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RESEARCH ARTICLE

RATS: An Alternative Solution for Tracking Open Field Locomotion of Rats with Spinal Cord Injury

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Abstract Changes in locomotion for animals under various health conditions including spinal cord injuries (SCI) provide insights on the disease and its recovery process. This can be achieved by analyzing their behavior in an open field test (OFT). Existing systems for OFT study are expensive and lack customization options, making it unsuitable for research projects with limited means. This study introduces Rodent Activity Tracking System (RATS), a new OFT system with automatic tracking and analysis to objectively assess the behavior of rats with SCI in comparison to healthy rats, focusing on distance traveled in the arena. 13 adults female Wistar rats were randomly divided into two groups – uninjured (7) and injured (6). For the injured group, contusion SCI was introduced on their T9/T10 thoracic area of the spinal cord using NYU Impactor device. The reliability of the newly developed system was compared with manual measurement, achieving a Cronbach's alpha of 0.9979 for internal consistency. The accuracy of the distance measured by the system showed no significant difference compared to the manual method, with an intraclass correlation coefficient (ICC) of 0.9909 and a Pearson correlation, r, of 0.9924. Bland-Altman analysis portrayed similar results with most measurements falling within 95% range. RATS demonstrates its capability by successfully measuring the average distance traveled by the rats, with the healthy rats covering on average 70% more distance than SCI-injured rats. This suggests that RATS is a reliable and valid tool for measuring the locomotor behavior of rats in OFT.

Keywords: Locomotion, open field test, rat, spinal cord injury, tracking.

Introduction

Spinal cord injury (SCI) research usually aimed at understanding the mechanism and extent of the injury as well as to develop treatment for it [1]. To do that, tools for measuring and quantifying locomotor changes due to the injury are required. Some changes in locomotion cannot be easily detected with naked eyes, thus a specific system that is capable of detecting the changes is required to be used to evaluate the injury condition or to assess the treatment's effectiveness. SCI has a negative impact on human quality of life. SCI occur in humans by numerous reasons including motor vehicle crashes and sport related incidents, with the former being the primary cause in Malaysia [2-4]. SCI is an extremely challenging condition with limited recovery rates, making it a remarkable field for further exploration [5]. Besides the patient, their families will have to bear consequences [6]. They need to provide special care and may potentially lose one of their family's breadwinners. Therefore, effort in understanding SCI in terms of its pathology, progression, effects, and treatment options are important.

Recognizing behavioral characteristics of patients with SCI can be done using a valid model that can replicate human's physiological and pathological processes [7]. Humans were not being used directly in

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this type of study as its ethical considerations are more challenging, with their various pre-existing health conditions that might affect the outcome of studies. On the other hand, small laboratory animals like rodent, dog, cat and monkey were reported to be useful to mimic human SCI, making them suitable to be the model for this type of study [8, 9]. However, laboratory rodents such as mice and rats remain the most common animal species studied [10, 11]. This is because they are the cheaper option and shown that they respond to SCI in similar characteristics to humans [12]. They are also easily bred and handled during studies, as well as having known genetic background [10, 13]. In this study, rats were chosen as the substitute. Besides being cheaper and anatomically well understood, it is also due to its size that is generally larger than mice, thus making it easier to be handled. In terms of the number of animal samples required, several studies have demonstrated the reliability of animal tracking systems using relatively small sample sizes of 10 to 20 animals. For example, Junior *et al.* and Gravel *et al.* successfully validated their respective systems with 12 and six rats, respectively [14, 15].

