Quantification Analysis of Behavioral Changes after Sciatic Nerve Ligation in Rats

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***Abstract*—Measuring behavior changes in humans and animals is ongoing research in both bioscience and neuroscience, due to its applications in nerve injury recovery. Previous studies focused on the sciatic nerve and its recovery process because partial damage to this nerve leads to paralyzed symptoms of entire lower body parts. Rats have been successfully used as a model for neuropathic pain such as partial sciatic nerve injury. Numerous behavioral tests have been used to measure pain behavior and determine the extent of damage in animals with induced nerve injury. However, data collection using behavioral tests is highly time-consuming and costly, because of visual inspection-based tests, manual labor, and the need for complicated equipment. The real-time video data can be obtained in animals' natural environment e.g. own cage, without the need for interacting with the animal, which potentially can influence the test results. DeepLabCut provides easy methods for observing and recording animal behavior in diverse settings using video data. In this study, to examine the behavioral changes in rats after sciatic nerve ligation, we (i) recorded videos of 9 rats during the first and fifth day after surgery, (ii) used DeepLabCut to track the rat's positions throughout the videos, and (iii) calculated behavior indexes based on spatio-temporal patterns that measure the behavioral changes in rats. The results in this study confirm our assumption that there were significant changes in the behavior of rats with partial sciatic nerve injury. These findings motivate us to investigate physiological processes accompanied by the recovery, and implement real-time monitoring and analysis for measuring behavior changes in rats.**

***Index Terms*—Animal behavior analysis, Partial sciatic nerve ligation, Rats, DeepLabCut**

I. Introduction

Measuring behavior changes in humans and animals is ongoing research in both bioscience and neuroscience, due to its applications in nerve injury recovery. The sciatic nerve, a large nerve in humans and other vertebrate animals, provides the connection to the nervous system for nearly the whole of the skin of the leg, the muscles of the back of the thigh, and those of the leg and foot. Partial damage to the sciatic nerve leads to weak knee flexion, foot movements, foot drop including sensation below the knee [1]–[3]. These symptoms affect the daily life of humans and animals. However, our body has the recovery process to relieve this problem [4]–[6]. The duration of this process depends on the level of sciatic nerve injury. The importance of the sciatic nerve and recovery process has driven many researches to explore various ways for improving and investigating its related disease such as neuropathic pain [7]–[10], and enhancing the recovery process in human and animals [6], [11], [12].

Previous studies selected the rat instead of a human in order to investigate the sciatic nerve and recovery process [10], [12]–[15]. According to Imamoto et al., the advantage of selecting rats as the subjects is that the duration of its recovery process is short [16].

However, their data collection and analysis processes were highly time-consuming and costly, because they were based on visual inspection, manual labor, or complicated equipment such as motion capture. Even if they used images-based observation such as video recording to deal with the issue, it is complicated to implement the program from scratch [17]–[20]. Using recent deep learning technology, DeepLabCut provides easy methods for observing animal behavior in diverse settings through video data, which saves experimental time and cost [21]. Due to the outstanding performance and user-friendliness, this tool also was used widely in animal behavior analysis.

In this study, to measure the behavioral changes in rats after sciatic nerve ligation, we (i) recorded videos of 9 rats on the first and fifth day after surgery, (ii) used DeepLabCut to track the rat's positions throughout the videos, and (iii) calculated behavior indexes based on spatio-temporal patterns that measure the behavioral changes in rats. The rest of this paper is organized as follows. Section II describes the methodology including the recording of free-moving rats, semi-automatic detection of rat's position, behavioral indexes to be analyzed, and statistical testing. In section III, we present the results from the experiment with the three groups of rats using our proposed behavioral indexes. Then, we describe the possible extensions and improvements of this study in section IV. Lastly, we conclude this study in Section V.

