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Original Research

Functional outcomes after spinal cord contusion injury in rats: the influence of age and key parameters

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Abstract

The complexity of sensory and motor dysfunctions following spinal cord injury (SCI) necessitates identifying key physical parameters that contribute to these outcomes. This paper examines the effects of critical parameters on behavioral and physiological outcomes after spinal contusion in preadolescent (7 weeks) and adult (30 weeks) male Sprague-Dawley rats. A standardized injury of 150-kilodyne force and 1-second dwell time was induced at thoracic level 10. Actual Force (152.6 ± 0.6 and 154.4 ± 1.2 kdyn) and Velocity (121 ± 0.4 and 120.7 ± 0.6 mm/s) were consistent between groups, although Displacement (981.5 ± 35.9 and 1048.6 ± 30.4 µm) varied. The preadolescent group showed more rapid body weight loss (POD 4 vs. POD 7) and quicker locomotion recovery (POD 16 vs. POD 22), while the adult group exhibited a faster onset of bladder dysfunction. By 40 days post-injury, all groups developed mechanical allodynia ($p < 0.05$), with thermal hyperalgesia significantly elevated in the preadolescent group ($p < 0.05$). Analysis of displacement subsets revealed that both Low and high-displacement groups had similar levels of mechanical allodynia; however, the high-displacement group experienced greater impairments in body weight, locomotion, and bladder function. These findings highlight age and spinal displacement as critical factors in evaluating consistent behavioral dysfunctions following SCI in rats.

Keywords: Age-related differences, behavioral dysfunctions, neuropathic pain, spinal cord injury, Sprague-Dawley rats

1. Introduction

Traumatic spinal cord injury (SCI) leads to sensory, motor, metabolic, and autonomic dysfunctions through progressive changes in neuroanatomy and microenvironments, both at the injury site and in remote regions (Brown et al., 2024; Ma et al., 2024; Willits et al., 2024). However, reports on dysfunctions following SCI in rodent models often show inconsistencies due to variations in experimental design, including differences in animal strains, devices, age, epidemiology, and methodologies (Sharif and Jazaib Ali, 2020; Steward et al., 2012).

Spinal contusion injury (SCI) in rodents closely mimics human SCI. The physical parameters of the injury are critical determinants of the severity, duration, pathophysiology, and recovery of neurological dysfunctions (Park et al., 2016; Sharif-Alhoseini et al., 2017; Walker et al., 2015). Mechanistically, contusion injuries are caused by a combination of physical factors, including injury intensity (force), velocity (impact speed), and spinal cord compression (displacement). These factors significantly influence the primary and secondary pathophysiological processes in the injured spinal cord and remote areas, leading to sensory, motor, metabolic, autonomic, and immune dysfunctions throughout the nervous system (Kang et al., 2020).

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Studies on SCI devices have highlighted the effects of physical parameters. For example, minor differences in injury severity have been reported among three different velocity groups using various contusion devices, such as 0.1 m/s with the Infinite Horizon (IH) device, 0.2 m/s with the Ohio State University device, and 0.4 m/s with the NYU device. These results suggest that small variations in injury force and device-specific velocity are not critical factors for assessing behavioral dysfunctions after SCI (Kim et al., 2009; Lam et al., 2014). Recent modifications of weight-drop devices have emphasized the importance of injury height and displacement in determining behavioral dysfunctions (Jarragh et al., 2023). Additionally, studies on spinal compression and behavioral outcomes have demonstrated a strong correlation, highlighting spinal compression as a key pathophysiological mechanism underlying neurological dysfunction (Batchelor et al., 2013). These findings collectively underscore the importance of spinal compression in evaluating behavioral dysfunctions in SCI models. Spinal compression is closely related to spinal damage and delayed functional recovery, irrespective of the applied injury force.

The IH Impactor device employs a force-controlled stepper motor to deliver pre-determined injury forces ranging from mild to severe SCI, along with controlled velocity (Scheff et al., 2003). This device, combined with pre-programmed software, enables relatively reproducible behavioral and cellular outcomes following SCI, such as loss and recovery of voluntary locomotion, neuropathic pain behaviors, increased neuronal excitability, receptor and intracellular protein kinase changes, and glial activation (Bannerman and Ghasemlou, 2022; Lauzadis et al., 2020; Scheff et al., 2003). However, these findings are largely derived from studies with narrow ranges of SCI parameters, typically involving a single age group, similar injury forces, and standardized experimental conditions, such as rat species, anesthesia, and postoperative care (Sparrey et al., 2016). A key criterion for spinal contusion studies is the stabilization and standardization of the injury environment, particularly spinal displacement, which strongly influences progressive neurological dysfunctions (Streijger et al., 2013; Zhang et al., 2008). Despite this, the IH Impactor does not precisely control spinal displacement, resulting in user-dependent variability, highlighting the need for further evaluation of displacement variability.

Characterizing reliable SCI parameters is crucial for understanding the impact of contusion factors on progressive behavioral and physiological dysfunctions. Such insights are essential for developing pharmacological strategies to treat SCI-induced dysfunctions in rodent models. In the present study, we investigated the effects of reliable injury parameters on physiological and behavioral dysfunctions and their recovery using pre-programmed settings on the IH Impactor device in both periadolescent and adult rat groups.