Open field locomotion is one of the most frequently used behavioral parameters [16], used to describe the effect of spinal injury on locomotion [17]. Locomotive changes can be quantified through behavioral analysis, which were applied in preclinical studies to provide quantitative behavioral assessment related to clinical assessments. Assessing locomotion in rats give insights to understand injured locomotor behavior [18]. Detected changes in motor function might be associated with disease or injury [19]. Changes in locomotion can be observed and assessed through monitoring their behavior and activity in open field test (OFT) [16, 19]. OFT is suitable because it can give behavioral information on the condition of the rat's locomotion [20, 21]. In examining the behavioral characteristics of SCI-injured rat, many tests and techniques are being used by researchers including observational based locomotor rating scale and analysis of video recordings of the subject, whether automatically or manually [22-24]. Basso, Beattie and Bresnahan (BBB) rating scale has long been accepted as valid measurement of hindlimb's functional assessment after SCI [23]. However, it involves subjective observation which might introduce biasness in the assessment. On the other hand, automated tracking systems as this study suggests can improve it by offering a more objective measurement. Moreover, precise locomotor parameters can be obtained and enhance the accuracy of the locomotor judgement. Therefore, this study is adopting evaluation by means of automated video analysis as this approach, either in the form of recorded or real time video frames, also offers great flexibility in analysis capabilities.

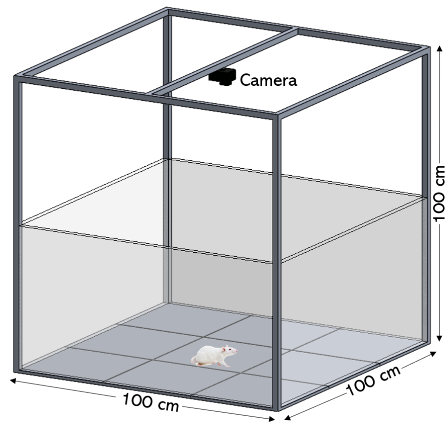
From previous reports, there are commercially available systems to monitor and analyze gait and behavior of rats or small animals from video and image frames such as Ethovision XT by Noldus and Actual-Track by Columbus Instruments [25]. However, commercial systems are often deemed too expensive, rendering them unavailable for low-budget studies. Furthermore, it lacks flexibility for the users [17, 26]. On the other hand, based on our knowledge, the non-commercial system such as the MouseWalker system developed by researchers from Columbia University [27, 28], also presents their own limitations to be used with full control in our study. Therefore, it is essential to develop a reliable and cost-effective custom system capable of quantitatively measuring the behavioral characteristics of SCI injured rats. Building a customized system grants the developer full control over its functionality, enabling solutions to limitations encountered during the development and testing phases of the system.

The main aim of this study is to build a system named RATS (Rodent Activity Tracking System). RATS is an inexpensive, uncomplicated and effective system that can be used objectively in quantifying SCI-injured rat's behavioral parameters in OFT. It is uncomplicated because it did not require a high level of programming skill from the user. It is also easy to use, and the algorithm is easy to understand. Moreover, the system's output was compared with measurement that was done manually to demonstrate the ability of the developed hardware and software to provide accurate and meaningful behavioral data for rats with SCI. This study specifically focused on one output parameter, that is the amount of distance traveled by rat in the arena. Besides being commonly measured in an OFT and able to inform about the difference in locomotor condition between healthy and injured rats, distance travel was chosen to be studied to demonstrate the ability of this system to provide meaningful analysis on the locomotion condition of SCI injured rats. Although only measuring total distance traveled, the system can be designed to also detect and analyze different meaningful output from an OFT. It can be programmed to do more analysis based on object detection in frames and frame manipulation like measuring rat's velocity and its entry into middle of arena [29-31].

