

RESEARCH ARTICLE

Influence of Sexuality in Functional Recovery after Spinal Cord Injury in Rats

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Abstract

Background: Spinal cord injury (SCI) is a major clinical condition and research is commonly done to find suitable treatment options. However, there are some degrees of spontaneous recovery after SCI and gender is said to be a contributing factor in recovery, but this is controversial. This study was done to compare the effects of sexual dimorphism on spontaneous recovery after spinal cord injury in Wistar Rats.

Methods: Spinal cord lesions were made by compressing the cord at T9 level and making a spinal cord contusion. Routine care of each rat was done daily. The LSS scoring system was used to measure the locomotion of these rats and to compare the recovery rate between male and female rats.

Results: The results suggested that there was no significant difference between the two sex in recovery.

Conclusions: To be female does not seem to be a prognostic factor for recovery after SCI. However, this preliminary study should be repeated in other animals and in larger cohorts.

Keyword: Gender, Rat, Spinal cord injury, Spontaneous recovery

Introduction

Spinal Cord Injury (SCI) belongs to the many exciting vast debilitating conditions and most of its victims are young in age. It is estimated that there are annually 130,000 new SCI cases in the United States (1). Along with the burden of the disease on the patients, relatives and governments must also endure the many related difficulties of caring for patients with SCI, which is known as a not-treatable disease that results in physical, emotional, and financial damage.

Efforts for treating the disease have yielded several issues in animal modeling and surveys on factors influencing the results of trials. One important factor in assessing the disease is the gender difference in spontaneous recovery after SCI (2-6). Data on this issue is controversial and some suggest that the female sex poses some opportunities to recover in higher degrees than their male counterpart (7, 8). Although this is not a constant finding and some authors did not find such a

relationship in their research (4, 9, 10).

Animal care for models of spinal cord injury, on the other hand, is arduous and these animals need more than usual care needed for any research, because they lack neurological function below the level of injury to the cord. In this way, bladder function is lost in these animals, and so the routine daily care of these animals includes manual bladder emptying using the Crede's Maneuver. This Maneuver includes compressing the bladder by hand so it becomes empty; however, the maneuver is easier to accomplish in female animals, because of shorter urethra in their bodies. For this reason, trends in performing experimental trials in the SCI field are toward female animals, and especially female rats (11). As mentioned above, there is suspicion about a higher degree of recovery in female rats after SCI, and the results about recovery in these experimental trials should be compared with male rats to ensure that selecting female rats for performing trials do not result in selection bias.

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In this study, functional recovery after SCI was compared in the two sexes of rats to have basic data on recovery so that further trials and interventions could be done.

Materials and Methods

Animal modeling: A total number of 50 Wistar rats were included in this study: 25 male and 25 female rats with a weight range of 250-300 grams and of the same age. The animals were kept in an animal room with a temperature of 22 ± 2 °C and day-night intervals of 12 hours. They were kept free to reach food and water and in groups of three to four rats in each cage.

Surgery and SCI induction: The surgery was performed under general anesthesia by a cocktail of Ketamine and Xylazine (60 mg/kg and 5 mg/kg, respectively). The hair on the back of the animals in the thoracic region was shaved and the skin was prepped by Povidone iodine. After draping the skin, a midline incision was made over the lower thoracic vertebrae. The fascia was opened sharply and the muscles over the T9 and T10 vertebrae were shaved. The inter-spinous ligament between these two vertebrae was identified and opened. Using clamps, the spinous process of the lower vertebra was kept so that the spinal column could take a kyphotic alignment and the interspace between the two vertebrae was widened.

Injury to the cord was induced by advancing the tip of a probe with two millimeters in diameter and compressing the cord for 30 seconds. The probe was removed and this compression was repeated after one minute.

The surgical site was washed thoroughly and the wound was closed in separate anatomic layers by simple sutures. The animals were kept in room temperature until complete recovery and then placed in their cages.

Animal care: All the animals took antibiotic injections for three continuous days after surgery (Ceftriaxone; 14 mg/kg and Cloxacillin; 25 mg/kg). The bladder was controlled at every daily care giving and emptied using the Crede maneuver until it become spastic. Rats with spinal cord injury harbor a strange complication in which they begin to eat their own feet, namely autophagia or self-mutilation. This condition results in loosing animals not only because of wound and infection, but also inability to assess the function of the mutilated lower limb. We, as described by Rahimi-Moavaghar, used saturated Picric acid to overcome and prevent this problem (12).

