording micturition frequency and volume.[2,3] Data were recorded and stored using data acquisition software (WINDAQ, DATAQ Instruments, Akron, OH, USA). The voided volume per micturition was compared between experimental groups.

Cystometry in conscious rats

At week 9 post injury, rats were anesthetized using isoflurane inhalation and the bladder exposed by a midline lower abdominal incision. A polyethylene catheter (PE-60, Clay-Adams, Parsippany, NJ, USA) was implanted into the bladder through the dome, as described previously.[2,3] The catheter was tunneled subcutaneously and exited through the skin on the back.

Following catheter implantation, rats were placed in a restraining cage (KN-326, Natsume, Tokyo, Japan) and allowed to recover from the anesthesia for 1–2 h. The bladder catheter was connected to a pressure transducer (BLPR, World Precision Instruments) and a microinjection pump (STC-523, Terumo, Tokyo, Japan). Room-temperature saline was infused at a rate of 0.1 ml min^{-1}. Micturition cycles stabilized and became regular after about 30 min of saline infusion. Three micturition cycles were collected after stabilization. The averages of maximal voiding pressure, post-void residual urine, bladder capacity and the frequency of non-voiding contraction in these micturition cycles were compared among groups. Fluid voided from the urethral meatus was collected to determine the voided volume. Residual fluid was first withdrawn through the catheter and then the bladder was expressed manually by applying pressure on the abdominal wall to collect the remaining intravesical contents. Bladder capacity was calculated as the voided volume plus residual volume. Non-voiding contraction was defined as rhythmic intravesical pressure increases greater than 5 mm Hg from baseline without a release of fluid from urethra.[2,3]

Tissue preparation

After cystometry, animals were anesthetized with intraperitoneal injections of sodium pentobarbital (100 mg kg^{-1}, Abbot Laboratories, North Chicago, IL, USA) and killed by intracardiac perfusion with 200 ml of 0.1 M, pH 7.4, phosphate buffer followed by 500 ml of ice-cold 4% paraformaldehyde fixative in phosphate buffer. The spinal cord was removed and postfixed for 24 h in the same fixative at 4 °C followed by cryoprotection in phosphate-buffered 30% sucrose solution for 3–5 days. Tissues were serially blocked, embedded in Optimal Cutting Temperature compound (Fisher Scientific, Pittsburgh, PA, USA) and kept at −80 °C before being cut into 30 μm coronal sections at the L6 level.

Lesion sections were evaluated for completeness of the injury in transection group or the extent of sparing of descending pathways in contusion group. Transections of the spinal cord were complete and contusion injuries were similar to those described previously.[2–4,8]

Projection patterns at the L6 spinal cord

Coronal sections at the L6 level were immersed in 0.1 M, pH 7.6, phosphate-buffered saline for free floating staining using the avidin–biotin complex method. Sections were permeabilized with 10% goat serum in phosphate-buffered saline for 2 h, then incubated with the appropriate primary antibody (serotonin (5-hydroxytryptamine; 1:50 000, ImmunoStar Inc., Hudson, WI, USA), dopamine-beta-hydroxylase (DβH; 1:1000, Protos Biotech Corporation, New York, NY, USA) for noradrenergic fibers, CGRP for small diameter primary afferents (1:6000, Peninsula Laboratories Inc., San Carlos, CA, USA)) and 2% goat serum in phosphate-buffered saline containing 0.3% Triton X-100 at 4 °C for 24–48 h, and finally reacted with a species-specific biotinylated secondary antibody and the ABC reagent (Vector, Burlingame, CA, USA), each for 2 h at room temperature. Staining was visualized with Sigma fast DAB (Sigma Chemicals, Perth, WA, Australia). Tissue sections were mounted on gelatin-coated slides, dehydrated in graded ethanol, cleaned in xylene and coverslipped. All sections were examined using bright-field microscopy and images were analyzed using NIH Image.[2–4]

Statistical analysis

Cystometric data and density of immunocytochemically positive-fibers were analyzed using one-way analysis of variance between groups. Voided volume per micturition in metabolic cage was analyzed using two-way analysis of variance between group and time, with time as a repeated measure beginning at 2 weeks post injury. *Post-hoc* analysis was performed using Fisher's *post-hoc* test. Data are presented as group mean ± standard error. Significance levels were set to 0.05 for all comparisons.