2. Materials and methods

2.1 Spinal contusion injury

Sprague-Dawley (SD) rats (n = 51, Harlan) were housed under reversed 12-hour light/dark cycles throughout the experimental period. All procedures conformed to the standards of the Institutional Animal Care and Use Committee and the guidelines of the National Institutes of Health (NIH). Periadolescent (7 weeks, 205 ± 0.7 g, n = 20) and adult (30 weeks, 492 ± 5.1 g, n = 21) rats underwent spinal contusion injury at thoracic level 10 using the IH Impactor device (Precision Systems Instruments, LLC, USA) under deep anesthesia (intraperitoneal

administration of sodium pentobarbital, 60 mg/kg). Contusion was induced by dropping an impact metal rod (2 mm diameter, rounded tip) from a height of 0.8 cm onto the dorsal dura mater, with a fixed force of 150 kilodyne (kdyn) and a dwell time of 1 second. This combination of force and dwell time was previously demonstrated to reliably induce neuropathic pain behavior and loss/recovery of locomotion compared to other combinations (Carter et al., 2016). The 2 mm diameter tip ensured uniform contusion across the spinal cord, avoiding partial or hemicontusion effects (Dietz et al., 2022; Khuyagbaatar et al., 2020).

During the procedure, the software (Precision Systems Instruments, IH spinal cord impactor, version 5.0.3, USA) automatically recorded the actual force (AF, kdyn), impact velocity (Velocity, mm/s), and spinal cord compression (Displacement, μm). After the injury, muscles and fascia were sutured, and the skin was closed with autoclips. Since SCI rats typically regained consciousness and began moving and drinking approximately 2 hours after the injury, they were kept on a heating pad during this time before being returned to their home cage.

Post-surgical care included ad libitum food and water, manual bladder expression twice daily until voluntary bladder control returned, and prophylactic antibiotic treatment (Baytril, 30 mg/kg, Bayer Animal Health, Leverkusen, Germany) for 5 days. None of the SCI rats exhibited unilateral paralysis of the hindlimbs, ensuring all underwent behavioral outcome measurements. Sham surgery (spinal laminectomy without contusion) was performed in age-matched rats as controls (n = 5 periadolescent, n = 5 adult). Three adult SCI rats were excluded due to urinary inflammation or unknown deaths during the study. To minimize variability, a single expert performed all SCI procedures and post-operative care.

2.2 Recovery of locomotion

Hindlimb locomotor function was assessed using the open-field BBB locomotor scoring system (Basso et al., 1995). BBB scores range from 0–7 (movement of hip, knee, and ankle joints), 8–13 (weight-bearing stance and coordinated stepping), and 14–21 (toe clearance, paw positioning, and trunk stability). Both hindlimbs were scored independently after contusion and sham surgeries.

2.3 Bladder dysfunction

SCI caused loss of spontaneous bladder control, with time-dependent bladder swelling until function recovered. Bladder size (diameter) was categorized into four arbitrary scores based on palpation:

1. Score 0: < 0.5 cm
2. Score 1: 0.5–1 cm
3. Score 2: 1–2 cm
4. Score 3: > 2 cm

Normal voluntary bladder function was defined as three consecutive days with a score of 0. Hematuria was not evaluated, as it was transient and not observed in all SCI groups.

2.4 Mechanically evoked pain

Mechanical sensitivity was assessed by paw withdrawal threshold (PWT) using von Frey filaments (VFFs) as described previously (Gwak et al., 2013). Rats were acclimated to a clear plexiglass testing apparatus (8 × 8 × 24 cm) for 30 minutes daily over 3 days before SCI to minimize stress. Testing was performed 40 days post-SCI, as motor recovery was incomplete by three weeks. Six VFF stimuli (log units: 3.61–5.18 g, starting at 4.31) were applied for 3–4 seconds to the center of the glabrous hindpaw skin, and the 50% PWT was calculated using the formula:

$$50\%\ g\ threshhold = (10^{[Xf-\kappa\delta]})/10{,}000$$

where Xf is the value of the final VFF (in log unit), κ is a correction factor, and δ is the average increment (in log units) between von Frey filaments (Chaplan et al., 1994).

2.5 Heat-evoked pain

Heat sensitivity was measured as paw withdrawal latency (PWL) to radiant heat stimuli (4.7 amps; UCSD Anesthesiology Research Engineering Core, USA) following Gwak et al. (2013). Rats were placed individually in Plexiglass cubicles (8 × 24 × 24 cm) over a light box. A radiant heat beam was directed onto the glabrous paw surface using a mirror. The light beam was automatically turned off upon paw withdrawal, allowing measurement of

latency. Two trials were conducted per paw with a 10-minute interval, and PWL scores were averaged. Testing occurred 40 days post-SCI due to incomplete motor recovery by three weeks.

2.6 Statistical analysis

Data were analyzed using paired *t*-tests (before vs. after within groups), unpaired *t*-tests (between groups), and one- or two-way analysis of variance (ANOVA) with Tukey's HSD post hoc tests. Analyses were conducted using SigmaPlot software (version 13.0, Systat Software Inc., UK), with statistical significance set at $p < 0.05$. Results are presented as mean ± standard error (SE).

3. Results

3.1 Outcomes of injury parameters

Following T10 spinal cord contusion injury, the average actual force in the periadolescent and adult groups was 152.6 ± 0.6 kdyn (range: 149–160 kdyn) and 154.4 ± 1.2 kdyn (range: 149–167 kdyn), respectively. The average velocity in the periadolescent and adult groups was 121 ± 0.4 mm/s (range: 117–124 mm/s) and 120.7 ± 0.6 mm/s (range: 117–127 mm/s). The average displacement in the periadolescent and adult groups was 981.4 ± 35.9 μm (range: 617–1340 μm) and 1048.6 ± 30.4 μm (range: 881–1322 μm) (**Table 1**).

Table 1. The outcomes of physical parameters following spinal cord contusion injury in rats.

