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Calibrated behaviors assessment in spinal cord compression injury model in rats

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***Abstract*---**Spinal cord injuries (SCI) are often associated with dire consequences resulting in the serious pathological conditions in SCI patients. These consequences have resulted in an increased interest of basic science researchers and healthcare professionals to investigate effective treatment of SCI. Among these initiatives, animal experimentation models emerged as a successful tool to study the SCI in detail. We created a standardized spinal cord compression injury model for this research and tested its effectiveness in examining the behaviors of rodents with SCI. We used 24 mature Sprague Dawley rats, weighing between 250 and 320g. Two sets, A and B, of rats were created. Under general anesthesia, Rat from group A underwent laminectomy and only had the T6–T8 vertebrae of their spinal arch removed. in contrast to the rats in group B underwent laminectomy as well as compression injuries using an aneurysm clip of 70 gm for 60 seconds. All the rats were observed at day 7, 14 and 28 following the experimentation. Using standardised techniques, the sensory-motor

abilities were evaluated. such as the Von Frey strands test, the Basso, Beattie, Bresnahan (BBB) test, and cold sensation test, Hot plate test and the tail flip test on days 7, 21, and 28 after post procedure of surgery. Following surgical procedures, all 24 rats were alive. except the two which died post op 07 day in group B. The findings from both groups' results were quantitatively examined by using Mann Whitney U tests. Overall, the group A showed no neurological abnormalities, whereas the group B exhibited a clear neurological deficit. The study's goal was to create a realistic and accurate model of spinal cord trauma. The findings showed that group B, which underwent compression damage using a 70 g aneurysm clip, had a good success in achieving these goals.

***Keywords*---**Thoracic level compression, spinal cord injury, and compression of aneurysm clip.

Introduction

A debilitating neurological disease known as a spinal cord injury (SCI), it has a major societal and financial effect on those who suffer from it as well as the healthcare system. These injuries can occasionally produce abrupt, traumatic spine lesions, with vertebral fractures or dislocations being the most frequent causes of SCI (Alizadeh et al., 2019). The spine sustains a primary injury when it is broken, when a disc is displaced, when a ligament is strained, or when a section of the spinal nerve is torn. Over ninety percent of SCI cases are traumatic in character and result from violent crimes, athletics, and other incidents (Wagner et al., 2018). According to the National Spinal Cord Injury Statistical Centre, 12,500 new instances of SCI are reported each year in South America.

The number of men to women was calculated to be 2:1, and it was more common in adults than in infants. While women are more susceptible during adolescence, males were most impacted during puberty. Individuals in above the age of sixty with SCI decline SCI have more easily than their peers in their teens (Choi et al., 2020). There could be either a complete or partial impairment of sensory and/or muscle function beneath the damage level depending on the position and degree of the injury lesion. While neck lesions typically result in a condition known teraplagia as lower chest lesions can induce paraplegia (Aimetti et al., 2019). For more than half of the times, C5 is involved while the lumbar area (11%) and thorax level (35%), respectively, make up all SCI. Thanks to recent medical advancements, patients frequently recover from these excruciating wounds and go on to live a long life (Kang et al., 2018). The life expectancy of SCI patients is significantly influenced by the severity of the damage and the degree of preserved skills. For instance, the American spinal cord association (ASIA) Impairment Scale (AIS) states that patients in the grade-D category have a life expectation of 75% or less if they need a wheelchair for daily activities, compared to up to 90% for patients who do not (Sengupta et al., 2020).

Despite recent improvements in the treatment of SCI, there is presently no full recovery from spinal cord injury that can produce the intended results. However,

some animal studies carried out in the field of neuroscience have shown promising results (Karsy and Hawryluk, 2019). These animal studies were based on a number of SCI models, which have shown value not only for researching a drug's therapeutic effectiveness a range of curative agents but also for understanding molecular pathways involved in the process of healing and recovery of Spinal Cord Injury (Verstappen et al., 2022).