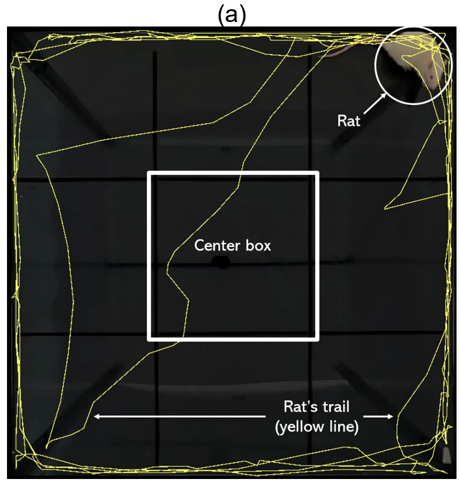
Methodology

System Hardware

The physical setup of the OFT are as shown in Figure 1(a). The open field test (OFT) equipment is a square shaped open arena with dimensions of 1000 mm (width) x 1000 mm (length) x 1000 mm (height), made using inexpensive transparent acrylic. The base of the arena is made of dark colored material to help with the image processing during post analysis. For OFT, the camera was centrally hung on top of the arena, 920 mm above the floor to clearly capture the whole showground. The OFT came with its own motion capture system. The required camera is capable of effectively capturing the motion inside the arena, as portrayed in Figure 1(b). This setup is using a GoPro Black 10 from GoPro Inc. GoPro camera was chosen because it is easily and wirelessly controllable using a smartphone, as well as it is also able to being accessed and controlled by the Python-based system developed. This is significant in this study as when the camera is fixed, it needs less disturbance to ensure the reliability of its video output. Moreover, lighting conditions inside the OFT is highly important during the experiment. Based on the pre-evaluation, normal to bright lighting conditions work well with this system. The frame rate for this OFT setup was set to 30 frames per second (fps) to meet the system's output requirements. Furthermore, the workstation is a computer with 11th Gen. Intel (R) Core i7-11800H @ 2.3 GHz, 32GB random access memory (RAM) and an NVIDIA GeForce graphic processing unit (GPU).



(a)



(b)

Figure 1. OFT system. (a) The camera was placed horizontally at the top-center of the arena for best frame. The floor was made dark to create a constant and contrasting background to help with video analysis as white rats were used. The wall prevented the rat from going out of the arena and jumping over it. Arena needs to be placed on a flat surface to avoid the effect of inclined surface on the clarity of the image and behavior of the rats. (b) Sample frame from a tracked rat's video. The yellow line represents the trail of the rat inside the arena

System Software

The primary purpose of developing this OFT system was to track and analyze the behavior of both healthy and injured rats during the study. From an OFT video, there are several behaviors that can be analyzed, including travel distance and its related speed, entry into the middle area, as well as its fecal count [21, 29]. However, in this study, the main parameter that was analyzed to prove its capability is travel distance. The rat activity inside the arena was recorded by the camera mounted at the top of the arena and was then sent to workstation for analysis. The videos from the camera first underwent editing to increase the efficiency of the analysis. This stage is necessary to remove any parts unrelated to or not contributing to the analysis. Unwanted and unused parts of the video, such as the audio and irrelevant areas in the frame, were carefully removed to ensure that no important information relevant to the analysis was disrupted. All data presented in this study were prepared using the analysis package, mainly developed using Python. The basis of the system developed is to track the body part of the rat in the video and explore how its changes contribute to the output parameter in two-dimensional (2-D) frames.

There are three main segments in the system: identifying and tracking of the body parts, as well as analyzing their respective outputs. In the workstation, the threshold value to define the color of the rat's body as well as to detect and differentiate it with the surrounding was defined and the system was informed about it before the analysis. When the video frames were extracted, the pixel location of the centroid of the rat's body in the frames were identified and recorded based on the color definition provided earlier. The centroid's pixel location, $(\bar{x}, \bar{y})$, was determined using image moment technique as shown in Equation (1). The centroid is computed by linking the first order spatial moments, M_{10} and M_{01}, in the image to the zeroth-order moment M_{00}, and calculated by summing their pixel intensities, as defined in Equation (2). *I(x,y)* is the intensity value at pixel location *(x,y)*. The images were changed to binary prior to the operation.