II. Methodology

A. Experiments and data collection

The experiments were performed using a total of 9 male Sprague-Dawley (SD) rats weighing 100-150 g (4-week-old at the time of operation). The rats were randomly divided into three groups: PSL model, sham-operated, and control groups. Three rats assigned to the PSL model group underwent partial sciatic nerve ligation (PSL) surgery to their right hind leg, according to the methods of Seltzer et al. [10], while the sciatic

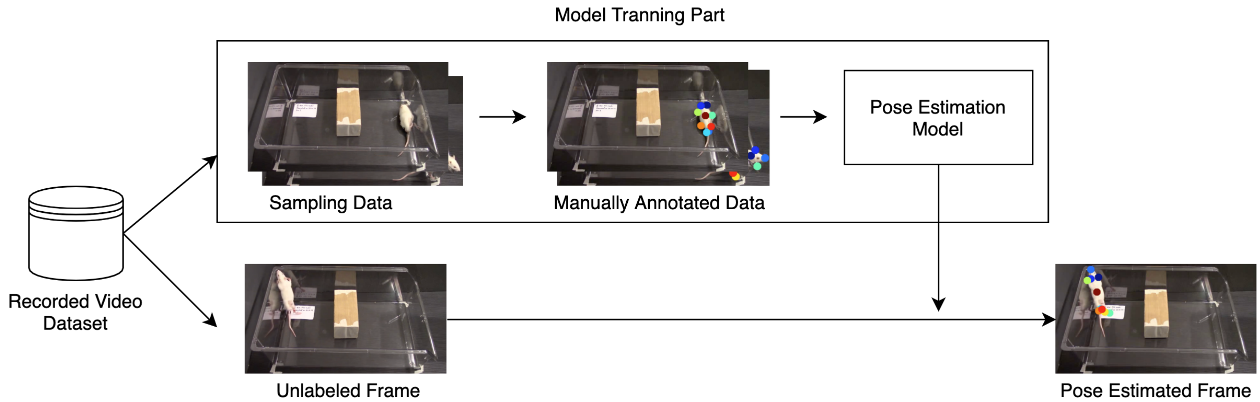


Fig. 1: Procedure of Semi-automatic detection via DeepLabcut

TABLE I: Dataset

	#rats	Video sessions	
		first-day	fifth-day
Control group	4	✓	-
PSL group	3	✓	✓
Sham group	2	✓	✓

nerve for two rats assigned to the sham-operated group was exposed but not ligated. Three rats assigned to the control group underwent no surgery at all and were used to evaluate the baseline activity in the normal condition.

For the PSL model and sham-operated groups, behavioral test sessions were performed one day and five days after the surgery. At the beginning of each session, the rats were placed in the bottom-left area of the open field apparatus consisting of a rectangular box ($55.6\times33.4\times19.5$ cm) with a rectangular obstacle ($19.0\times9.5\times9.5$ cm) as shown by snapshots of Figure 1. We then filmed the rats freely moving around the open field for approximately 15 minutes at the frame rate of 50 fps, using a Canon LEGRIA HF R47 fixed above the open field. For the control group, the same behavioral test sessions were performed only on one day. All the movie data filmed in this way were used for our later analysis (See also the summary of our dataset in Table I.)

B. Semi-automatic detection of rat's position

To analyze the behavioral changes from the videos, we need to track the position of the rat first. For this task, we used DeepLabCut, a supervised CNN-based tool for animal posture quantification [21]. This tool allows us to automatically annotate the positions of objects in entire videos, once their positions are manually provided in a few of the video's frames. As shown in Figure 2, we used this tool to track 10 points on the subject's body: the tips of the ear, the tip of the nose, tail base, tips of four legs, and center of the body, as well as the right hind leg where the surgery is performed.

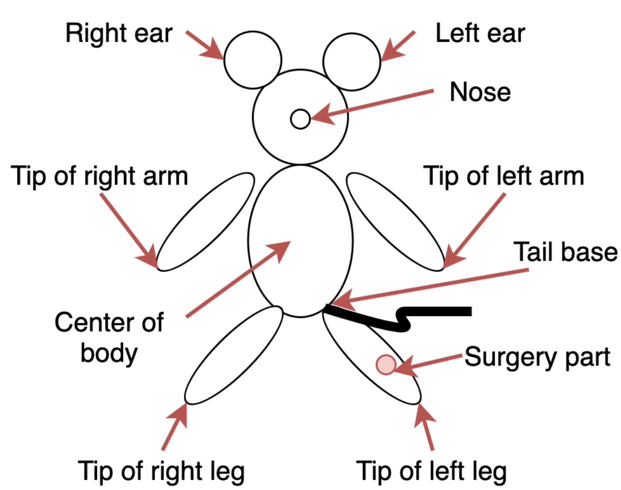


Fig. 2: Tracked body parts of rat

As DeepLabCut is based on supervised learning, using a ResNet50 model for predicting positions, it requires a sample of our videos for training its model. To create the training data set, we randomly selected 20 frames per video, and manually annotated the positions of 10 body parts through the provided user interface. Once trained, the tool then can be used to estimate the positions throughout the remaining frames. All the estimated part positions were used in our analysis which will be described in the next section.