Rats, which are commonly used as laboratory animals, are now frequently used to predict various kinds of behavioral studies that evaluate the neural processes, particularly in injury models. Behavioral testing can prove modifications to the spinal cord physiology and neural function following a scheduled operation of SCI at the desired location. In order to assess the effects of SCI on various neural pathways and fasiculi of the sensory motor systems, fundamental studies are conducted after any medical intervention (Cregg et al., 2014).

Additionally, there are numerous methods to create a spinal cord injury model, but the most popular ones are compression models for rodents using calibrated forceps, clips, and balloons10. To determine the roles and histopathological outcomes at the thorax level, the clip compressing model is thought to be more comparable and accurate (Raineteau and Schwab, 2001). The non-transaction compressing model, for example, was developed in 1978 and has been widely used for rodent experiments (Hahmann and Schroeter, 2010, Riento and Ridley, 2003).

Researchers are always searching for models that better match real injuries. Until now many studies are conducted in which different models used are compression, contusion, distraction, dislocation and transaction models. Among them the most commonly used models are compression-stimulating impaction and contusion-simulating the bruise (Sharif-Alhoseini et al., 2017, von Euler et al., 1997, Gruner et al., 1996).

The compression models include prolonged cord compression, whereas contusion models are created by placing a load on the spinal cord. This is the main distinction between the two models. Some of these models are merged to create contusion-compression models, where the cord becomes compressed for a considerable amount of time following a significant collision. This particular SCI model closely resembles the SCI process seen in fracture dislocations and rupture fractures (All and Al-Nashash, 2021). Numerous compression models, such as clip compression as well, balloon compression, and spinal column strapping methods, have been created for researching SCI (Forgione et al., 2017, Paterniti et al., 2018).

The present experiment's objective was to create rat SCI compression model, which can be utilized to not only calibrate the behavioural aspects of CSI but can also be used to validate the histopathological findings occurring in a SCI compression model in the future studies.

Materials and Method

The goal of the present research was to develop a model of animals for investigating the mechanisms underlying spinal cord damage and its behavioural effects. After receiving permission from Khyber Medical University's ethical committee in Peshawar, the experimental research was carried out. twenty-four healthy adult Sprague Dawley rats (SD rats) measuring 250–300 gm and averaging 8–10 weeks of age were included in our research; however, any diseased or unwell animals, juvenile rats (rats less than 8 weeks of age), and elderly (rats older than 1 year of age) rats were removed. All of the rodents were split into categories A and B. Laminectomy was performed on the animals in group A, but there was no constriction. The Dural sac at T7 level, which contains the spinal cord and was made visible by laminectomy of the T6 or T8 spine, was subjected to an aneurysm clip with a 70 g closure force on the group B animals for a duration of one minute. The clip was removed at the conclusion of the operation, and operative sealing of the wound was carried out in stages with silk as shown in Fig (1).

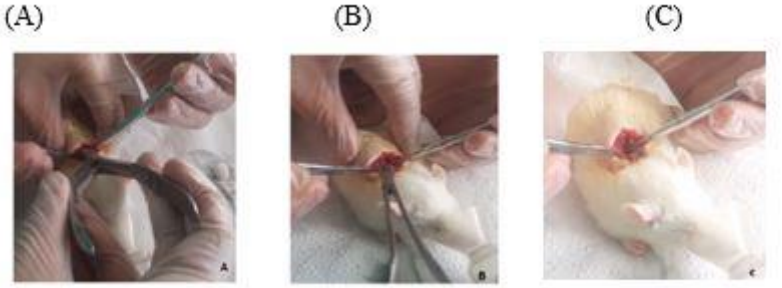

Figure 1. (A) Laminectomy of the T6-T8 vertebrae; (B) & (C)Compression injury model using aneurysm clips

Following surgery, the attitude assessments were administered on days 7, 14, and 28. A sensory and locomotor shift study was part of the behaviour evaluation. The methodological framework used for behaviour assessment is shown in Fig (2).