$$\bar{x} = \frac{M_{10}}{M_{00}}, \bar{y} = \frac{M_{01}}{M_{00}} \quad (1)$$

$$M_{ij} = \sum_x \sum_y x^i y^j I(x, y) \quad (2)$$

$$d_a = \sqrt{(x_{a+1} - x_a)^2 + (y_{a+1} - y_a)^2} \quad (3)$$

$$distance\ traveled = \sum_{a=0}^{n} d_a \quad (4)$$

The pixel locations in subsequent frames were used to calculate the distance traveled between them, as being used by [32] in their research. Referring to Figure 2, if (x_a, y_a) is the rat body's centroid coordinate at frame a, while (x_{a+1}, y_{a+1}) is the rat body's centroid coordinate for the consecutive frame, $a + 1$, then the distance traveled by the rat in between the consecutive frames, d_a were calculated using Equation (3), while total distance traveled, which sums all the distance traveled by a rat in between frames was defined by Equation (4).

In Equation (4), *n* is defined as the total number of video frames in the video. These were all written in the code, as defined by Figure 3. At the end of the analysis, the output parameters which are the distance traveled by healthy and injured rats in the OFT were used to conclude its findings on the system's effectiveness in performing the analysis.

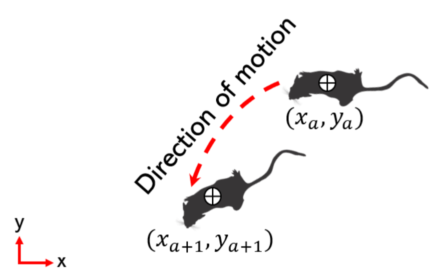
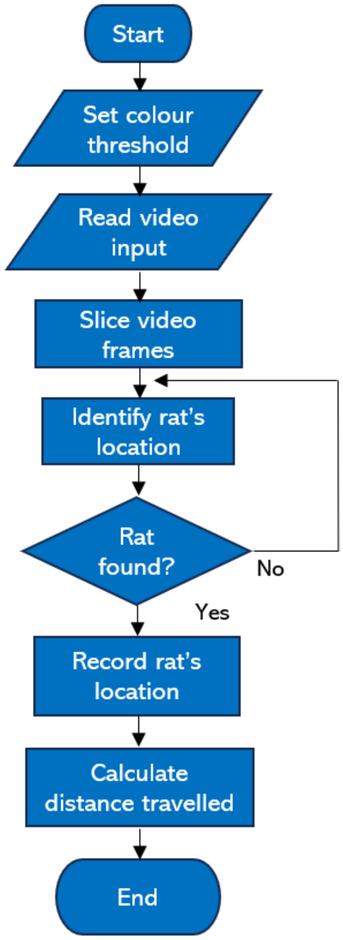


Figure 2. Calculation of distance traveled by the rat is based on difference in rat's centroid location in subsequent video frames. In each of the frames, the rat's body was identified, and its centroid's pixel location was recorded




```
Program start
Initialize colour threshold
Read video input
Extract video frames
for frame 1st until frame last
      Find rat
      if rat not found, repeat 'Find rat'
      Record rat's location
Calculate distance travelled
Program end
```

Figure 3. Flowchart of the system. The system was programmed to identify the rat's centroid position based on its color. Changes in the centroid's location between consecutive frames were tracked, and the differences were calculated as the distance traveled by the rat. The individual distance is calculated by comparing the centroid's location in the current frame with the centroid location of the rat's body in the next frame. To calculate the total distance traveled during the entire experiment, each of the individual distances was summed up