Using DeepLabCut, we found that the estimated positions of some frames have low log-likelihood, and thus low predicted probability. These erroneous positions will become a cause of wrong analysis. To deal with this issue, we set a condition that positions with log-likelihood lower than 0.8 were excluded from our analysis.

C. Behavioral indexes to be analyzed

Once the position of body parts have been estimated, we extracted spatio-temporal patterns from the positions to analyze certain behaviors of the rats. To describe these behaviors, we calculated the exploration level, preference of left/right position, traveling distance, velocity, climbing activity, and walking/standing state as behavioral indexes in our experiment. These behavior indexes were extracted from both sessions of the PSL and Sham groups. For the control group, the indexes on the fifth day were assumed to be the same as the first-day session. We expect the subjects to have different behaviors between the first- and fifth-day because of the difference in recovery duration. We defined these 6 behavior indexes as the following:

1) Exploration level: This behavioral index represents whether the subject is more likely to wander around or adhere to certain locations. A behavior of walking from place to place within the experiment field could be described as *unpredictable*, as opposed to only walking in a limited, predictable route. Following this concept of predictability, we define the exploration level to be the statistical entropy of the rat's position. The statistical entropy is defined as:

$$H(X) = -\sum_{i=1}^{n} P(x_i)\log P(x_i) \tag{1}$$

where n is the number of possible positions in the experiment field, and $P(x_i)$ is the frequency that our rats are located in position i.

2) Preference of left/right positions: This index represents the tendency of the rats to stay in the left or right half of the experiment field more often than the other. Considering the rat's positions at each frame, this index is simply the ratio of occurrences of the rat being located in the left half of the video, over the total occurrences of being located in either half. This index is simply the ratio of occurrences of the rat being located in the left half of the video, over the total occurrences of being located in either half. Given the video s containing N frames, the ratio is the following:

$$P(s) = \frac{\sum_{s_i}^{N} \mathrm{left}(s_i)}{\sum_{s_i}^{N} \mathrm{left}(s_i) + \sum_{s_i}^{N} \mathrm{right}(s_i)} \tag{2}$$

where $\mathrm{left}(s_i)$ is equal to 1 when the rat's position in frame s_i is in the left half, otherwise 0. On the other hand, if the rat's position in frame s_i is in the right half, $\mathrm{right}(s_i)$ is equal to 1, otherwise 0. Note that a ratio of 0.5 implies that the rat is equally likely to be on either side.

3) Traveling Distance: This index represents the total distance that the rat travels. To obtain the index, we track the center position of the rat in every frame, calculate the Euclidean distance between consecutive frames, and sum up the distances. As some videos were longer than others, we normalized the index with a division by the video's duration. Formally, this behavior index is defined as:

$$T(s) = \frac{1}{N} \sum_{i=1}^{N} \mathrm{Euclidean}(c_i - c_{i-1}) \tag{3}$$

where c_i is the coordinate (x_i, y_i) of the rat's center position at frame i, and N is the total number of frames in the video.

4) Climbing Activity: This index represents how often the rat climbs over the obstacles and walls in the experiment field. As climbing is a movement that involves more usage of the rat's hind legs, such as stretching, we expect that sciatic nerve ligation would affect its ability to climb. For this index, we mainly track the nose position to see whether its nose extends over the obstacles or walls. This works for all walls except the bottom, where the rat's nose may appear to cross the border even though it is still walking behind the wall. To solve this, we additionally check other positions, such as the legs and ear tips, to determine whether it is actually climbing the bottom wall. Again, we count the frames in which the rat is climbing, and divide it by the total number of frames to obtain a ratio of climbing activity. As such, this index is defined as:

$$\mathrm{Climb}(s) = \frac{1}{N} \sum_{i}^{N} f(P_i) \tag{4}$$

where P_i is the set of positions at frame i, and $f(P_i)$ is 1 when the rat is climbing in frame i, and 0 when it is not.