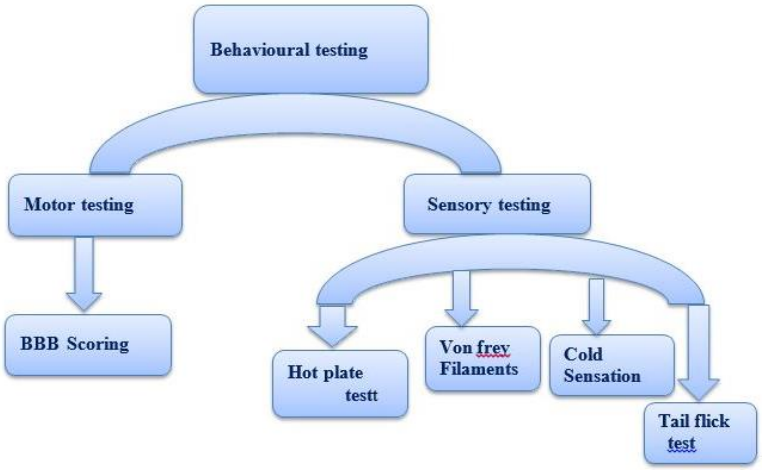

Figure 2. Methodological framework used for behaviour assessment

Statistical analysis

The mean as well as the standard deviation were calculated using descriptive statistical methods. The test known as the Mann-Whitney U test was employed to analyze continuous data within each group for statistical inference.

Results

Recovery of rats

Every single one of the experiment's animals recovered from their laminectomy, but two rats in group B passed away after surgery after seven days. Rats in groups A and B were killed at various times after surviving post-operatively for 28 days. Four rodents from group B got retained urine. Each rodent received a twice-a-day bladder massage (Crédé's manoeuvre) (Linsenmeyer et al., 2006) until they resumed their usual behaviour. Later, even in the seriously paralysed or tetraplegic rodents, the urge to void returned. On the seventh following surgery day, the rodents' incisional locations had fully recovered.

Behavioral results

In comparison to the injured animals in group B, the rodents in group A demonstrated positive behavioural outcomes. The sensorimotor exam was used to analyse the behaviour processes (Tarlov and Klinger, 1954).

BBB Scoring

6 hours after operation, the rodents in group A had BBB scores of 21 points, meaning there were no neurological problems because the anaesthetic impact had worn off (Basso et al., 1995). On the seventh day after surgery, two rodents with compression injury (70 g compressing force) perished in captivity (BBB score 0). Following operation, the compression group rodents either developed

quadriplegia or had their hamstrings severed (BBB scores varying from zero to sixteen points with a mean value at various periods, i.e. 2, 7 and 12 points, respectively).

Hot Plate Test

The results of the heated plate test on medullar and brain responses were used. Significant effects of procedures among the two groups showed marked difference in the mean between group A and B. (sig.=1.3 and 1.8 respectively). Correspondingly, the hot plate test in group B showed functional defects when compared with group A (Fig. 3&4).

Von frey filaments

According to the Von Frey analyses, there has been a noticeable increase in sensory processes. Tables show the behavioural results reflected as Von Frey filament values. (1 to 3). Using the test known as the Mann-Whitney U test, significant variations were discovered at all survivor periods longer than 14 days after the SCI.

Cold Sensation Test

This test's results revealed the chilly responses. At the restricted area, observations of acetone vaporisation were made after 30, 60, and 90 minutes. In group B, but not in group A, there was a substantial decrease in the amount of time required to react to acetone. Statistically it indicates no significant effects of the difference in different time intervals as shown in the tables (1 to 3).

Tail Flick test

The result of this test was used to gauge the medullary responses' temperature sensitivity. At various points during behavioural tests, group A rodents displayed more tail flick responses than group B. Using the Mann-Whitney U test, the results indicate that the lesion has no statistically meaningful impact on the data, but the time periods do not i.e. Mann Whitney U teat is same in 7 and 14 post op days as compared to 28 post op day. Therefore, the tail flick experiments revealed that the compression group B had a reduced capacity to react to thermal stimulation.

