Evaluating the Relationship Between Locomotion Speed and Gait Patterns in SCI-Injured Rats

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***Abstract*—Analyzing changes in gait patterns is a common method for evaluating injury severity and progression during rehabilitation, including in rats with contusion spinal cord injury (SCI). This study aims to examine the correlation between gait parameters and locomotor speed in rats with contusion SCI using a newly developed walkway system. Thirteen female Wistar rats (7 uninjured and 6 injured) were selected for this experiment. All of them walked through a walkway and a camera below the walkway recorded their motion. Their gait and speed data were analyzed to provide insights into post-injury locomotion. The system output shows that injured rats exhibit slower speeds, shorter stride lengths, and wider stance widths compared to the uninjured, proving that their gait and speed of the injured rats were altered due to the injury. Additionally, Pearson correlation analysis revealed a strong positive correlation (r = 0.746) between speed and stride length for uninjured rats, while injured rats showed a moderate correlation (r = 0.471). Furthermore, stance width demonstrated a moderate negative correlation with speed for both uninjured (r = -0.503) and injured rats (r = -0.488). These relationships, although not statistically significant due to sample size limitations, suggest meaningful trends and provide a foundation for future research where the relationship between the speed and gait parameters can be used to further justify the condition of SCI in rats. Future studies with larger sample sizes might strengthen these findings and clarify the role of speed-related gait changes in SCI rehabilitation process.**

Keywords—gait parameters, locomotion speed, rat, spinal cord injury, walkway system

I. Introduction

Motor vehicle crashes and sports related accidents are among the leading causes for spinal cord injury (SCI) in Malaysia [1], [2], [3]. It negatively impacted both the individuals and their families [4]. Spinal cord serves as the means of transferring information between the central nervous system (CNS) and peripheral nervous system (PNS) [5]. Damage to the spinal cord, either partially or entirely, disrupts communication between the brain and body, leading to impaired limb movement and locomotion [6], [7].

Locomotion is one of the behaviors than can be used to understand the impact of SCI on human. By analyzing locomotion changes through gait analysis, researchers can gain insights in understanding the severity of the injury and the treatment progression a patient undergoes. For ethical reasons, scientists study on gait abnormalities due to SCI with valid animal substitutes that can replicate human biological traits, such as rats [8], [9]. Rats were chosen for this research because they are relatively easy to handle and exhibit similar responses to SCI as human [10].

From previously reported studies, gait analysis is an effective tool for assessing injury conditions in rats with SCI [11], [12]. Besides assessing injury severity, it can also be used in monitoring rehabilitation progress or recovery efforts, as reported from the studies by Zheng et al. and Guo et al. [13], [14]. Gait analysis needs specialized apparatus that is capable to capture and analyze them, such as the commercially available CatWalk system by Noldus. Due to cost constraints, this study built an affordable yet reliable walkway system. This walkway system records videos of the rats in motion and analyzes it to determine the gait parameters. From the parameters, conclusions about the injury condition of the rat can be drawn. SCI-injured rats adjust their gait to compensate for limitations and difficulties resulting from the injury. By monitoring these changes in gait patterns, the injury condition and progression can be assessed and provides meaningful information.

In addition to gait parameters, the locomotion speed of the injured rats will also be affected. However, most studies have focused on gait analysis and have not extensively explored on this aspect when reporting their findings. There are studies reported by Danner et. al which indicates that different injuries can lead to changes in rats' speed with variations depending on the type of injury, including contusion SCI [7]. This proves that speed can be another dimension that need to be investigated when analyzing gait parameters of SCI-injured rats. Hence this study also take speed into account in its analysis.

The primary objective of this study is to analyze changes in gait parameters, namely the stride lengths and stance width of the rats with contusion SCI using the newly developed walkway system and relate them with their locomotor speed. For comparisons, gait of healthy, uninjured rats was also analyzed. By investigating these parameters, the relationship between speed and gait in both groups can be explored.