5) Velocity: Inspired by Nakamura et al., this index represents the rat's speed at a given moment in our experiment field [15]. After a recovery period, we expect some change in the rats' speed between both the first- and fifth-day sessions. As we discovered that the rat's speed per frame can swing wildly and lead to inaccurate analysis, we calculated this index per second instead. For each second of the video, containing 50 frames, we discard the frames whose position predictions have low log-likelihood, concatenate the remaining frames, and take the middle frame's position as the current second's position. Then, we used the positions to calculate the speed at each second, and used the average of these values as the behavioral index. Given a video s, we define this index as:

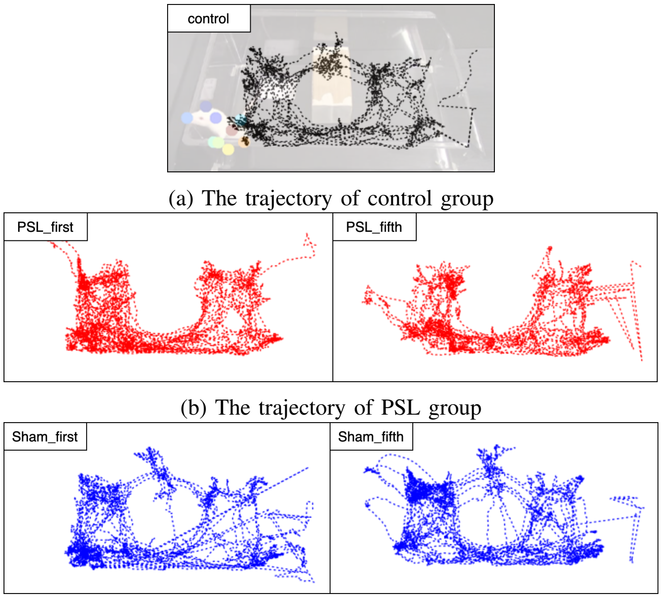
$$V(s) = \frac{1}{N} \sum_{i}^{N} (p_i - p_{i-1}) \tag{5}$$

where p_i is the position of the rat at second i in video s as described above, and N is the total number of seconds in the video. Note that we omit the division by 1 second because the position difference is already per second.

6) Walking/standing state: This index represents the tendency of the rats to walk or stand more often. This is also inspired by Nakamura et al. [15]. Once we obtained the velocity at each second, we set a minimum velocity threshold to define whether the rat is walking or standing. From that, we define the ratio of walking/standing state as:

$$\mathrm{Walk}(s) = \frac{1}{N} \sum_{i}^{N} C(p_i - p_{i-1}) \tag{6}$$

where $C(v)$ is 1 if the velocity v is higher than the selected threshold, otherwise is 0. In this study, we set the threshold value to 2cm/s according to prior work.



(a) The trajectory of control group

(b) The trajectory of PSL group

(c) The trajectory of Sham group

Fig. 3: The trajectories of control, PSL, and group

D. Statistical Testing

In order to statistically validate our results, we used a paired sample t-test to test our null hypothesis that the rats' behaviors at the first- and fifth-day sessions are equal. In this evaluation procedure, first, we took the behavioral indexes from different two days in the same rat group as the two observation groups. After that, we calculate the t value of each behavioral index i:

$$t_i = \frac{m_i}{s_i/\sqrt{n}} \tag{7}$$

where n is the number of pairs in our data, while m_i and s_i are the mean and the standard deviation, respectively, of the difference of each sample d_i:

$$d_i = f_i(s_{\text{fifth}}) - f_i(s_{\text{first}}) \tag{8}$$

where f_i is the function for calculating index i, and s_{first} and s_{fifth} are the samples from the first and fifth days, respectively.

Once we had determined the t value, we used the t-test table to examine the critical value of Student's t distribution with $n-1$ degrees of freedom corresponding to the selected significance level α at 0.1. If $|t_i|$ is greater than the critical value, then there is a significant difference between the paired subjects in the index i.

