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Deficits in bladder function following spinal cord injury vary depending on the level of the injury

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ABSTRACT

Loss of bladder function is an important consequence of a spinal cord injury (SCI) but is rarely assessed in animal studies of SCI. Here, we use a simple outcome measure (volume of retained urine) to assess bladder dysfunction over time following moderate contusion injuries at 3 different thoracic levels (T1, T4, or T9) and complete crush injuries (T1 vs. T9). The volume of urine retained in the bladder was measured daily for fourteen days post injury by anesthetizing the animals with isoflurane, expressing the bladder, and weighing the urine. To compare bladder deficits with the degree of impairment of hindlimb motor function, locomotion was assessed using the BBB open field rating scale. Rats with contusions at T4 and T9 exhibited bladder impairments reflected by increased urine retention from 1 to 12 days post injury. In contrast, rats with contusions at T1 exhibited minimal deficits (smaller volumes of retained urine). Lesion size and overall functional impairment were comparable between groups based on quantitative assessments of lesion area at the epicenter and BBB locomotor scores. Moreover, a sector analysis of sparing of different portions of the white matter revealed no differences in sparing of different funiculi between the groups. Injections of Fluorogold into lumbar segments led to retrograde labeling of a larger number of neurons in the pontine micturition center (PMC) following T1 injury when compared to T4 or T9. Thus, moderate contusion lesions at T1 spare a critical descending pathway able to mediate at least reflex voiding in rats.

Introduction

The dual functions of the urinary bladder to store and periodically eliminate urine (de Groat and Yoshimura, 2006) are mediated by pathways that span the neuraxis. The circuitry involved includes afferent and efferent pathways in the periphery that include elements of the viscerosensory, sympathetic, parasympathetic, and somatic motor systems, local circuitry in the lumbosacral spinal cord, ascending projections to the brainstem, and descending pathways back down to the lumbosacral spinal cord (de Groat and Yoshimura, 2006; Shefchyk, 2002; Sugaya et al., 2005). Voluntary voiding also involves pathways from the cortex to the brainstem, although these are less well-defined than the pathways to and from the brainstem and spinal cord.

Depending on the level and severity, spinal cord injuries can disrupt either the ascending or descending tracts or the local circuitry at the segmental levels that are important for bladder function. In humans, spinal cord injuries (SCI) in the lumbosacral region disrupt local reflex circuitry causing bladder areflexia (Abdel-Azim et al., 1991; Kaplan et al., 1991). Lesions above the level of the lumbosacral spinal cord disrupt ascending and descending pathways causing characteristic symptoms that evolve over time. At early post-lesion intervals, reflex contraction of the bladder detrusor muscle is impaired. The result of this is urinary retention which, left untreated, can be life-threatening (Grundy and Russell, 1986). Over time, changes occur in circuitry mediating bladder reflexes that lead to other functional alterations including detrusor sphincter dyssynergia, in which bladder contractions occur at the same time that lack of detrusor activation blocks urine outflow (de Groat et al., 1990). This produces pathological intra-bladder pressures that induce bladder hypertrophy and can damage the urinary tract. Under the best of circumstances, the bladder of an individual with a spinal cord injury rarely empties completely creating conditions that foster the development of urinary tract infections (UTIs). Indeed, prior to the development of penicillin, UTIs greatly shortened the life expectancy of individuals with SCI. For this reason, individuals with SCI regard the recovery of bladder function as one of their highest priorities (Anderson, 2004; Estores, 2003; Rosenzweig and McDonald, 2004).

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Experimental models investigating the effects of both partial and complete SCI at the thoracic level have documented the same phenomenon of urinary retention in both rats and mice (Engesser-Cesar et al., 2005; Keirstead et al., 2005; Pikov and Wrathall, 2001; Zinck et al., 2007). Interestingly, however, recent studies reporting on the development of new models of SCI at the cervical level in rodents have reported minimal if any bladder deficits (Anderson et al., 2007). This is in contrast to the situation in humans with incomplete cervical injuries where bladder dysfunction is a major concern (Kaplan et al., 1991; Weld and Dmochowski, 2000).

Although these previous studies hint at possible differences in the extent of bladder function following cervical vs. thoracic level injuries, there have been no direct comparisons using similar lesion models and methods of bladder assessment. Accordingly, the primary goal of the present study was to directly assess whether there were differences in bladder dysfunction following comparable partial contusion injuries at different spinal levels. We report here that histologically similar lesions at T4 or T9 produce impairments in bladder function whereas lesions at T1 produce minimal deficits.

One explanation for the relative lack of bladder dysfunction following contusion lesions at the cervical or high thoracic level is that the lesion spares different white matter regions that contain projections important for bladder control. Thus, the second goal of the present study was to assess the degree sparing of different portions of the white matter following lesions at T4 and T9 that produce substantial impairment of bladder function, vs. lesions at T1 that produce minimal impairment. In addition, we use retrograde labeling techniques to assess the degree of sparing of projections from the pontine micturition center following lesions at different levels to assess whether sparing of descending projections was related to sparing of bladder function.

