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Locomotor recovery and mechanical hyperalgesia following spinal cord injury depend on age at time of injury in rat

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Abstract

We tested the effect of age at the time of spinal cord injury (SCI) on locomotor recovery, in open field tests, and mechanical hyperalgesia, using paw withdrawal frequency (PWF) in response to noxious mechanical stimuli, in male Sprague–Dawley rats after spinal hemisection at T13 in young (40 days), adult (60 days) and middle-age (1 year) groups. Behavioral outcomes were measured weekly for 4 weeks in both SCI and sham groups. Following SCI, the young and adult groups recovered significantly more locomotor function, at a more rapid rate, than did the middle-age group. The PWF of the young group was significantly increased, the adult group was significantly decreased, and the middle-age group showed no significant change in fore- and hindlimbs when compared to other age groups, pre-injury and sham controls. These results support age-dependent behavioral outcomes after SCI.

Keywords: Age; Paw withdrawal frequency; Hemisection; Locomotor; Spinal cord injury

Following spinal cord injury (SCI), central neuropathic pain-like behavior, such as allodynia (non-noxious stimulus becomes noxious) and hyperalgesia (noxious stimulus becomes more noxious), appears to be influenced by exogenous factors such as the injury method, location, and intensity [15] and may be influenced by endogenous factors such as sex, age, diet, and genetic differences [6,16]. Among those factors, the age at the time of SCI may be an important determinant in the development of behavioral outcomes such as degree of locomotor recovery and development and maintenance of chronic central pain, perhaps because the localization of pattern generators, synaptic efficacy and organization, and the distribution of spinal neural circuits vary with age [11,13]. Previously, we observed that animals spinally injured at 1 month of age appeared to develop more robust cutaneous mechanical hyperalgesia than those a few weeks older or months older. Thus, we hypothesized that behavioral outcomes following SCI may be affected by age at the time of SCI. In order to rigorously test the relationship between behavioral outcomes (locomotor recovery and mechanical hyperalgesia) and the effect of age at the time of SCI, we measured open field locomotion skills and responses to cutaneous noxious mechanical stimuli applied to the paw in three different age groups of rats following spinal cord hemisection.

Male Sprague–Dawley rats were divided into three age groups depending on reproductive competency: young (164.6 ± 5.46 g, 40 days, $n = 7$), adult (273.2 ± 8.09 g, 60 days, $n = 8$), and middle-age (546 ± 12 g, 12 months, $n = 8$). The rats were obtained from Harlan Sprague-Dawley, Inc., housed with a light/dark cycle of 12/12 h, and fed ad libitum. In relation to reproductive competency, the young group are rats that are 'juvenile and pre-puberty', the adult group are rats that demonstrate the presence of mature spermatozoa in the vas deferens, and the middle-age group are rats that are in the end period of spermatozoa production [5]. Experimental procedures were reviewed by the UTMB Animal Care and Use Committee and were consistent with the NIH Guide for the Care and Use of Laboratory Animals. Spinal hemisection was induced by transverse unilateral cut of the spinal cord at T13 [8]. Under general anesthesia (sodium pentobarbital, 50 mg/kg, i.p.), the spinal cord was exposed and hemisected just cranial to the L1 dorsal root

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entry zone with a #11 scalpel, taking care to avoid major dorsal vessels or vascular branches. To ensure complete unilateral hemisection, a tuberculin syringe, with 28 gauge needle, was placed dorsoventral at the midline of the cord and the syringe was pulled laterally. The hemisection lesion was confirmed histologically post-sacrifice as unilateral and included the dorsal column, Lissaur's tract, lateral and ventral column, and gray matter. Additionally, in a unilateral hemisection, the ipsilateral hindlimb is initially paralyzed while the contralateral limb is unimpaired. Animals with contralateral hindlimb involvement, a demonstration of over hemisection or ischemic complications, were excluded. The ipsilateral limb partially recovers by 2 weeks [3]. To ensure unilateral hemisection and the ability to measure paw withdrawal response, locomotor function recovery was evaluated using open field locomotor assessment with the Basso, Beattie, and Bresnahan (BBB) open field test scale as described elsewhere [3]. BBB scores were measured prior to injury (before) and on post-operation day (POD) 1, 7, 14, 21, and 28. Only the scores from the hindlimb on the hemisected side (ipsilateral side) were reported since there were no observable differences in locomotor function for any other limbs. To examine behavioral outcome following SCI, paw withdrawal frequency (PWF) in response to repeated mechanical stimuli (ten trials, 0.2 Hz) was measured using a noxious intensity of mechanical stimulus (204.14 mN, Von Frey filament) to the glabrous surface of the forelimbs and hindlimbs. In this test, PWF was quantified by measuring the frequency of brisk paw withdrawals in response to the normally noxious mechanical stimuli, an accepted measure for cutaneous mechanical hyperalgesia [15]. The PWF was measured prior to injury and at POD 7, 14, 21, and 28. Data are expressed as the change in percentage of PWF which were calculated as the percentage of paw withdrawals before injury subtracted from the percentage of paw withdrawals after injury. The positive % of mean PWF indicates an increased number of withdrawals (hyperalgesic behavior), whereas the negative % indicates a decreased number of withdrawals (hypoalgesic behavior). The PWF scores of both ipsilateral and contralateral sides were combined because there was no significant difference between sides. Statistical analysis of mechanical responses was performed using the one-way (for comparisons between pre-injury and post-injury within each group) or two-way (for comparisons between groups) analysis of variance (ANOVA) with repeated measures over time as a factor followed by Duncan's test for multiple comparisons, using the SAS program (version 8.0). An alpha level of significance was set at 0.05 for all statistical tests. Data are expressed as means ± standard error (mean ± SE).