Animal Model

This study involves the use of animals. Therefore, it has complied with all the relevant regulations and policies for the care and use of animals, in accordance with the approved protocol by the UiTM Committee on Animal Research & Ethics (UiTM CARE), with the reference code UiTM CARE 2/2023/(412/2023). In this report, thirteen female Wistar rats (two months old, 200-230 grams/rat) were randomly divided into two groups: uninjured (n=7) and injured (n=6). Initially there are 15 rats, but two rats died due to complication from surgery. The number of rats used aligns with established sample sizes in previous research [14, 15]. The injured group were introduced with contusion SCI on their T9/T10 thoracic region after laminectomy using the NYU Impactor device (WM Keck Center for Collaborative Neuroscience, USA). Contusion injury models are often used by previous studies in gaining insights into SCI mechanisms as well as studying the locomotive pattern and behavior of SCI-injured rats [12, 33, 34]. The height of the drop was set to 25 mm. A successful contusion SCI can be observed through the jolting or jerking of the rat's hindlimbs during the procedure, specifically when the impactor hit its spinal cord [35]. Figure 4 displays the images during the surgery procedures. To support their recovery, the injured rats were provided with proper post-surgery care as stated in [36]. On the 28th day post-injury, all uninjured and injured rats were euthanized humanely using an overdose of carbon dioxide inhalation.

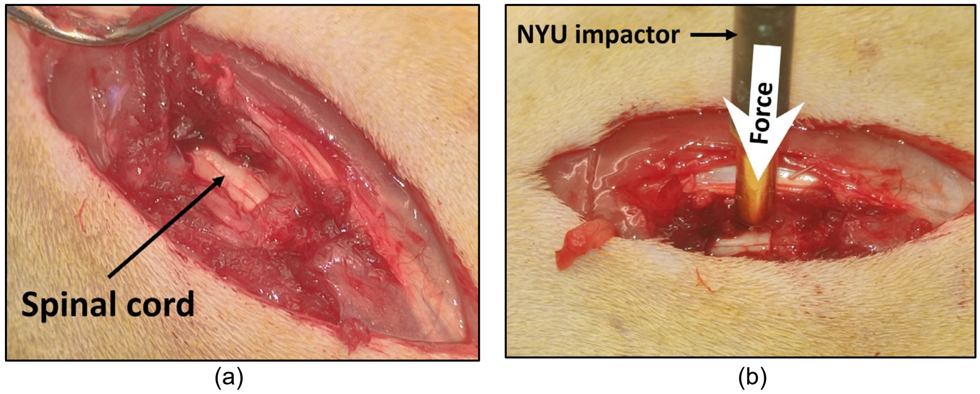


Figure 4. During contusion SCI, the (a) spinal cord of the rat was exposed through laminectomy and (b) the NYU Impactor was dropped at the height of 25 mm above the exposed cord to produce the required force for the contusion

Testing Procedure

OFT was used to assess the behavioral parameters of rats. In this study, the system is demonstrated in analyzing the distance traveled by the injured rats inside the arena, in cm, and comparing them with the healthy rats. Prior to the experiment, the rats were let free inside the open field arena regularly for one week to make them familiar with its environment. Each rat was released for a minimum of two minutes inside the arena every time they were let free. The test was conducted over four consecutive weeks after the injury, specifically on the 4th (Week 1), 11th (Week 2), 18th (Week 3) and 25th (Week 4) days post-injury. During the experiment, each rat was released separately inside the arena and their motion inside the arena was fully recorded by the top-mounted camera. After each test, the rat was returned to its cage, and the arena was cleaned with disinfectants before the next rat was introduced.