RESULTS

LUT function

Micturition behavior in metabolic cage. Voided volume/micturition gradually increased, indicating recovery from spinal shock. Although an increase of voided volume/micturition seemed to be slightly earlier in contusion group, there was no statistically significant difference between contusion and transection groups (Figure 1).

Cystometry. Urodynamic parameters from cystometry, including micturition pressure, non-voiding contraction, bladder capacity and post-void residual urine volume, were markedly increased in both

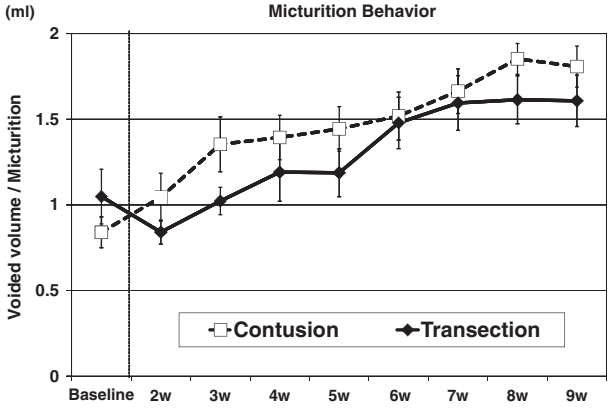

Figure 1 Micturition following contusion or transection of the spinal cord. Voided volume/micturition was gradually increased in both contusion and transection models, indicating recovery from spinal shock. w, week.

Table 1 Summary of urodynamic parameters in cystometry

Parameters	*Normal*	*Contusion*	*Transection*
Micturition pressure (cm water)	19.5 ± 0.8	36.1 ± 2.9[a]	33.3 ± 1.5[b]
Number of non-voiding contraction (/micturition)	0	7.0 ± 1.1[a]	5.9 ± 1.1[b]
Bladder capacity (ml)	0.44 ± 0.05	1.54 ± 0.07[a]	1.53 ± 0.14[b]
Post-void residual urine volume (ml)	0.03 ± 0.01	0.16 ± 0.04[a]	0.20 ± 0.06[b]

[a]$P<0.05$, normal vs contusion.
[b]$P<0.05$, normal vs transection.

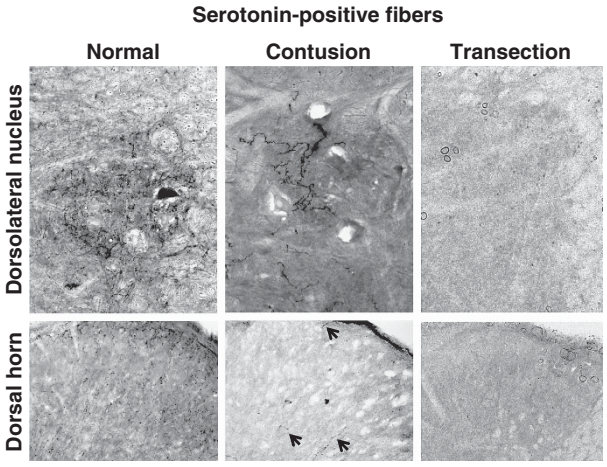

Figure 2 5-HT-positive fibers in the lumbosacral spinal cord. Some serotonin-positive fibers identified in contusion group, although the density of serotonin-positive fibers was low compared with unoperated rats. There were no fibers of these descending pathways in transection group. Arrows in contusion: serotonin-positive fibers.

contusion and transection groups compared with normals ($^{\dagger}P<0.05$, normal vs contusion, $^{\ddagger}P<0.05$, normal vs transection). There was no significant difference between contusion and transection groups in these parameters (Table 1).