III. Results

A. Trajectory of rats

As a preliminary assessment, we analyze the trajectories of the three rat groups. Visualizations of the trajectories are provided in Figure 3. As a typical example, only one session in the control group is shown. For the PSL and Sham groups, trajectories from the first- and fifth-day sessions are displayed side-by-side. The control trajectory covers most of the experiment field, including the obstacle area in the middle. In contrast, the PSL trajectories from the first-day session show denser trajectories in certain areas, compared to the fifth-day session trajectories, which indicates fewer movements. We also found that both PSL and Sham groups enter the obstacle area less than the control group. These trajectories show that there are some differences between their behaviors in both sessions that can be analyzed further.

TABLE II: The trending of PSL and Sham groups for each behavioral index

Behavioral Index	PSL group	Sham group
Entropy	$\searrow$	-
Preference of left/right positions	$\downarrow$	-
Traveling Distance	$\searrow$	-
Climbing	-	$\nearrow$
Velocity	$\downarrow$	-
Walking state	-	-

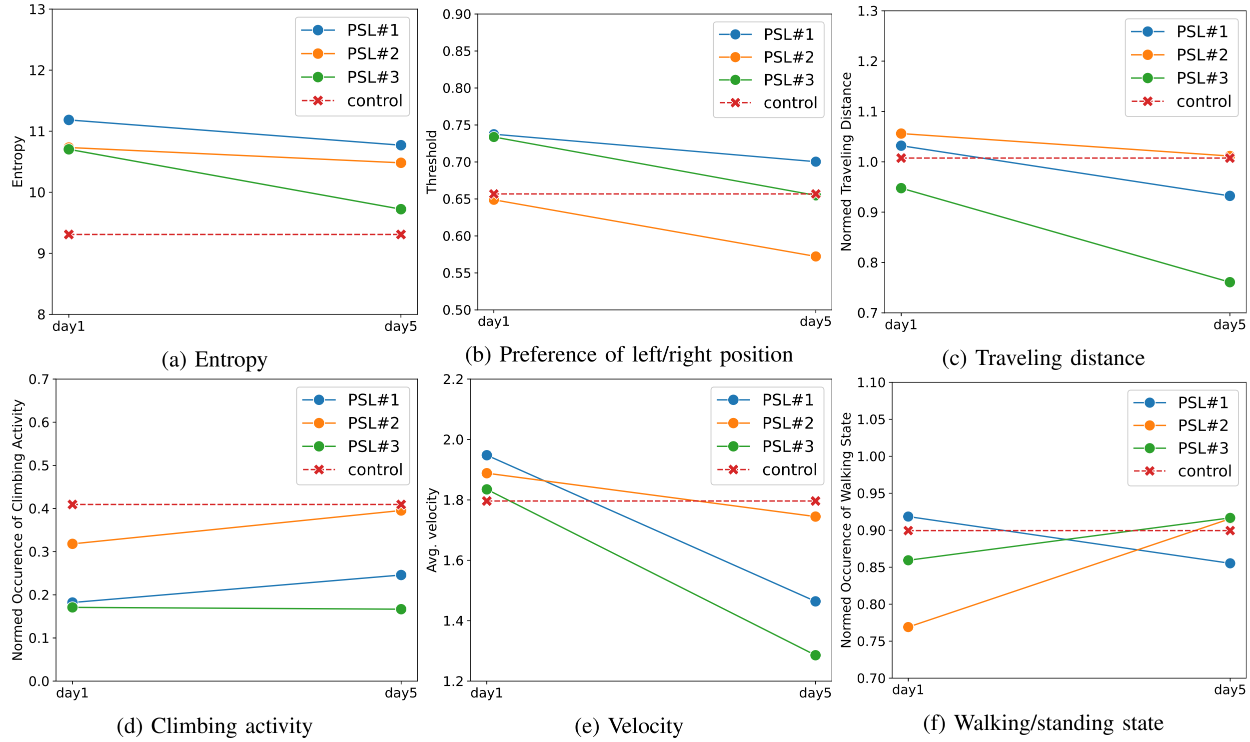
B. Behavioral Indexes Comparison and Statistical Analysis

Figure 4 shows the behavioral indexes of all three PSL rats across the first- and fifth-day sessions, along with the average values from the control group for comparison. It shows that by measuring the difference between both sessions, all the behavioral indexes can indicate the rat's behavior changes after a recovery process. Most behavior indexes, such as entropy, preference of left/right positions, traveling distance, and velocity, have similar decreasing trends by the fifth-day session. Additionally, some indexes tend to approach the control group's average values over time.