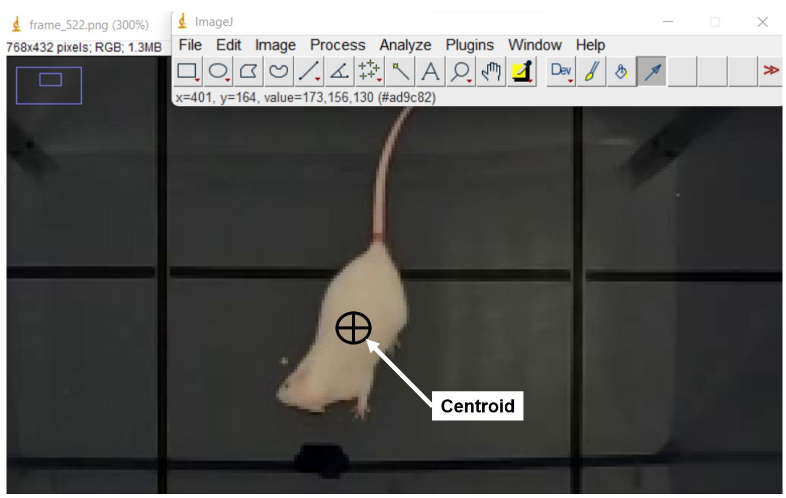


Figure 5. Manual marking using ImageJ. This image shows a rat's body and how the centroid of the body was determined manually. The pixel location of the body's centroid was recorded for assessment

Uninjured rats and rats with contusion SCI was used to test this newly developed system. The duration for each test session inside the OFT was set at 240 seconds or four minutes. However, for the validity test of the system in this report, one video from the seven healthy rats was randomly selected and only the first 120 seconds from the recorded video were tracked and verified. For verification purposes, only the first half of the session was prioritized to ensure high quality validation within the available resources. In addition, our initial quality checks confirmed that the tracking results for the remaining 120 seconds maintained consistent performance metric. The two minutes duration from the video was sampled for every 1 seconds or approximately every 29 frames as the frames per second (fps) for the video is 29.97, making the number of data points, d_p=120. As mentioned, the entire experiment was done over specific days within a four-week duration. However, the data presented in this report is from Week 1 of the experiment. Additional data collected from Week 2 until Week 4 will be presented in a separate report. To validate the reliability of tracked distance by the new system, a comparison was made with a manual method. In the manual setup, the rat's location in each frame was determined manually as shown in Figure 5 and marked using ImageJ version 1.54d, a Java-based image analysis software released by the United States National Institutes of Health (NIH) [37]. The rat's location in each frame was recorded and used to calculate the distance it traveled in between consecutive frames.

Results and Discussion

To address the first concern, the entire system, including a personal computer, costs less than RM20,000. In comparison, an OFT system can cost over RM50,000 based on the current exchange rate of 1USD = RM4.70. Next, the data obtained by the RATS was compared to the manually obtained output. This approach of comparing automated obtained data with manually gained ones is similar to that used in studies by [15, 38]. Prior to comparing the RATS system with manual marking, both manual marking and automated analysis were repeated three times for all 120 frames. This is to assess the repeatability and internal consistency of the data. The outputs from each marking method were compared for consistency, as shown in Table 1. For manual marking, the three measurements done showed excellent internal consistency with a Cronbach's alpha of 0.9979, indicating good intra-observer repeatability. Moreover, they are also positively correlated, with their intraclass correlation coefficient (ICC) and Pearson coefficient, *r*, were calculated to be more than 0.99 in each pair. Hence, the average of the three manual measurements was made as reference and compared with the outcome from the RATS. As for RATS, the three outputs demonstrated perfect consistency (alpha = 1.0) as its results were automatically identical.

Distance traveled by the rat between each of the 120 consecutive frames was compared between manual and automatic measurements. Figure 6(a) illustrates the Pearson correlation results between the output parameters obtained from RATS and those from the manually marked method. The ICC was determined to be 0.9909, while the Pearson coefficient, *r*, was calculated as 0.9924. The ICC value between the methods, as detailed in Table 2, was evaluated to be 0.9909.

Moreover, Figure 6(b) illustrates the Bland–Altman plots to assess the agreement between RATS and the manually marked output in measuring distance traveled by the rat in the OFT. Bland–Altman analysis provides a reliable method for assessing concordance and identifying potential bias between two systems under comparison [39, 40]. In Bland-Altman plot, the x-axis represents the mean of the two measurements, while the y-axis denotes the difference between them. The mean difference between the two methods was calculated to be 0.4086 cm, while the upper and lower limits of agreement were determined to be 2.3036 cm and -1.4864 cm, respectively. It was observed that the mean difference indicates a small average positive bias of the RATS compared to the reference or manual method. Additionally, about 5.8% of the output fell outside of the 95% limits of the agreement. These outliers suggest that there are situations where the methods disagree with each other. Despite this, most of the measurements still fell within the 95% appropriate range. This indicates good to strong agreement between the two methods, with a few exceptions that may require further analysis.