Anatomical changes

Projection patterns of modulatory descending projections at the L6 spinal cord. At the L6 level in normal group, serotonin-positive fibers were observed in the DL nucleus and the DH, DβH-positive labeled fibers, indicating noradrenergic axons, were identified in the DL nucleus and the SPN. Some serotonin- (Figure 2) and DβH- (Figure 3) positive fibers were identified in contusion group, indicating sparing of some descending fibers (arrows). As expected, no such labeling was seen in transection group, indicating no other sources for these descending modulators in the spinal cord.

Primary afferent projections at the L6 spinal cord. In normal group, CGRP-positive fibers project to the superficial layers of the DH. Following injury, CGRP immunoactivity was modestly denser in both contusion and transection groups compared with normals (Figure 4a), and some entopic fibers extended into the deeper layers of the DH. Densitometric analyses showed that CGRP immunoreactivity in the DH was significantly denser in contusion and transection groups than in normals (transection vs normal: $P<0.05$, contusion vs normal: $P<0.05$), but there was no significant difference between transection and contusion groups (Figure 4b). These results suggest increased sprouting of small caliber dorsal root axons to the DH in both the transection and contusion groups compared to normals.

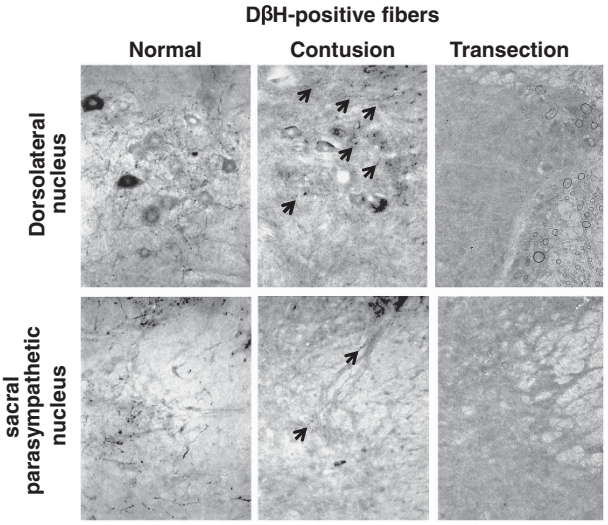

Figure 3 DβH-positive fibers in the lumbosacral spinal cord. Some DβH-positive fibers identified in contusion group, but none in transection group. Arrows in contusion: DβH-positive fibers

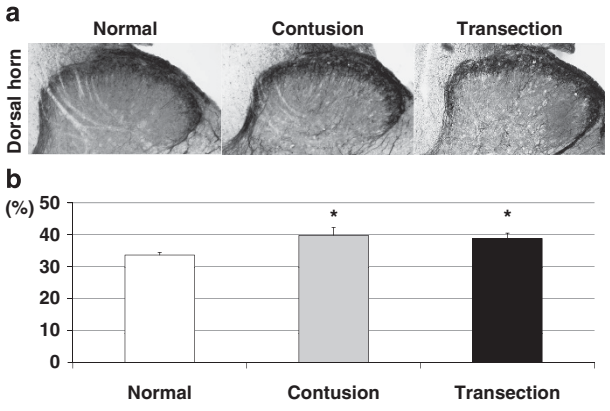

Figure 4 CGRP-positive fibers in the lumbosacral spinal cord. CGRP-positive fibers were modestly denser in the superficial dorsal horn and some entopic fibers were seen in deeper layers in both contusion and transection groups compared with unoperated normal rats (**a**). CGRP immunoreactivity in the DH was significantly denser in transection and contusion groups than in normal rats (**b**). *$P<0.05$.

DISCUSSION

In animal models, SCI produces an initial period of bladder areflexia, followed by the slow re-emergence of involuntary reflex micturition and detrusor hyperactivity mediated by spinal reflex pathways. Coordinated function between the bladder and urethral sphincter is disrupted after SCI, and the degree of dyssynergia is related to the severity of spinal injury.[6] This loss of coordination leads to functional bladder outlet obstruction identified by urinary retention and increased micturition pressure. Non-voiding contractions, manifested as phasic bladder contractions during urine storage, result in urinary incontinence and high intravesical pressures, leading to bladder hypertrophy and deterioration of the upper urinary tract. Non-voiding contractions are likely to reflect hyperactivity of the primary afferent projection. In the present study, the lack of significant differences in LUT function in micturition behavior or cystometry indicates that the injuries were functionally equivalent between contusion and transection.