Periadolescent	B.W. (g)	A.F. (kdyn)	Velo. (mm/s)	Displ. (㎛)	Adult	B.W. (g)	A.F. (kdyn)	Velo. (mm/s)	Displ. (㎛)
rat 1	205	152	122	804	Rat 1	562	155	117	1270
rat 2	204	149	124	1023	Rat 2	480	158	124	1322
rat 3	199	152	120	1289	Rat 3	507	149	122	1111
rat 4	206	152	122	1340	Rat 4	460	154	117	1040
rat 5	212	153	120	970	Rat 5	490	151	122	987
rat 6	199	151	120	1040	Rat 6	489	156	122	1236
rat 7	208	149	120	617	Rat 7	497	150	119	886
rat 8	205	152	122	793	Rat 8	490	152	122	894
rat 9	210	154	122	987	Rat 9	460	157	120	881
rat 10	205	151	120	970	Rat 10	490	149	120	1040
rat 11	205	153	122	934	Rat 11	485	167	122	1005
rat 12	203	156	120	846	Rat 12	478	164	127	934
rat 13	210	151	124	917	Rat 13	495	150	117	1058
rat 14	204	153	117	987	Rat 14	493	153	118	1140
rat 15	202	160	122	934	Rat 15	498	150	120	947
rat 16	210	150	122	952	Rat 16	503	155	121	1081
rat 17	207	153	120	1058	Rat 17	486	149	122	988
rat 18	205	157	117	1087	Rat 18	501	160	120	1055
rat 19	203	150	122	1111					
rat 20	207	154	122	970					
20 rats	205.4±0.8	152.6±0.6	121±0.4	981.4±35.9	18 rats	492.4±5.1	154.4±1.2	120.7±0.6	1048.6±30.4

Following spinal contusion injury, all physical parameters showed no significant differences between periadolescent and adult groups. B.W.; body weight, A.F.; actual force, Velo.; velocity, Displ.; displacement.

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Previous studies suggested that differences in displacement from 300 to 500 µm significantly affect behavioral and histological outcomes, with extended loss of neurons and increased tissue damage in the ventral and dorsal white matter (Behrmann et al., 1992; Sjovold et al., 2013). Given the nearly twofold difference in displacement values between the lowest (881 µm) and the highest (1322 µm), we divided the groups into Low Displacement (below average) and High Displacement (above average) based on mean displacement values.

For the periadolescent group, the mean displacements were 882.4 ± 33 µm (Low, n = 11) and 1102.4 ± 42.56 µm (High, n = 9). For the adult group, the mean displacements were 960.2 ± 19.2 µm (Low, n = 10) and 1159.1 ± 36.5 µm (High, n = 8). Significant differences in displacement were observed within each age group (*p < 0.05 for periadolescent; #p < 0.05 for adult, **Figure 1**).

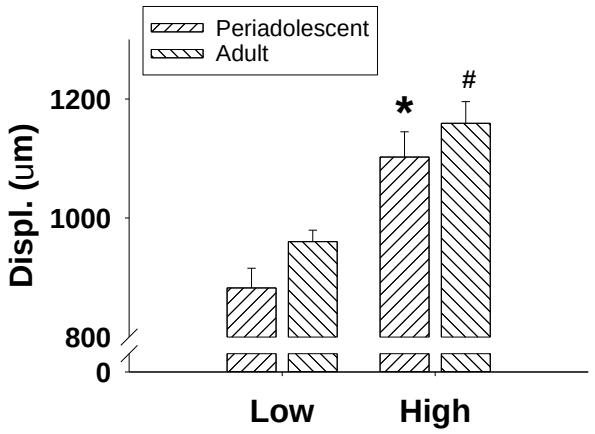

Figure 1. Significant differences between Low and High Displacement groups in periadolescent and adult rats following Spinal Contusion Injury. Displacement values were categorized into Low and High Displacement groups based on the average values within each age group. In the periadolescent group, the mean displacement was 882.4 ± 33 µm for the Low Displacement group (n = 11) and 1102.4 ± 42.56 µm for the High Displacement group (n = 9). In the adult group, the mean displacement was 960.2 ± 19.2 µm for the Low Displacement group (n = 10) and 1159.1 ± 36.5 µm for the High Displacement group (n = 8). Both age groups showed a significant difference between Low and High Displacement values (p < 0.05 for periadolescent; #p < 0.05 for adult).

3.2 Body weight changes

Prior to SCI, the average body weight of the periadolescent and adult groups was 205.5 ± 0.8 g and 492.4 ± 5.1 g, respectively (**Table 1**). Following SCI, the periadolescent group exhibited a significant decrease in body weight on POD 4 (174.3 ± 2.6 g) that persisted until POD 16 (*p < 0.05, **Figure 2A**). Similarly, the adult group showed a significant decrease in body weight on POD 7 (466.1 ± 5.3 g) that persisted until POD 16 ($^{\#}p$ < 0.05, **Figure 2A**). In contrast, sham controls displayed a gradual increase in body weight without any significant decreases (**Figure 2A**).

The lowest body weight for the periadolescent and adult groups was 170.5 ± 2.6 g (POD 7, 17 ± 1.2% decrease) and 465.3 ± 5.5 g (POD 7, 5.5 ± 0.4% decrease), respectively. The percentage change in body weight was significantly different between the periadolescent and adult groups (*p < 0.05, **Figure 2B**).

In Displacement groups:

i. Periadolescent group: Both Low (*p < 0.05) and High Displacement (#p < 0.05) groups showed a significant decrease in body weight on POD 7. High Displacement groups exhibited delayed recovery compared to Low groups (POD 13 vs. POD 10, **Figure 2C**).
ii. Adult group: High Displacement groups experienced a faster decrease in body weight (POD 1 vs. POD 4) and delayed recovery (POD 22 vs. POD 13, **Figure 2C**).