II. Methodology

A. Animal Preparation

This study used 13 adults female Wistar rats weighing approximately around 200-250g each. They were randomly subjected to two groups: 7 uninjured and 6 injured. Access to food and water was given ad libitum. All rats were purchased from Laboratory Animal Facility and Management (LAFAM), UiTM and all procedures were approved by UiTM Committee on Animal Research and Ethics (UiTM CARE) (reference code2/2023/(412/2023). The injured rats were subjected to contusion SCI injury using NYU Impactor Device (WM Keck Center for Collaborative Neuroscience, USA), as shown in Fig. 1 below. Surgical procedures and animal care post-surgery was done delicately as outlined by Yusof et. al [15]. For 28 days post injury (dpi), the rats were closely monitored while performing the required tests. At the 28 dpi, they were all euthanized through overdose of carbon dioxide inhalation.

B. Walkway System

The walkway system records rat's gait data by capturing video footage from below the walkway and analyzing the parameters. The walkway was built using transparent acrylic with dimensions of 80mm (width) x 900mm (length) x 80mm (height) and 10mm thickness as shown in Fig. 2(a). The ceiling and floor of the walkway were equipped with controllable light emitting diodes (LED) strips to illuminate the rats' paws when walking on and to control the amount of light inside the walkway during test. Dark backdrops were used to enhance the paws from the background. The motion capture system for the walkway consists of a camera and a workstation. The camera is a GoPro Black 10 camera from GoPro Inc, while the workstation is a personal computer powered with 11th Gen. Intel Core i7. Frame frequency of 60 frames-per-second (fps) was chosen for the walkway test at it is the optimum condition to balance between processing time and to avoid image losses.

The captured videos were edited and sent for analysis using the Python code developed specifically for this task to track and calculate the gait parameters and the speed. Stride length measures the distance covered between the point of initial contact of a foot with the ground and the next contact of the same foot with the ground again. Stance width, on the other hand, is the average distance between left and right hindlimb during walking, while walking speed is the ratio of distance over time the rat took to walk from the first stride to the final stride inside the walkway. To identify and track the motion of the rat, a Python-based open-source software developed by Dr Mackenzie Mathis named DeepLabCut (DLC) was used. DLC is a software tool for marker-less pose estimation that can track body parts such as the paws without initially setting a physical marker [17], [18]. This makes the software suitable for dealing with active and small animals like the rats. Using this software, the location of the paws in each frame in the videos were identified and marked. The paws of the left hindlimb were selected to measure the rats' stride, while both hindlimbs were tracked for the stance width data. Fig. 2(b) displayed the captured frame from a video of an injured rat. From the image, the location of the hindpaw was identified and tracked for analysis. The hindlimbs were selected because they are among the body parts that receive significant impact from the contusion injury [13].

To identify which paw is touching the floor of the walkway at a particular time, each of the four paws were marked with different color thresholds by the DLC. The pixel location of the markers at both the left and right hindlimb of each frame was identified and recorded before the stride length, stance width and speed of the rats were calculated. From Fig. 2(c), if (x_b, y_b) is the coordinate for the centroid of a left hindlimb's paw at position b, and (x_{b+1}, y_{b+1}) as the coordinate for the same paw's subsequent position, then the stride length of the left hindlimb was calculated using (1). Subsequently, for the stance width, if (x_b, y_b) is the coordinate for the centroid of the left hindlimb's paw at position b, and (x_c, y_c) is the coordinate for the centroid of the right hindlimb at position c, then the stance width of the hindlimb of the rat was calculated using (2).