Prior to hemisection, BBB scores were 21 in all three SCI age groups: young, adult and middle-age. After hemisection, the ipsilateral hindlimb BBB scores at POD 1 were 0 in all three SCI age groups, whereas the three other limbs and the sham control scores were at 21. However, by POD 7, the ipsilateral hindlimb BBB scores of the young and adult groups were 11.29 ± 1.84 and 11.5 ± 2.06, significantly greater than the scores of the middle-age group at 1.63 ± 0.56 ($P < 0.05$, Fig. 1). The significant differences in locomotor recovery persisted over the entire test period; however, the BBB scores did not recover to pre-surgical values in any SCI group. The mean PWFs for the forelimbs on POD 28 were significantly increased in the young group to 35 ± 7.5%, whereas the adult group displayed significantly decreased PWF, −16.3 ± 5.5%, when compared to pre-injury and sham control values (Fig. 2A, $P < 0.05$). However, the mean PWF of the middle-age group following SCI and those of the age-matched sham control groups did not display a significant change. In addition, it should be noted that the mean PWF of the young group (35 ± 7.5%) was significantly higher than the mean PWF of the adult (−16.3 ± 5.5%) and middle-age (−8.1 ± 7.5%) groups, respectively ($P < 0.05$). In the hindlimbs, the mean PWF at POD 28 of the adult group was significantly decreased to −43.1 ± 6.4% when compared to baseline and sham control values (Fig. 2B, $P < 0.05$), whereas the young and middle-age groups did not display a significant change. In addition, in between group comparisons, the mean PWF at POD 28 of the adult group, −43.1 ± 6.4%, was significantly lower than scores of the young and middle-age groups at 9.3 ± 5.8% and −3.1 ± 3.1%, respectively (Fig. 2B, $P < 0.05$). However, sham controls did not display a significant change between groups.

Our present data demonstrate that younger rats displayed faster locomotor recovery and greater hyperalgesic behavior following SCI than adult and middle-age rats. Although we do not know the mechanisms that are responsible, we

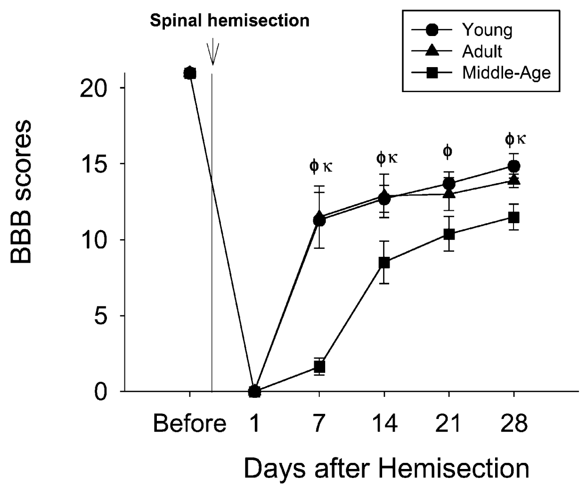

Fig. 1. Time courses of locomotor recovery of ipsilateral hindlimbs following spinal hemisection among the three different SCI age groups of rats. From POD 7 to 28, the young (circle, $n = 7$) and adult (triangle, $n = 8$) groups displayed significantly increased locomotor recoveries compared to the middle-age (square, $n = 8$) group. However, the BBB scores of all groups did not recover to pre-surgical values. Before: BBB scores prior to injury. ϕ and κ indicate significantly different BBB scores of the young and adult groups from corresponding scores of the middle-age group ($P < 0.05$). Data are expressed as means ± SEs.