The lowest body weights were as follows:

i. Periadolescent Low: 171.9 ± 3 g (16.8 ± 1.4% decrease)
ii. Periadolescent High: 168.7 ± 4.8 g (17.3 ± 2.2% decrease)
iii. Adult Low: 453 ± 3 g (6.3 ± 0.5% decrease)
iv. Adult High: 480.8 ± 9.4 g (4.6 ± 0.6% decrease)

A significant difference was observed between Low and High Displacement groups in the adult group (*p < 0.05, **Figure 2D**).

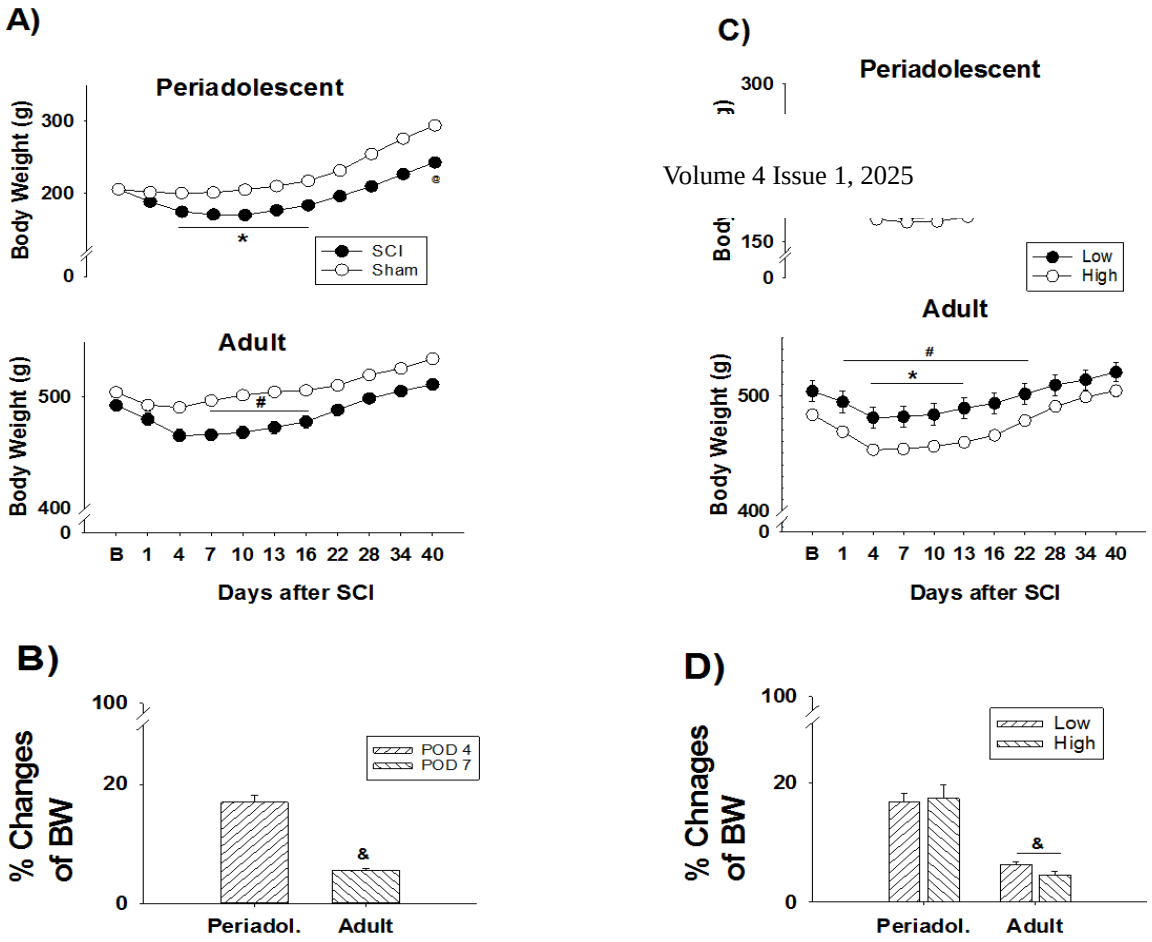

Figure 2. Changes in body weight after spinal contusion injury. (A) The average body weight of periadolescent group showed a faster decrease than the adult group (4 days vs. 7 days after SCI) while the sham control (n=5, each group) showed a gradual increase of body weight without a significant decrease during the entire test period. (B) The changes of percentage of body weight on the lowest body weight of both periadolescent (POD 4) and adult groups (POD 7) showed a significant difference ($^{\&}p < 0.05$). (C) The adult group showed a faster decrease and delayed recovery of body weight in both Low ($^{*}p < 0.05$) and High ($^{\#}p < 0.05$) Displacement groups than the periadolescent group. (D) The adult group showed a significant difference in the change of percentage body weight in both Low and High Displacement groups compared with the periadolescent group ($^{\&}p < 0.05$). However, both age groups did not show a significant difference between Low and High Displacement.

3.3 Recovery of locomotion

Prior to SCI, the average BBB score for both age groups was 21 ± 0 (**Figure 3A**). On POD 1, both groups displayed a BBB score of 0, indicating complete loss of locomotion in both hindlimbs (**Figure 3A**).

i. Periadolescent group: BBB scores significantly increased on POD 16 for both the left (*$^{*}6.9 \pm 1.2, p < 0.05$*) and right (*$^{*}7.6 \pm 1.0, p < 0.05$*) sides (**Figure 3A**).

ii. Adult group: BBB scores significantly increased on POD 19 for both the left (4.2 ± 0.5, $^{\#}p < 0.05$) and right (4.8 ± 0.56, $^{\#}p < 0.05$) sides (**Figure 3A**).