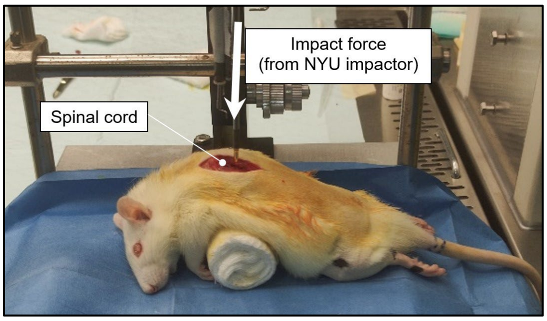
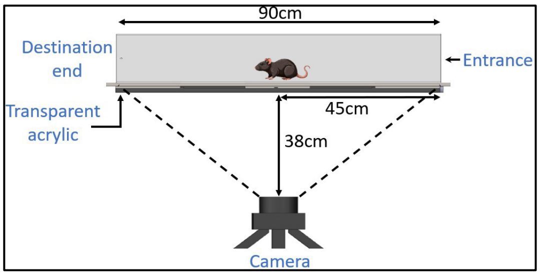
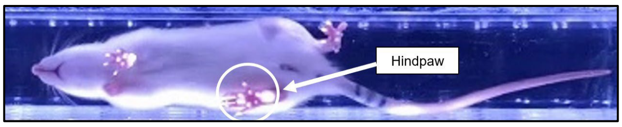


Fig. 1. Image depicts the NYU impactor delivering a 25mm drop onto the exposed T9/T10 vertebrae during surgical procedure. A successful contusion SCI can be visually confirmed through the jerking of the rat's hindlimbs when the impactor hits rat's spinal cord. [16].



(a)



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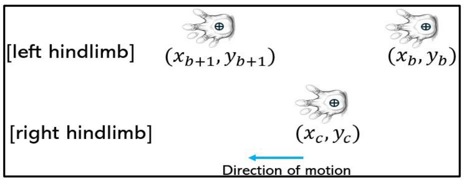


(c)

Fig. 2. (a) Setup of the walkway system during experiment. The camera was placed at 0.38m below the walkway for best view. Every time the paws touch the floor, light from the LED strips brightens up the rat's paw. (b) Camera view from the bottom of the walkway. (c) Definition of stride length and stance width.

$$SL = \sqrt{(x_{b+1} - x_b)^2 + (y_{b+1} - y_b)^2} \quad (1)$$

$$SW = y_c - y_b \quad (2)$$

The output of the walkway system (stride length of left hindlimb and stance width) was validated by comparing it to manual measurements obtained by identifying the location of each paws' placement in the respective frames by using ImageJ, a JAVA based image analysis software developed by United States National Institutes of Health [19]. The location of each paw was recorded, and the outputs were calculated. The Pearson correlation coefficient, r, for the stride length was obtained to be 0.995 and 0.990 for the uninjured and injured group, respectively. Similarly, for stance width, the r-values were found to be 0.991 and 0.941 for the uninjured and injured rats. These results demonstrate the reliability of the walkway system to be used in measuring gait parameters for rats in this study.

C. *Experimental Procedures*

From their cage, the rats were be released at the entrance of the walkway as marked in Fig. 2(a) and walked spontaneously through to the other end. During this event, the camera placed below the walkway will record rat's movement. After each test, the rats was transferred back to the respective cage and the walkway was cleaned with disinfectants before the next rat was released inside it.