Assessment of function: There are many scales to assess the function after SCI in rats and the BBB score is the most familiar one among the researchers, but this

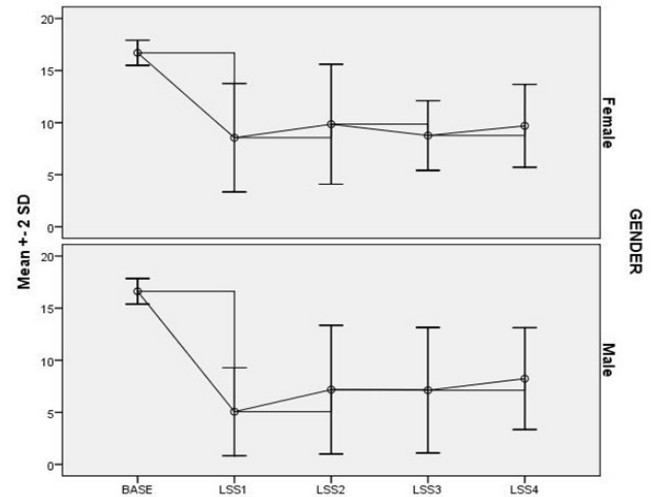


Figure 1. LSS Score in Male and Female Rats ($P > 0.05$).

test is observer dependent and could produce unequal scores among the different observers (13, 14). The other test in this regard is LSS (Losville Swim Scale) that is more documented and is based on the swimming skill of the rats and gives a number between 0 and 17 to the records so it could be used during the study (15). Hence, the LSS score of each rat was recorded the day before the surgery as the basal score, and again, it was gained at weeks: one, three, five, and seven after the surgery.

Data analysis: Data processing was performed by the SPSS 22 for Windows (SPSS, Inc., Chicago, IL). Average values were presented as mean \pm standard deviation, and a P value of < 0.05 was considered statistically significant. Characteristics between the two experimental groups were compared by using the unpaired student's t test for parametric data and by using the Pearson's test for categorical data.

Results

There were 19 rats that survived until the end of the study in each group. The LSS scores of the two groups are shown in Table 1. Data analysis revealed that functional recovery after SCI does not differ significantly between male and female rats [Table 1; Figure 1].

The results suggest that the spinal cord could have plasticity to recover after SCI, but it never reaches normal levels (16). This recovery reached a plateau on day 21 after injury and no significant recovery happened on later surveys. This is consistent with findings from

Table 1. LSS Score of male and female rats (Mean \pm SD)

	BASE	LSS1	LSS2	LSS3	LSS4
MALE	16.63 \pm 0.48	5.39 \pm 2.48	7.16 \pm 2.91	7.88 \pm 2.67	7.22 \pm 2.87
FEMALE	16.55 \pm 0.56	8.34 \pm 2.56	9.61 \pm 2.47	9.82 \pm 2.22	10.37 \pm 1.9

BASE: LSS Score before the surgery, LSS 1: The LSS Score 1 week after surgery, LSS 2: The LSS Score 3 weeks after surgery, LSS 3: The LSS Score 5 weeks after surgery, LSS 4: The LSS Score 7 weeks after surgery

other studies (16).

Discussion

Many studies compared the effects of sexual dimorphism on recovery after SCI (2-5, 7, 8, 17-19). Basic researches have shown that estrogen and progesterone could have neuro-protective effects (20-27). It has also been shown that sex hormones are effective in relieving inflammation of CNS after Multiple Sclerosis (28). Accordingly, although MS is more prevalent among women, exacerbation attacks are more severe and debilitating in men (29). Experimental trials have shown that progesterone results in diminishing brain edema, improving function, and minimizing secondary neuron loss (25, 30).

Recent studies suggest that estrogen and progesterone could improve functional recovery after injury to the spinal cord and brain (26, 27, 31-35). Anti-inflammatory effects, increasing blood supply to damaged tissue, enhancing BCL-2 (anti-apoptosis), and lowering intracellular calcium are some of the proposed mechanisms for this neuro-protection (33).

Studies about the effects of sexual dimorphism on recovery after SCI have had controversial results. Some studies showed that female rats could have better recovery than the male (7) however, this relationship has not been shown in other studies (9).

Studies on humans, however, did not show any difference in recovery after SCI between men and women (5, 6). Fisher et al. did not show any relationship between sexual dimorphism and recovery in patients with complete quadriplegia (10). One study showed that men could have better outcomes than females with injuries on the same level and with the same severity (5). Sexuality, in general, does not seem to have an effect

in recovery after SCI, although there are significant differences regarding complications (4, 17).

This study did not show any difference in recovery after SCI in locomotion during 7 weeks and this is compatible with findings in other studies.

There was no difference in recovery after SCI between male and female rats in this study, which is similar to some other data in experimental and clinical trials. Further studies are needed to assess the effects of sexual dimorphism and related hormones in neuro-protection for recovery. Our study also shown that spontaneous recovery maximizes on day 21 after injury in rats.

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