Sham groups maintained a BBB score of 21 throughout the test period (data not shown).

In Displacement groups:

i. Periadolescent group: Both Low ($^{@}p < 0.05$) and High ($^{\$}p < 0.05$) groups showed significant recovery of bilateral locomotion on POD 19.

ii. Adult group: High Displacement groups ($^{@}p < 0.05$) recovered faster than Low groups ($^{\$}p < 0.05$, POD 16 vs. POD 19, **Figure 3B**).

However, no significant differences were observed between Low and High Displacement groups within each age group (**Figure 3B**).

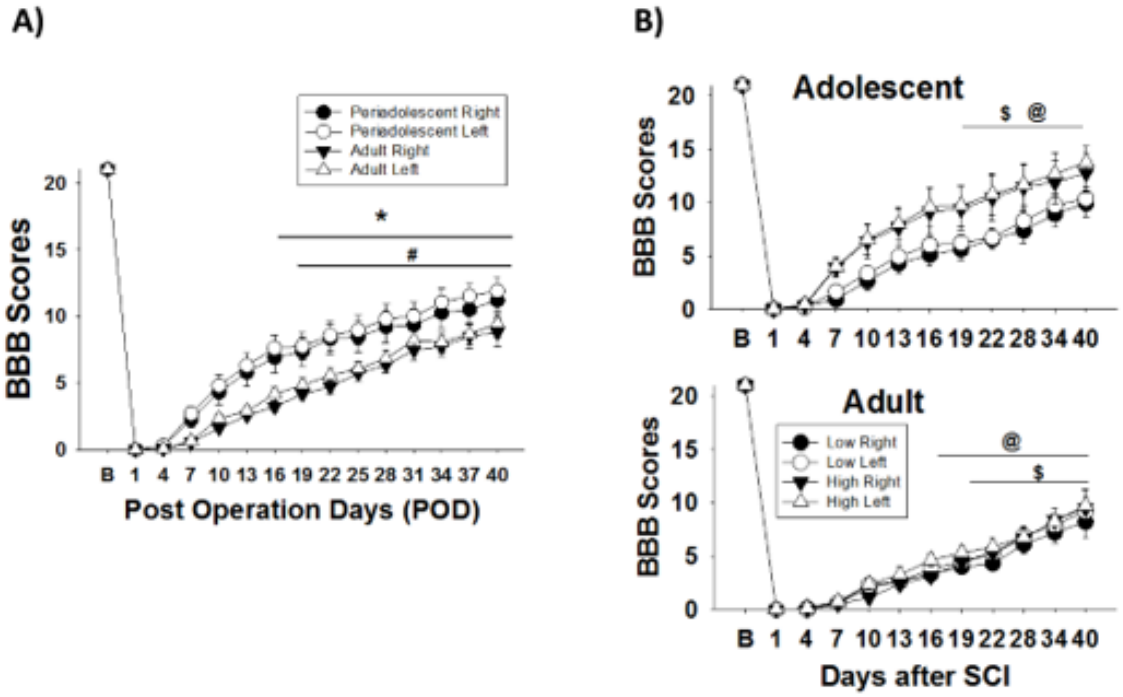

Figure 3. Locomotion dysfunction following spinal cord contusion injury. (A) One day after the contusion injury, both age groups exhibited complete loss of locomotion, with a BBB score of 0. A significant recovery of BBB scores was observed in the periadolescent group on POD 16 for both hindlimbs ($^{*}p < 0.05$), while the adult group showed a significant increase on POD 19 ($^{\#}p < 0.05$). (B) Within the Displacement groups, the periadolescent group demonstrated significant recovery of BBB scores on POD 19 in both the Low Displacement ($^{@}p < 0.05$) and High Displacement ($^{\$}p < 0.05$) groups. The adult group with High Displacement exhibited faster recovery of BBB scores compared to the Low Displacement group (POD 16 vs. POD 19). However, no significant differences were observed between the Low and High Displacement groups within either age group. Sham groups consistently maintained BBB scores of 21 throughout the test period for both the periadolescent and adult groups (data not shown).

3.4 Bladder dysfunctions

Prior to SCI, the average bladder size in both the periadolescent and adult groups was approximately zero (**Figure 4A**). On POD 4, the periadolescent group exhibited a significant increase in bladder size (1.25 ± 0.16), which persisted until POD 10 ($^{*}p < 0.05$, **Figure 4A**). Similarly, the adult group showed a significant increase in bladder size on POD 1 (1.1 ± 0.18) that lasted until POD 10 ($^{\#}p < 0.05$, **Figure 4A**). On POD 7, bladder sizes peaked at 1.7 ± 0.1 ($^{*}p < 0.05$) in the periadolescent group and 2.4 ± 0.2 ($^{\#}p < 0.05$) in the adult group, with a significant difference between the two groups ($^{\&}p < 0.05$, **Figure 4B**). Despite these differences, both groups exhibited similar recovery patterns in bladder function over time. The sham groups did not exhibit any bladder dysfunction in either the periadolescent or adult groups (**Figure 4A**).

In the Displacement groups, Low and High Displacement groups in both the periadolescent and adult groups showed no significant differences in bladder dysfunction recovery (**Figure 4C**). However, the periadolescent group exhibited significant bladder dysfunction from POD 4 to POD 10 ($^{*}p < 0.05$), while the adult group displayed bladder dysfunction from POD 1 to POD 16 ($^{\#}p < 0.05$), indicating that the adult group experienced a faster onset of bladder dysfunction but a delayed recovery.