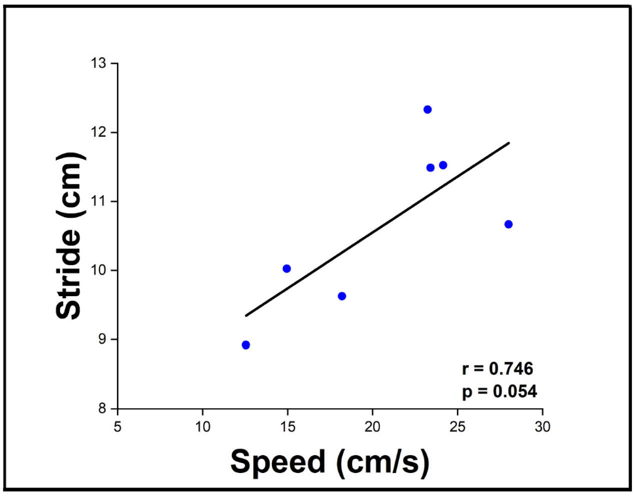
III. Results and Discussion

Table I displays the average speed, stride length and stance width of the uninjured and injured rats obtained during the period of this study. It is clearly seen that uninjured group exhibit faster average speed, longer average stride and shorter stance width when compared to the injured group. These are expected as they are showing similar results with what was observed in previous studies [7], [20]. Uninjured rats typically have longer stride lengths than the injured rats because they have better communication between the brain and the spinal cord, thus leading to a better coordination in their limb movement. Furthermore, in terms of the stance width, injured rats tend to have it wider to overcome their instability due to the SCI injury [20]. The differences recognised in Table I between the two groups indicate the injury is impacting their locomotion ability. The speed and gait parameters of the injured rats were altered to overcome the adjustments after the effective SCI injury.

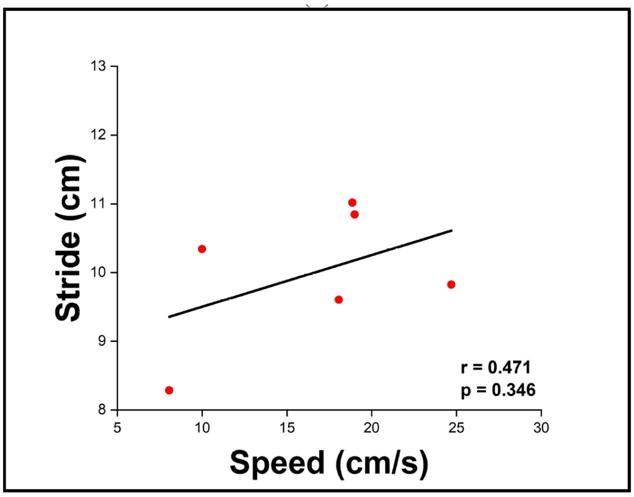
Fig. 3 investigates the relation between the first gait parameter in this experiment, the stride length, and the speed. In both groups of uninjured and injured rats, increased in speed generally results in an increase in average stride length, as seen from the plots and positive Pearson's r value. This is because as the rats move faster, they take longer strides to cover more distance is less time.

TABLE I. Average Speed, Stride Length and Stance Width of The Uninjured and Injured Rats During Week 1 of The Experiment

Output Parameters	Uninjured Rats	Injured Rats	Difference (%)
Speed (cm/s)	20.644	16.445	25.53
Stride (cm)	10.655	9.986	6.7
Stance width (cm)	2.957	3.454	14.39



(a)

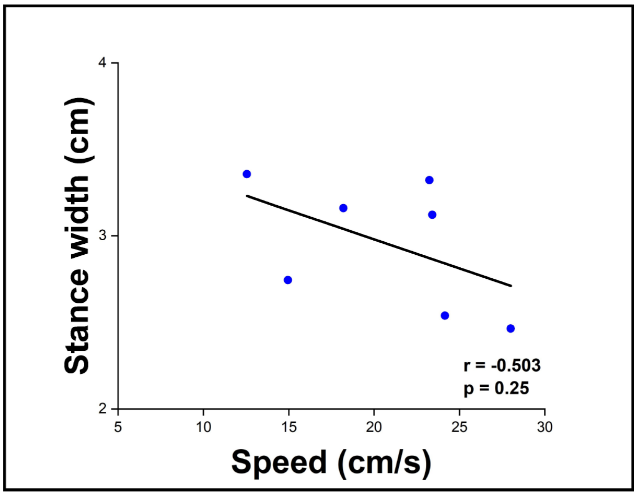


(b)