Table 1. Reliability test results for manual marking and RATS. Measurement pairs were compared using ICC and Pearson coefficient, *r*, while intra observer repeatability was tested using Cronbach's alpha

Measurement method	Measurement pairs	d_p	ICC	r	alpha
Manual	1&2	120	0.9959	0.9959	0.9979
	2&3		0.9930	0.9928	
	1&3		0.9928	0.9930	
RATS	1&2		1.0000	0.9999	1.0000
	2&3		1.0000	0.9999	
	1&3		1.0000	0.9999	

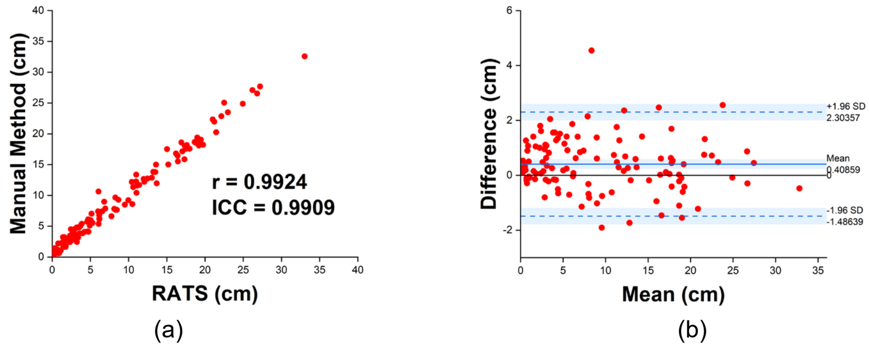


Figure 6. (a) Correlation between the analysis output and (b) Bland-Altman plot for the manual method and RATS

Table 2. Correlation test result for manual method and the RATS and comparison between total distance traveled from both methods

ICC	Pearson (r)	Total distance traveled	Difference
0.9909	0.9924	Manual: 1130.4519 cm RATS: 1081.4210 cm	4.3373%

Besides the distance traveled by the rat between consecutive frames, the total distance traveled during the 120 second duration was calculated by summing up the individual distance between frames. The manual method calculated this to be 1130.4519 cm, while the RATS calculated it to be 1081.4210 cm. This corresponds to a difference of 4.3373%, which is lower than the targeted 5% difference. Furthermore, Figure 7 shows the overlay plot of the manually marked location of the rat compared to the automated location tracked by the RATS. The red line indicates the connected rat locations in consecutive frames from the manual method, while the blue line indicates those tracked by the RATS.

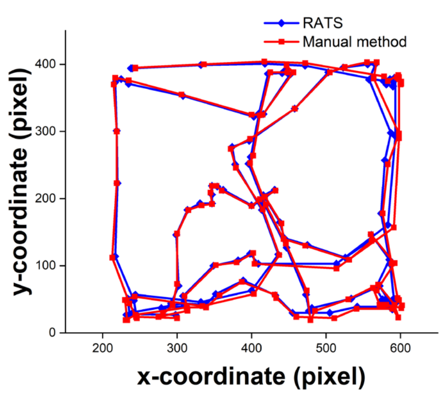


Figure 7. Overlay plotting of location of the rat inside the arena tracked using the manual method and the new system, RATS

Based on the high ICC value, strong Pearson correlation, and the Bland-Altman analysis, it can be concluded that there is a strong agreement and a strong linear relationship between the measurements obtained from the two methods. This indicates that RATS is as effective as manual measurement in yielding reliable outcomes when measuring the distance traveled by rats in the OFT, making it an acceptable method for distance measurement in this study.