The average bladder size in the Low and High Displacement groups within the periadolescent group was 1.91 ± 0.1 and 1.56 ± 0.3 (POD 7 and POD 10), respectively. In the adult group, both Low and High Displacement groups showed bladder sizes of 2.4 ± 0.2 and 2.4 ± 0.3 on POD 7, indicating more severe bladder dysfunctions. Notably, the Low Displacement group in the adult group showed a significant difference compared to the periadolescent group, whereas the High Displacement groups did not display significant

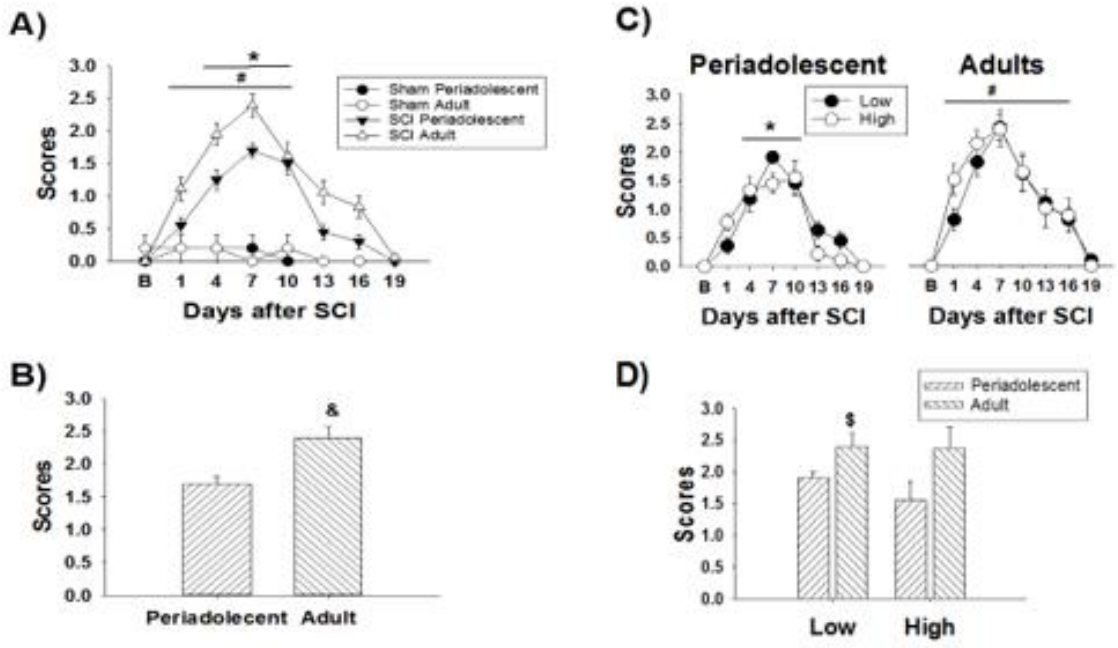

Figure 4. Bladder dysfunction following spinal contusion injury. (A) Both the periadolescent and adult groups exhibited a significant increase in bladder size on POD 4 ($^{*}p < 0.05$) and POD 1 ($^{\#}p < 0.05$) compared to pre-injury levels. However, both groups followed a similar recovery trajectory, returning to normal bladder size by POD 19, comparable to pre-injury levels. (B) On POD 7, both groups reached their peak bladder size, with the adult group showing a significantly larger bladder compared to the periadolescent group ($^{\&}p < 0.05$). Sham groups in both age categories did not exhibit any changes in bladder dysfunction throughout the experimental period. (C) In the Displacement groups, no significant differences in bladder dysfunction recovery were observed between Low and High Displacement groups within each age group. However, the adult group showed a delayed recovery of bladder dysfunction compared to the periadolescent group. (D) In the Low Displacement group, the adult group exhibited significantly greater bladder dysfunction compared to the periadolescent group ($^{\$}p < 0.05$). In contrast, the High Displacement groups did not show significant differences between the two age groups.

3.5 Mechanical allodynia and thermal hyperalgesia

Prior to SCI, the paw withdrawal thresholds (PWTs) to von Frey filament (VFF) stimulation in the periadolescent group were 14.3 ± 0.4 g (left hindpaw) and 14.0 ± 0.5 g (right hindpaw). Forty days post-SCI, the PWTs significantly decreased to 4.2 ± 0.6 g (left) and 4.4 ± 0.8 g (right) compared to pre-SCI values ($^{*}p < 0.05$, **Table 2**). Similarly, in the adult group, pre-SCI PWTs were 15.0 ± 0 g for both hindpaws, which decreased significantly to 6.9 ± 1.0 g (left) and 6.4 ± 0.7 g (right) on POD 40 ($^{\#}p < 0.05$, **Table 2**). Both groups showed no significant differences in PWTs between left and right hindpaws.