Table II shows the trends of PSL and Sham groups between first- and fifth-day sessions. $\downarrow$ and $\uparrow$ indicate the decreasing and increasing trend with a significant difference, respectively, while $\searrow$ and $\nearrow$ indicate the decreasing and increasing trend with a non-significant difference, respectively. $-$ means there is no clear trend among the subjects. We found that the PSL group has decreasing trends in entropy, preference of left/right positions, traveling distance, and velocity index. In the Sham group, only their climbing activity has an increasing trend. Based on a statistical t-test, we also found a significant difference ($p < .05$) in preference of left/right positions between both sessions in our experiment. The velocity index between both sessions also has a significant difference ($p < .10$). Even though other behavior indexes do not have significant differences statistically, the trends between them are usable for investigation.

IV. Discussion and Future Works

Our results suggest significant behavior changes of the PSL group caused by ligation at the sciatic nerve, and surgery at the right hind leg. The analysis was carried out using



(a) Entropy (b) Preference of left/right position (c) Traveling distance

(d) Climbing activity (e) Velocity (f) Walking/standing state

Fig. 4: Result of behavioral indexes for all rats in the PSL group

video data from individual animals in a cage, one and five days after the surgery. Operated animals are usually singly housed in cages for a smoother recovery. Single housing is necessary for preventing bites of the surgical wound by a cage mate. Even though the number of rats in the PSL group is 3, we also found the same trend of behavior changes for entropy, preference of left/right positions, traveling distance, and velocity indexes. Furthermore, these indexes also show significant differences between first- and fifth-day sessions. For other behavior indexes that are not significantly different, with more data it might be possible to confirm whether it is significant or not.

To confirm that behavior changes in the PSL group were mainly caused by ligation at the sciatic nerve, we discuss the result of the Sham group in this section. As with the PSL group, we had only two subjects for the Sham group. Since we did not perform sciatic nerve ligation on the Sham group, the behavior changes should be caused by only surgery at the right hind leg. Most indexes showed an inconsistent trend among two rats; thus one rat showed an uptrend while the other showed a downtrend. The comparison of trajectories between PSL and Sham rats shows that despite the surgery, the pattern of Sham animals resembled those of controls. Therefore, it is likely that behavioral changes in the PSL group could be caused by ligation at the sciatic nerve.

Scientifically, the reproducibility of the results should be confirmed in our future study to use them as reliable indexes for the recovery process, and help us to investigate this process in humans. In the case that we can explore the behind of the recovery process in humans, it is possible to create the drug to reduce the duration for recovering.

From the engineering viewpoint, we aim to improve the current system to achieve real-time monitoring and analyzing behavioral changes in rats. The real-time system will increase the speed and potential for experiments related to observe the behavior changes in rats. Furthermore, we also plan to apply the system to other animals such as the dog.

V. Conclusion

This paper presents the behavior in rats is changed after sciatic nerve ligation, through observing the selected behavioral indexes in first- and fifth-day sessions. Our data demonstrated that, based on different trends between PSL and Sham groups, the behavior changes observed in the PSL group could be caused by ligation at the sciatic nerve. Since the main limitation of this study is the small sample size, the reproducibility of our results should be validated by increasing the number of subjects for all groups in further studies.

Validated reliable behavioral parameters may help to detect changes in pain behavior and nerve damage both in animals and humans. The PSL-model is well established animal model and it can be successfully used to develop real-time behavioral monitoring and analyzing methods and establish parameters that can be used to follow recovery. Well-defined parameters can be used in future studies to better characterize the recovery process and for assessing the efficacy of drugs used or developed for treating neuropathic pain.

Future studies should include developing behavioral ethograms for each animal species and disease model, comparing data obtained from behavioral tests and real-time monitoring and analyzing methodologies. Additionally, this method should be implemented by using other disease models such as neurodegenerative animal models.

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