However, differences in extent of innervation of the lumbar spinal cord by the serotonergic and noradrenergic axons and in the density of CGRP-labeled dorsal root projections to the DH were observed between operated groups. The L6 spinal cord contains preganglionic parasympathetic neurons in the SPN that innervate the ganglia supplying the smooth muscle of the bladder wall,[5] and the DL nucleus, which contains somatic motor neurons that innervate the external urethral sphincter and coordinate the activity of the bladder and the urethra.[5,6] Our previous studies suggested that the greater density of descending serotonergic and noradrenergic projections, elicited by the neuroprotective effects of the cellular grafts transplanted into a contusion lesion site, ameliorates of the dyssynergia between the bladder and the urethral sphincter[2–4] by providing some descending control over spinal nuclei and greater descending control over sensory transmission in the DH.[9–12] The density of bladder afferent projections, that is, CGRP-positive fibers in the DH, is increased after severe SCI[13–15] and this has been implicated in detrusor overactivity that develops following SCI.[2–4] Bladder afferents in the lumbosacral spinal cord also project to the DH, SPN and dorsal commissure in the lumbosacral spinal cord,[16,17] which was induced by disruption of the descending projections. These findings provide greater descending control over sensory transmission in the DH.[9–12]

Some serotonin- and DβH-positive fibers were preserved in contusion group at the L6 spinal cord, but none were present in transection group. CGRP-positive fibers were denser in both contusion and transection groups than in normals. The discrepancy between similar urodynamic parameters in metabolic cages and in cystometry and differences in projection patterns is not uncommon. A likely explanation is that the sparing of descending projections was insufficient to permit measurable recovery. In fact, LUT function spontaneously recovers following a mild contusion injury of the spinal cord and more severe contusion injury induces permanent LUT dysfunction.[6] Our previous study showing that transplantation of cellular grafts after similar contusion lesions improved LUT function compared with SCI animals without treatments. This was attributed to the greater sparing of descending modulatory pathways because of the neuroprotective properties of the transplanted cells and indeed was demonstrated immunocytochemically. We show here that sprouting/sparing occurs even without a graft but is less pronounced, and is not associated with recovery of LUT function.

In LUT dysfunction after SCI, effects of administration of agonists or antagonists may differ according to the injury type. The appropriate medication is likely to differ between contusion and transection groups, as a low density of serotonin- and DβH-positive fibers was preserved in the spinal cord of contusion group. Our previous studies suggest that a difference in response to a contusion injury treated with a cellular graft into the lesion site, compared with a contusion injury without a cellular graft, showed a greater response to alpha 1-adrenergic blockers in urodynamic parameters.[3,18] This indicated that there was possibility of different sensitivity for alpha 1-adrenergic receptor subtypes. We suggest that there will be different pharmacological responses with serotonergic agents between contusion and transection groups as well.[19] Further, alterations in NMDA receptors involved in recovery of urethral sphincter coordination[6] suggest a potential clinical application using glutaminergic agonists.[1,20] Thus, if pharmacological data from animal studies are applied to clinical treatments, the preclinical studies should be done in both contusion and transection injuries.