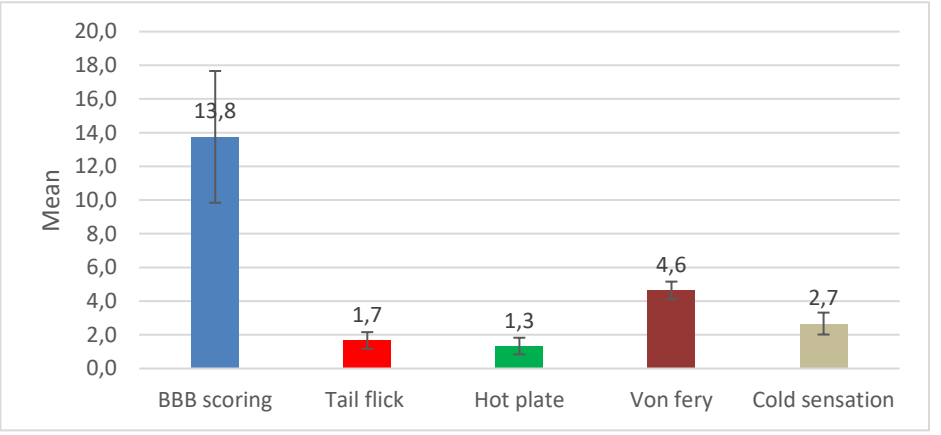

Figure 3. Mean and Standard deviation of the sensorimotor test for Group A

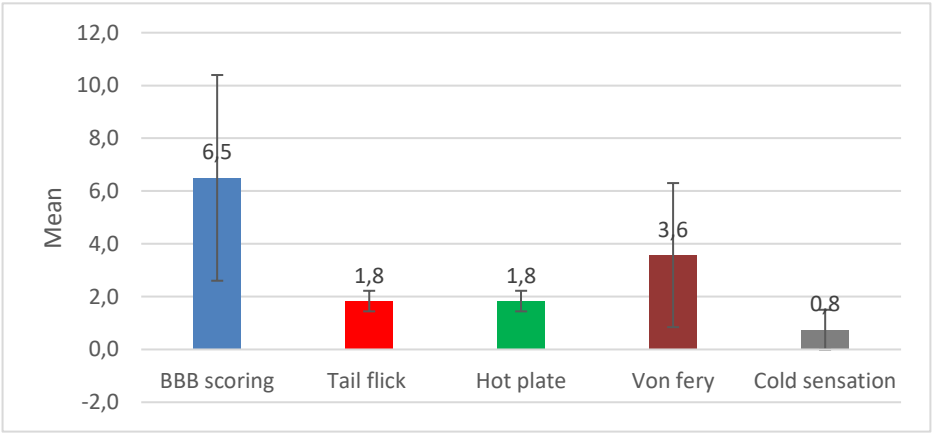

Figure 4. Mean and Standard deviation of the sensorimotor test for Group B

Table 1. Group's comparison for interval of 7 days

Variable	Intervals	N	Mean Rank	Mann-Whitney U	Sig.
BBB scoring	7 days A	4	6.50	0.00	.020
	7 days B	4	2.50		
Tail flick test	7 days A	4	4.50	8.00	1.000
	7 days B	4	4.50		
Hot plate test	7 days A	4	3.50	4.00	.127
	7 days B	4	5.50		
Von fery test	7 days A	4	6.50	0.000	.014
	7 days B	4	2.50		
Cold sensation test	7 days A	4	6.50	0.000	.013
	7 days B	4	2.50		

Table 2. Group's comparison for intervals of 14 days

Variable	Intervals	N	Mean Rank	Mann-Whitney U	Sig.
BBB scoring	14 days A	4	6.50	0.000	.019
	14 days B	4	2.50		
Tail flick test	14 days A	4	4.50	8.000	1.000
	14 days B	4	4.50		
Hot plate test	14 days A	4	3.50	4.000	.127
	14 days B	4	5.50		
Von fery test	14 days A	4	2.50	0.000	.020
	14 days B	4	6.50		
Cold sensation test	14 days A	4	6.50	0.000	.011
	14 days B	4	2.50		