Results from OFT

To demonstrate the system's ability, the distance traveled for the seven uninjured and six injured rats four days after the contusion surgery was analyzed. As being reported by [41], injured rats tend to explore less in an OFT compared to healthy rats, resulting in shorter travel distances. Similar results were obtained in this study. Figure 8 shows the results from OFT for Week 1 for the uninjured and injured rats. As expected, the average distance traveled by healthy rats was 1848.05 cm, while the injured rats traveled significantly less, at 1084.07 cm, during the four minutes of OFT. This corresponds to the healthy rats covering 70% more distance than the SCI-injured group. This is not an uncommon situation for contusion SCI model since similar results were presented by previous studies [35, 42]. The shorter distance traveled by the injured rats might be attributed to the effective contusion SCI sustained by the rats four days earlier [43]. Successful contusion SCI usually impairs the hindlimb function of the rats, preventing them from lifting or supporting their body weight properly until the hindlimbs recover. If compared to rats with other types of SCI, such as complete transection injuries where the spinal cord is completely detached, resulting in more severe impairment, the rats in this contusion model of SCI traveled a greater distance.

Based on the results from the OFT, consistent deficiencies attributable to the surgery are evident in the output parameters. This demonstrates the capability of the developed OFT system to reliably measure behavioral differences in both normal and SCI-injured rats. While this article focuses solely on travel distance as its parameter of interest, analysis of the rat's video-recorded position enables examination of other relevant variables. These include the number of entries into the middle area, which correlates with rat's anxiety, and its travel speed in the arena, providing deeper insights into its behavior.

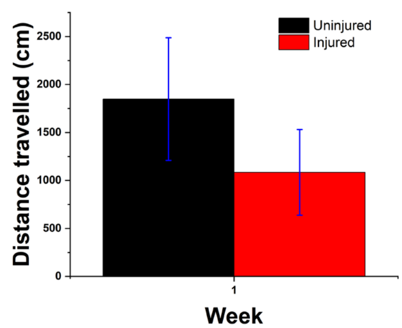


Figure 8. Results from the new OFT system developed show average distance traveled by healthy and injured rats. Error bars represent standard deviations. The injured rats traveled distance in Week 1 was shown to be shorter than the uninjured, indicating the presence of injury to its spinal cord

Conclusions

This article presented a newly developed system, RATS, consisting of its own hardware and software setup for automatic measurement of distance traveled by healthy and contusion SCI injured rats in an OFT. The cost to develop this system was found to be less than 40% of the commercially available system in the market, making it significantly more cost-effective. Moreover, since the system was developed in-house, it provides us with total control on the design of the hardware and software part of the system. Any issues that arise could be addressed without limitation from external parties. Furthermore, based on the ICC and Pearson correlation coefficient, *r*, as well as the Bland-Altman analysis of the system's outcomes, RATS can be deemed reliable as its output parameters aligned with the reference values which were obtained through manual measurements. Additionally, the system successfully measured the distance traveled by the seven healthy and six injured rats in Week 1. From the output, there are clear indications of injury in the injured group, with a shorter average distance traveled by the injured rats. Besides suggesting that there exists clear agreement between the output from the RATS and the reference values, it is worth noting that from the results, there are about 5% exceptions that require further investigation to improve the system.

This system is also suitable to be used with other types of rodents besides Wistar and for studying diseases other than SCI, as its working principles are not limited by rodent size or injury type, expanding its usefulness. Moreover, besides travel distance, other important parameters from an OFT such as time spent in center of the arena can also be possibly determined from this system as their detection principles are similar. It is hopeful that this study can contribute to behavioral research of SCI injured rats and in enriching the community within this field of research. The success of this research will indirectly contribute to the preclinical studies into SCI injured individuals. Although the hardware and software of the system are believed to be working reliably, as the results suggest, continuous improvements are still required to enhance its ability.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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