Materials and methods

Experimental groups

Adult female Sprague–Dawley rats weighing 190–230 g were used. Rats were group-housed (five per cage) in a room with controlled temperature, humidity, and light cycle. Rats had access to food and water *ad libitum*. All animals were handled for one week prior to injury.

In six separate experiments, a total of 81 rats received a spinal cord injury at one of three different thoracic levels: T1, T4, or T9. In the first three experiments (labeled as experiments 1, 2, and 3), a total of 30 rats received moderate contusion injuries at T1, T4, or T9. A follow-up experiment with 16 rats (experiment 4) then repeated a direct comparison between groups with contusion injuries at T1 vs. T4 that were run at the same time (8 rats per group). An additional experiment with 28 rats (experiment 5) directly compared bladder function following lesions at T1, T4, or T9, and then injected the retrograde tracer Fluorogold into the lumbar enlargement (T12) at day 15 after injury (7 rats per group) to retrogradely label any surviving pathways from the bladder control centers in the brainstem. This experiment included a group of seven control rats that received no injury, but underwent identical animal care, behavioral monitoring, and Fluorogold injection. Finally, an experiment with 14 rats (experiment 6) directly compared groups of rats that received crush injuries at T1 and T9. These rats also received Fluorogold injections into the lumbar enlargement 15 days after injury (7 rats per group).

Surgical procedures: contusion injury

Rats were anesthetized with an intraperitoneal injection of Ketamine and Xylazine (100 mg/kg and 10 mg/kg, respectively). Laminectomies were performed at T1, T4, or T9. Moderate contusion injuries were produced using the Infinite Horizon impact device (Precision Systems & Instrumentation [PSI], Lexington, KY). Contusion force was 200 kdyn; dwell time was 0 s. The average displacement was 1252 μm; the average velocity was 119 mm/s.

Postoperatively, rats were housed in cages with Alpha-Dri bedding and were placed on a water-jacketed heating pad at 37 °C for the first night following injury. Bladders were expressed every 12 h following injury until animals were killed humanely 2 weeks later. Additionally, rats received subcutaneous injections of Baytril (0.5 mg/kg), Buprenorphine (0.01 mg/kg), and lactated ringers (10 ml) following the morning bladder expression. The rats were maintained on the Baytril and Buprenorphine for the first 10 days post injury (dpi), and received saline for the entire 14 day survival interval.

Surgical procedures: crush injury

Rats were anesthetized as described previously and received laminectomies at either T1 or T9. Injuries were produced by crushing the spinal cord with #5 Dumont forceps for 3 s. Post-operative care was as described previously.

Retrograde labeling

The 42 rats from the final two experiments were anesthetized as described previously and received laminectomies at T12. Fluorogold (4% w/v in saline, Fluorochrome, LLC) was injected into the exposed spinal cord with a 10 μl Hamilton syringe with a pulled glass tip. Injections were delivered stereotactically at three sites in the spinal cord: at midline (depth: 0.6 mm) and 1.1 mm lateral to midline (depth 1.1 mm), bilaterally. All animals received 0.3 μl per site resulting in a total volume of 0.9 μl injected into the spinal cord.

Assessment of bladder function

Urine was collected and weighed during the morning bladder expression. For this purpose, each rat was individually anesthetized with isoflurane gas (1.5 l/min at a concentration of 3.0% in oxygen) for 5 min. Previous studies have demonstrated that the amount of urine manually expressed under anesthesia is more highly correlated with the actual amount of urine in the bladder, as detected using ultrasonography, than when bladders are expressed in unanesthetized rats (Keirstead et al., 2005).

The amount of retained urine was determined by collecting the expressed urine and weighing it. A grid was placed under the animal in the anesthesia induction chamber to collect urine that was voided while the rat was being anesthetized (this was usually a small amount). The urine collected during induction of anesthesia was combined with the urine collected during manual expression. Rats recovered from anesthesia within 2 min following bladder expression.

Assessment of hindlimb locomotor function

All rats were assessed with the Basso, Beattie, Bresnahan Locomotor Rating Scale (BBB) (Basso et al., 1995) on days 2 and 13 after injury in order to relate bladder dysfunction with locomotor ability. The BBB is a 21-point scale designed to assess hindlimb locomotor recovery following thoracic spinal cord injury. A BBB score of 0 indicates no hindlimb movement. A BBB score of 1 through 8 indicates joint movement, but no weight support. A BBB score of 9 through 20 indicates an ability to support weight and use the limb for locomotion but with some degree of abnormality. A BBB score of 21 corresponds to the locomotion of a normal rat. Bladders were expressed 10 min prior to locomotor testing.