Table 3. Group's comparison for intervals of 28 days

Variable	Intervals	N	Mean Rank	Mann-Whitney U	Sig.
BBB scoring	28 days A	4	6.50	0.000	.019
	28 days B	4	2.50		
Tail flick test	28 days A	4	3.50	4.000	0.13
	28 days B	4	5.50		
Hot plate test	28 days A	4	3.50	4.000	0.13
	28 days B	4	5.50		
Von fery test	28 days A	4	4.75	7.000	0.76
	28 days B	4	4.25		
Cold sensation test	28 days A	4	6.50	0.000	0.01
	28 days B	4	2.50		

Figure 5. Several different behavioural tests, including the Hot Plate test, the Tail Flick test, and the BBB scoring device

Discussion

For the good of the patients, their confinement to a wheelchair and the numerous medical consequences for years or even for the rest of their lives due to the traumatic lesions of their spinal cords are denoted by more than ten years of study. In the majority of instances, the specified therapy and the medical services for patients with SCI were greeted with dissatisfaction (Dryden et al., 2005; Hejl et al., 2015). Continuous study studies and its advancement in this

area have led to the suggestion that SCI will ultimately be reversible and convincing (Tchvaloon et al., 2008).

The use of animal models in study is crucial for developing experimental strategies intended to restore functions lost as a result of SCI (Rowland et al., 2008). Rat experimental models are currently the most widely used due to their ease of availability, care, expense, accessibility, and the presence of an established functional analysis method (Silva et al., 2014).

By compressing the spinal cord using an aneurysm clip, forceps, or balloon compression working through the intact dural mater, the compression model in SCI is first presented. By varying the crushing force and time, this model can also be used to produce SCI in varying degrees (Stokes and Jakeman, 2002). Additionally, it can imitate the neuropathological states that people experience (Vanick et al., 2001).

The capacity to collect real, accurate, and practical behavioral data is the cornerstone of any SCI research. The 21-point BBB locomotor measure is the exam that is most useful for evaluating motor abilities following a SCI. The BBB score points reflect a particular collection of traits that rats with various degrees of spinal cord injury exhibit during spontaneous open field movement when using their hind limbs for locomotion 30. The evaluation of this exam will determine how the BBB score will be used in our research to compare the results between group A and group B.

The heated plate and chilly sensation tests were most responsive measures to reflex function for detecting of sensorimotor shortage following SCI. because the evaluation is straightforward, repeatable, and qualitative. The current research uses the tail flick and Von Frey filaments because they both demonstrated comparable feelings. The von Frey test reflects the supraspinous process of nociceptors, while the tail flick test also represents organised spinal responses.

The outcomes shown in sensorimotor tests are in accordance and shows reliable and cognitive results as conducted by the different researchers at different studies. Thus, the evaluation of rare limbs study event and evaluation findings offers a delicate sign of the imitate impact of SCI on various periods. The most accurate methods of detecting behavioural abnormalities brought on by surgery are random grooming, raising, and movement.

Conclusions

The use of laboratory rodents in SCI experiment models is generally recognised. For SCI research, they are commonly employed. This research supports the re-creatibility and reliability and sensitivity experiments in spinal cord compression model in rodents. The behavioural feature suggests that BBB and other sensory tests are comparable to medullary disease in both people and various experimental animals.

The compression model can demonstrate that a novel therapeutic approach is suitable for preclinical testing to regulate and restore the neuropathological

process involved in secondary injury mechanisms and to ameliorate its aftereffects in paralysed or quadriplegic patients. Successful clinical study reveals novel treatments for SCI, but these treatments require appropriate experimental designs. However, the 3Rs should be taken into account for morality, animal care, and the approval of its results (Hubrecht and Carter, 2019).

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