The average reduction in PWTs was 10.1 ± 0.6 g (left) and 9.6 ± 0.9 g (right) in the periadolescent group, compared to 8.1 ± 1.0 g (left) and 8.6 ± 0.7 g (right) in the adult group. However, there were no significant differences in PWT reduction between the two age groups. Sham-operated animals showed

In the Displacement groups:

1. Periadolescent group: Pre-SCI PWTs were 14.2 ± 0.6 g (both hindpaws) in the Low Displacement group and 13.7 ± 0.9 g (left) and 14.8 ± 0.5 g (right) in the High Displacement group. On POD 40, PWTs in the Low group significantly decreased to 4.2 ± 0.7 g ($^{*}p < 0.05$, left) and 3.7 ± 0.7 g ($^{*}p < 0.05$, right), while the High group showed reductions to 4.6 ± 1.4 g ($^{\#}p < 0.05$, left) and 4.8 ± 0.8 g ($^{\#}p < 0.05$, right) (**Table 2**).
2. Adult group: Pre-SCI PWTs in both Low and High Displacement groups were 15.0 ± 0 g for both hindpaws. On POD 40, PWTs in the Low group decreased to 5.9 ± 0.9 g ($^{*}p < 0.05$, left) and 6.4 ± 1.4 g ($^{*}p < 0.05$, right), while the High group showed reductions to 7.0 ± 1.1 g ($^{\#}p < 0.05$, left) and 7.6 ± 1.4 g ($^{\#}p < 0.05$, right) (**Table 2**).

The average reduction in PWTs for the Low Displacement group in the periadolescent group was 9.6 ± 0.9 g (left) and 10.1 ± 0.6 g (right), while in the High group, it was 9.2 ± 1.6 g (left) and 9.6 ± 0.9

g (right). For the adult group, the Low group showed reductions of 9.1 ± 0.9 g (left) and 8.9 ± 1.1 g (right), while the High group showed reductions of 7.9 ± 1.1 g (left) and 7.4 ± 1.4 g (right) (**Table 2**). Despite these differences, there was no significant difference between periadolescent and adult groups.

3.7 Thermal hyperalgesia

Prior to SCI, the paw withdrawal latencies (PWLs) to radiant heat stimulation in the periadolescent group were 15 ± 0.7 s (left hindpaw) and 16.9 ± 1.2 s (right hindpaw). Forty days post-SCI, the PWLs decreased to 13 ± 1 s (*$p < 0.05$) and 14.7 ± 0.9 s ($p = 0.195$), respectively, indicating a significant reduction only on the left side (**Table 3**).

In the adult group, pre-SCI PWLs were 16 ± 1.1 s (left) and 18.1 ± 1.1 s (right). On POD 40, PWLs were 16.7 ± 1 s (left) and 16 ± 0.9 s (right), with no significant differences observed compared to pre-SCI values (**Table 3**).

Table 2. Changes in paw withdrawal thresholds (PWTs) of hindpaws following spinal contusion injury.

Mechanical Allodynia (Age Group)					
	Sham			**SCI**	
Periadolescent	**Left (g)**	**Right (g)**	**Periadolescent**	**Left (g)**	**Right (g)**
Before	14.7 □ 0.3	15.0 □ 0	Before	14.3 □ 0.4	14.0 □ 0.5
After	14.7 □ 0.3	15.0 □ 0	After	4.2 □ 0.6*	4.4 □ 0.8*
Adult			**Adult**		
Before	15.0 □ 0	15.0 □ 0	Before	15.0 □ 0	15.0 □ 0
After	15.0 □ 0	14.7 □ 0.3	After	6.9 □ 1$^{\#}$	6.4 □ 0.7$^{\#}$
Mechanical Allodynia (SCI Displacement Group)					
Periadolescent	**Left (g)**	**Right (g)**	**Adult**	**Left (g)**	**Right (g)**
Low			**Low**		
Before	14.2 □ 0.6	14.2 □ 0.6	Before	15.0 □ 0	15.0 □ 0
After	4.2 □ 0.7*	3.7 □ 0.7*	After	5.9 □ 0.9*	6.4 □ 1.4*
High			**High**		
Before	13.7 □ 0.9	14.8 □ 0.5	Before	15.0 □ 0	15.0 □ 0
After	4.6 □ 1.4$^{\#}$	4.8 □ 0.8$^{\#}$	After	7.0 □ 1.1$^{\#}$	7.6 □ 1.4$^{\#}$

$*p < 0.05$: Significant difference compared to the sham group in the periadolescent group. $^{\#}p < 0.05$: Significant difference compared to the sham group in the adult group.

Table 3. Changes in paw withdrawal latencies (PWLs) of hindpaws following spinal contusion injury

Thermal Hyperalgesia (Age Group)					
	Sham			**SCI**	
Periadolescent	**Left (s)**	**Right (s)**	**Periadolescent**	**Left (s)**	**Right (s)**
Before	16.6 □ 1	14.5 □ 0.9	Before	15.0 □ 0.7	16.9 □ 1.2
After	14.3 □ 1.5	15.4 □ 1.3	After	13.0 □ 1*	14.7 □ 0.9
Adult			Adult		
Before	17.2 □ 1.2	18.1 □ 1.7	Before	16.0 □ 1.1	18.1 □ 1.1
After	19.2 □ 1.4	16.9 □ 1.5	After	16.7 □ 1	16.0 □ 0.9
Thermal Hyperalgesia (SCI Displacement Group)					
Periadolescent	**Left (s)**	**Right (s)**	**Adult**	**Left (s)**	**Right (s)**
Low			Low		
Before	17.6 □ 1.8	14.6 □ 1	Before	18.2 □ 1.5	14.9 □ 1.3
After	14.1 □ 1	13.6 □ 1.2	After	14.4 □ 0.9	16.6 □ 1.5
High			High		
Before	16.0 □ 1.6	15.5 □ 0.9	Before	18.0 □ 1.6	17.4 □ 1.7
After	15.4 □ 1.5	12.4 □ 1.6$^{\#}$	After	18.0 □ 1.6	16.8 □ 1.4

Paw withdrawal latencies (PWLs) were recorded in seconds as the time between the start of the radiant heat beam and the paw withdrawal response. An automatic photocell was used to measure this latency. Significant difference compared to the sham group in the periadolescent group (*$p < 0.05$). Significant difference compared to the sham group in the adult group ($^{\#}p < 0.05$).