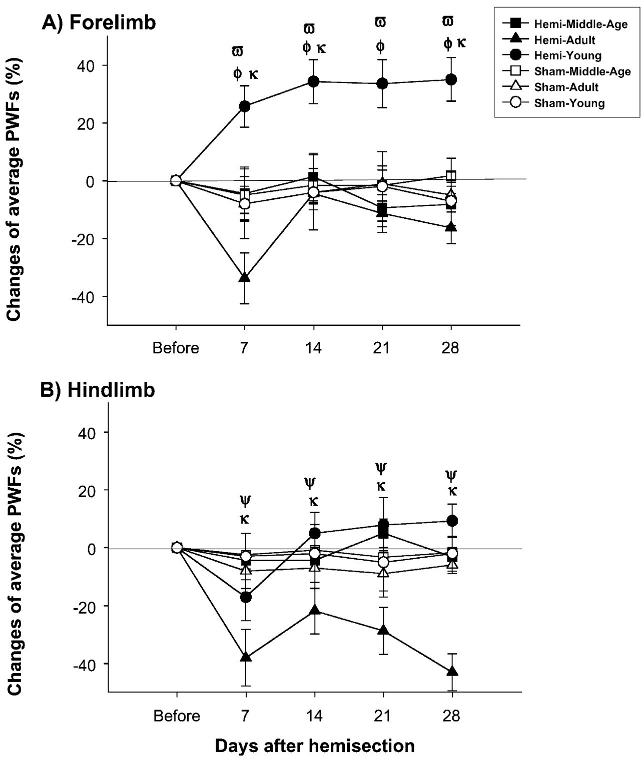

Fig. 2. Paw withdrawal responses to mechanical stimuli of 204.14 mN on both forelimbs (A) and hindlimbs (B) among the three different SCI age groups of rats with spinal hemisection (hemi-) or sham (sham) surgeries. The mean ± SE PWF (%) of the hemi-young group (filled circle) displayed significantly increased responses in forelimbs (A), whereas the hemi-adult group (filled triangle) displayed significantly decreased PWF in both limbs compared to pre-surgical values (A,B). However, the hemi-middle-age group and all sham groups displayed no significant changes compared to pre-surgical values. PWFs are combined scores of both ipsilateral and contralateral sides of both forelimbs and hindlimbs. Data are expressed as percentage change of PWFs from baseline (B), which was recorded before surgery, as means ± SE. Before: the mean PWF prior to injury. ϕ and κ indicate significant difference between pre-injury and post-injury of young (ϕ) and adult (κ) groups. ϖ indicates a significant difference between young and both adult and middle-age groups. ψ indicates a significant difference between adult and both young and middle-age groups ($P < 0.05$).

speculate that ontological differences in neural circuits are partly responsible. For example, the recovery of locomotor function after SCI is improved by both activation of descending monoamine pathways [1,2,19] and spontaneous exercise with physical activities [17]. Any disruption of the monoamine pathways (dopamine, noradrenaline and serotonin) following spinal hemisection would be expected to result in alterations in locomotor function. Age-related differences of locomotor recovery may be due to known age-related differences in the monoamine system, motor activity level (young > adult), other enzymatic levels, and the ability to sprout and form new neural connections. For example, the activity of enzymes such as tyrosine hydroxylase (rate limiting enzyme in the production of dopamine) and dopamine decarboxylase (enzyme in the conversion of dopamine to noradrenaline) is reported to decrease with age [14,18]. In addition, monoamine levels are lower in middle-age rats compared to young rats [7,10,12]. Future studies to determine monoamine age-dependent contributions to recovery by use of monoamine blockers would be interesting to pursue [1].

The pattern of PWF in response to noxious punctate stimuli displayed a surprising trend with age; younger rats displayed increased withdrawal responses (hyperalgesia) in forelimbs whereas adult rats displayed decreased withdrawal responses (hypoalgesia) in both forelimbs and hindlimbs. The differences in somatosensory behavior following SCI may be influenced by the degree to which the sensory system can respond to the same injury, minor sensory impairment (allodynia and/or hyperalgesia) and major sensory deficits (hypoalgesia) [4]. The somatosensory compensatory response to injury appears to be age-dependent. In addition, noxious mechanical stimulation is dependent on the properties of C- and A-fiber nociceptors and their projection circuits [20]. Thus, our data suggest reduced C- and A-fiber nociceptor input in the middle-age group before and after SCI. Spinal hemisection appears to result in diminished C- and A-fiber nociceptor processing in the adult group whereas C- and A-fiber nociceptor processing in the young group appears to increase, presumably due to sprouting of fine primary afferents which is known to occur in young but not older rats [9]. These results suggest that the change of behavioral output following spinal hemisection differentially develops with advancing age.

In summary, our data indicate that behavioral outcomes such as locomotor recovery and development of cutaneous hyperalgesia differentially develop and are dependent on the age at the time of SCI, with younger rats displaying better locomotor recovery and more robust neuropathic pain-like behaviors than older rats. Thus, age at time of injury may be a risk factor for developing mechanical hyperalgesia after SCI.

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