In conclusion, although a significant difference was not found in LUT function, supraspinal projections to the lumbosacral spinal cord were significantly different between contusion and transection groups. This suggests that anatomical plasticity occurs following both complete and incomplete injuries and that the benefits of pharmacologic treatments may be different in two lesion models.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

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1 Angeli CA, Edgerton VR, Gerasimenko YP, Harkema SJ. Altering spinal cord excitability enables voluntary movements after chronic complete paralysis in humans. *Brain* 2014; **137**: 1394.
2 Mitsui T, Neuhuber B, Fischer I. Acute administration of AMPA/Kainate blocker combined with delayed transplantation of neural precursors improves lower urinary tract function in spinal injured rats. *Brain Res* 2011; **1418**: 23.
3 Mitsui T, Shumsky JS, Lepore AC, Murray M, Fischer I. Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and motor functions, decreases thermal hypersensitivity, and modifies intraspinal circuitry. *J Neurosci* 2005; **25**: 9624.
4 Mitsui T, Fischer I, Shumsky JS, Murray M. Transplants of fibroblasts expressing BDNF and NT-3 promote recovery of bladder and hindlimb function following spinal contusion injury in rats. *Exp Neurol* 2005; **194**: 410.
5 Marson L. Identification of central nervous system neurons that innervate the bladder body, bladder base, or external urethral sphincter of female rats: a transneuronal tracing study using pseudorabies virus. *J Comp Neurol* 1997; **389**: 584.
6 Pikov V, Wrathall JR. Coordination of the bladder detrusor and the external urethral sphincter in a rat model of spinal cord injury: effect of injury severity. *J Neurosci* 2001; **21**: 559.
7 Westlund KN, Bowker RM, Ziegler MG, Coulter JD. Noradrenergic projections to the spinal cord of the rat. *Brain Res* 1983; **263**: 15.
8 Nothias JM, Mitsui T, Shumsky JS, Fischer I, Antonacci MD, Murray M. Combined effects of neurotrophin secreting transplants, exercise, and serotonergic drug challenge improve function in spinal rats. *Neurorehabil Neural Repair* 2005; **19**: 296.
9 Millan MJ. Descending control of pain. *Prog Neurobiol* 2002; **66**: 355.
10 Tsuruoka M, Maeda M, Nagasawa I, Inoue T. Spinal pathways mediating coeruleospinal antinociception in the rat. *Neurosci Lett* 2004; **362**: 236.
11 Ueyama T, Arakawa H, Mizuno N. Central distribution of efferent and afferent components of the pudendal nerve in rat. *Anat Embryol (Berl)* 1987; **177**: 37–49.
12 Suzuki R, Morcuende S, Webber M, Hunt SP, Dickenson AH. Superficial NK1-expressing neurons control spinal excitability through activation of descending pathways. *Nat Neurosci* 2002; **5**: 1319.
13 Krenz NR, Meakin SO, Krassioukov AV, Weaver LC. Neutralizing intraspinal nerve growth factor blocks autonomic dysreflexia caused by spinal cord injury. *J Neurosci* 1999; **19**: 7405.
14 Zhou Y, Wang Y, Abdelhady M, Mourad MS, Hassouna MM. Change of vanilloid receptor 1 following neuromodulation in rats with spinal cord injury. *J Surg Res* 2002; **107**: 140.
15 Hains BC, Willis WD, Hulsebosch CE. Temporal plasticity of dorsal horn somatosensory neurons after acute and chronic spinal cord hemisection in rat. *Brain Res* 2003; **970**: 238.
16 Jancso G, Maggi CA. Distribution of capsaicin-sensitive urinary bladder afferents in the rat spinal cord. *Brain Res* 1987; **418**: 371.
17 Steers WD, Ciambotti J, Etzel B, Erdman S, de Groat WC. Alterations in afferent pathways from the urinary bladder of the rat in response to partial urethral obstruction. *J Comp Neurol* 1991; **310**: 401.
18 Miyazato M, Oshiro T, Chancellor MB, de Groat WC, Yoshimura N, Saito S. An alpha1-adrenoceptor blocker terazosin improves urine storage function in the spinal cord in spinal cord injured rats. *Life Sciences* 2013; **92**: 125.
19 Chang HH, Havton LA. Serotonergic 5-HT(1?A) 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* 2013; **239**: 210.
20 Liao JM, Yang CH, Cheng CL, Pan SF, Chen MJ, Huang PC *et al*. Spinal glutamatergic NMDA-dependent cyclic pelvic nerve-to-external urethra sphincter reflex potentiation in anesthetized rats. American journal of physiology. *Renal Physiol* 2007; **293**: F790.