The average differences in PWLs between pre- and post-SCI were 2 ± 1.2 s (left) and 2.2 ± 1.6 s (right) The average differences in PWLs between pre- and post-SCI were 2 ± 1.2 s (left) and 2.2 ± 1.6 s (right) in the periadolescent group, whereas the adult group exhibited changes of -0.6 ± 1.7 s (left) and 2.1 ± 1.4 s (right) (**Table 3**). No significant differences in PWLs were detected between periadolescent and adult groups or between sham-operated groups (**Table 3**).

In the Displacement groups:

1. Periadolescent group: Pre-SCI PWLs were 17.6 ± 1.8 s (left) and 14.6 ± 1 s (right) in the Low Displacement group and 16.0 ± 1.6 s (left) and 15.5 ± 0.9 s (right) in the High Displacement group. On POD 40, PWLs in the Low group decreased to 14.1 ± 1 s (left) and 13.6 ± 1.2 s (right), while the High group exhibited PWLs of 15.4 ± 1.5 s ($^{\#}p < 0.05$, left) and 12.4 ± 1.6 s (right), showing a significant difference (**Table 3**).

2. Adult group: Pre-SCI PWLs in both Low and High Displacement groups were 15 ± 0 g for both hindpaws. On POD 40, PWLs remained consistent with pre-SCI values in both displacement groups, with no significant differences observed (**Table 3**).

4. Discussion

This study demonstrated that spinal cord contusion injury produced consistent outcomes for Injury Force and Velocity, while Displacement exhibited greater variability. High Displacement was associated with more severe and delayed recovery of behavioral dysfunctions, highlighting its critical role in SCI outcomes. Previous studies have established a positive correlation between graded Injury Force and Velocity with biochemical and neuroanatomical changes, including lymphocyte infiltration, cystic degeneration, and extended spinal damage (Lam et al., 2014). However, in our study, a pre-set force of 150 kdyn and velocity from an 8 mm height produced reliable outcomes without significant variability. Additionally, a report indicating that velocity does not significantly influence spinal damage suggests that the impact of velocity may depend on the type of injury device and other associated parameters (Frantsuzov et al., 2023). This suggests that investigating a broader range of impact heights may be necessary to fully understand force- and velocity-dependent dysfunctions.

4.1Body weight changes

Both age and displacement significantly influenced body weight recovery after SCI. The lowest body weights occurred approximately one-week post-injury, with recovery to pre-SCI levels by three weeks, consistent with prior findings (Spann et al., 2017). This weight loss is likely linked to gastrointestinal dysfunction following SCI, as previously reported (Primeaux et al., 2007). High Displacement groups exhibited faster initial weight loss and delayed recovery compared to Low Displacement groups, with adults displaying more pronounced effects than periadolescents.

4.2 Recovery of locomotion

Age and displacement also impacted on locomotor recovery. The adult group exhibited delayed recovery compared to the periadolescent group, consistent with previous findings that reduced spontaneous axon sprouting and increased age-related oxidative stress, inflammation, and demyelination impede recovery (Taub and Woolf, 2024; Jaerve et al., 2011). Interestingly, adults in the High Displacement group showed faster locomotor recovery compared to the Low Displacement group. This finding may be explained by the removal of spared long ascending propriospinal neurons (LAPNs), which can impede recovery, as suggested by Shah et al. (2012) and Shepard et al. (2021).

4.3 Bladder dysfunction

Both age and displacement influenced bladder dysfunction recovery. Peak dysfunction occurred on POD 7, with a return to normal function between two- and three-weeks post-injury. These results align with studies indicating that CRF and serotonin levels in the lumbosacral spinal cord are critical for bladder recovery (Hayashi et al., 2010; Studeny and Vizzard, 2005). Adult rats experienced faster onset and delayed recovery of bladder dysfunction compared to periadolescents, likely reflecting age-related differences in bladder control mechanisms.

4.4 Mechanical allodynia and thermal hyperalgesia

SCI induced significant mechanical hypersensitivity in all injury groups, with both periadolescent and adult groups showing an approximate 8 g reduction

in PWTs. This finding highlights mechanical allodynia as consistent outcome of SCI. In contrast, thermal hyperalgesia was only significant in the periadolescent group. Previous studies suggest that detecting significant differences in thermal sensitivity may require larger sample sizes or younger animals (Kim et al., 2014). Taken together, these findings suggest that mechanical allodynia is a more robust indicator of SCI-induced sensory dysfunction than thermal hyperalgesia, which appears more influenced by age and experimental design. The consistent induction of mechanical allodynia across all groups underscores its importance in evaluating SCI outcomes.

5. Conclusions

This work highlights the importance of aging and spinal displacement as critical factors in evaluating behavioral dysfunctions following SCI. The first-week post-injury is identified as a pivotal window for assessing and addressing behavioral dysfunctions, emphasizing the need for timely and acute interventions. Reducing variability in spinal displacement through precise vertebral stabilization and consistent contusion positioning is vital for enhancing experimental reliability and reproducibility. Furthermore, age-specific considerations should be integrated into the design of SCI studies to optimize therapeutic strategies for addressing behavioral and physiological dysfunctions in rodent models.

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JK analyzed the data and drafted the manuscript. YSG performed the experiments and revised the manuscript. Both authors read and approved the final version of the manuscript for submission.

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The authors have no conflicts of interest to declare

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