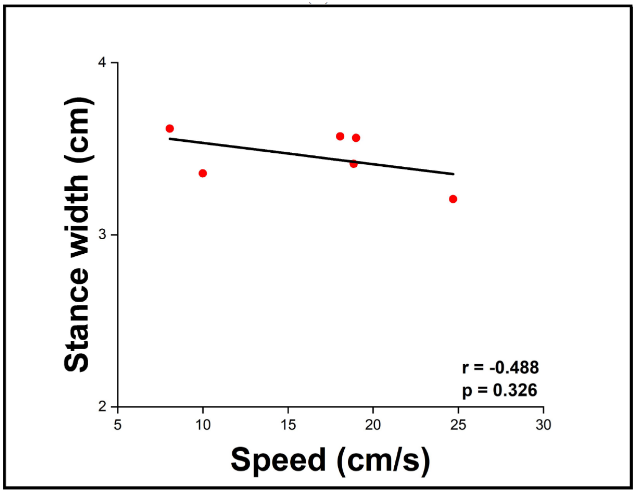
Fig. 3. Correlation between locomotion speed and average stride lengths for (a) uninjured and (b) injured rats.

In healthy rats, the relationship is stronger between locomotor speed and stride length (r = 0.746) as compared to the moderate correlation in injured group (r = 0.471). This is in line with other findings that states stride length increased as speed increases [7]. In SCI-injured rats, although the trend is like the uninjured rats, the relationship is not as strong. This might be due to the existence of SCI-induced impairments that limits the ability of the rats to extend their stride length even at higher speeds. On the other hand, the p-values is insignificant due to some possible reasons, including the small sample sizes, where only seven and six samples each for the uninjured and injured group. Although the p-value does not allow for a statistically solid conclusion, the data still provides valuable information about the strength and direction of the relationship between speed and stride lengths for injured rats.

From Fig. 4, stance width and speed in general is having moderate negative correlation. This negative correlation between speed and stance width was clearly reflected in the negative Pearson coefficient value for both groups, contrary to the relationship between stride length and speed. The trend is similar for both the healthy and injured rats: as the speed increases, the stance width decreases, with uninjured rats' r value is slightly stronger than the injured's



(a)



(b)

Fig. 4. Correlation between locomotion speed and average stance width for (a) uninjured and (b) injured rats.

r at -0.503 and -0.488, respectively. Similar to the strides for the injured rats, slightly weaker correlation between the speed and stance width suggests significant locomotor impairment of the SCI-injured rats. However, the trend is not conclusive because the correlation is not statistically significant (p-value > 0.05). Similar to stride length, small sample size might contribute to this. In this situation, with the stance width for injured rats have moderate correlation when compared to the walking speed while the stance width of the injured rats is larger than the uninjured, it can be concluded that this data still provides significant information regarding the relationship between walking speed and stance width of injured rats.

Small sample size was used in this study due to logistic and financial constraints. Despite the limitation, the analysis of 13 rats – 7 uninjured and 6 injured – provides meaningful observations into the relationship between speed and gait parameters for SCI-injured rats, enhancing understanding on the subject. The lack in statistical numbers in the results is likely attributable to limited sensitivity from the small sample sizes rather than without any important relationships. Future studies with larger sample sizes will be necessary to confirm these findings and establish more definitive conclusions.

IV. Conclusion

This research article presented the analysis of locomotion speed, stride length and stance width of healthy and injured rats using a walkway system four days after a contusion SCI surgery. From the results, SCI-injured rats experience slower speed and shorter stride length, while showing larger stance width when compared to the uninjured rats, proving that their SCI-injury is impacting their locomotion ability. Furthermore, when investigating the relationship between speed and the two gait parameters, it is shown that stride length have positive correlation with speed, while the stance width have negative correlation. Although the correlation trend is similar for both uninjured and injured rats, the weaker correlation observed in the injured rats indicates significant locomotor impairment, despite the small sample size. In summary, from this study, the relationship between the speed and gait parameters involved can be used to further justify the presence of SCI in rats. This correlation also has the potential to be used to measure both injury severity and its progression during rehabilitation exercise. Future research with larger sample sizes is required to further validate these findings and draw more solid inferences.

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