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CHAPTER 3

2D-digital video ankle motion analysis for assessment of function in the rat sciatic nerve model

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ABSTRACT

Background Ankle motion analysis may provide a better method to assess function in the rat sciatic nerve model than the standard method, the sciatic function index (SFI), but it is not widely used in experiments on nerve regeneration, possibly because of complicated analysis.

Methods In this study, we investigated the practical use of a 2D digital video motion analysis system. Reproducibility was investigated in normal rats. Recovery of ankle motion was analyzed after sciatic, tibial, and peroneal nerve crush injury. Results were compared with scores for the SFI.

Results 2D digital video motion analysis proved to be reproducible with no significant difference in results from animal to animal and day to day. Interobserver variability was also small. In the analysis of recovery after separate nerve crush injuries, subtle differences in ankle plantar flexion and dorsiflexion could be detected. The method was also more sensitive than the SFI: whereas scores for the SFI had returned to normal 4 weeks after sciatic nerve crush injury, the ankle angle at mid stance was still significantly different from that in sham-operated animals 6 weeks after the injury.

Conclusion 2D digital video ankle motion analysis is a practical method to assess function in the rat sciatic nerve model that is more sensitive than the standard method of the SFI.

INTRODUCTION

The rat sciatic nerve model is the most commonly used model in experiments on nerve regeneration. Several methods can be used to evaluate the results of regeneration, including electrophysiology and nerve and muscle morphometry [1]. These methods are useful for evaluating different aspects of the regeneration and reinnervation process, but results do not necessarily correlate with the recovery of nerve function [2-6]. Functional analysis eventually may be the most important evaluation method before introducing experimental treatments of nerve repair techniques into patients.

At present, the standard method of analyzing recovery of function in the rat sciatic nerve model is the sciatic function index (SFI) [7]. Introduced in 1982 by de Medinaceli et al [8] and later modified by Bain et al [9], the SFI is based on the measurement of footprints in walking tracks for different parameters of print length, toe spread and intermediate toe spread (Figure 1A). Footprints have been acquired using paper and paint, radiographic film, and photographic paper [7]. A disadvantage is that footprints sometimes can not be measured because of contractures [10], autotomy [11] or smearing. Therefore, video-based footprint analysis techniques have been developed [12-14]. Rats are thereby filmed in a transparent runway that has a mirror placed below the track at a 45° degree angle [12], or rats at

rest are filmed from below in a transparent box to determine the static sciatic index [14]. Although these video-based methods have increased the number of measurable footprints, problems of contractures and autotomy of the foot still remain [7, 10, 11, 15].

Alternative methods to assess function in the rat sciatic nerve model have been investigated, including, analysis of the ankle angle during the stance and swing phases [16-19]. Ankle motion analysis has not been widely adopted in experiments on nerve regeneration possibly because of technical difficulties in acquiring images and analysis of the ankle angle from these images. We investigated the use of two-dimensional (2D) digital video motion analysis to assess ankle kinematics in the rat. The same system is in use in the evaluation of patients with neurologic deficits [20]. In this study we investigated the ease and reproducibility of the method in normal rats. We also determined its sensitivity to detect subtle differences in ankle plantar and dorsiflexion after sciatic, tibial, and peroneal nerve crush injuries. Results were compared with scores for the SFI, the tibial functional index (TFI), and the peroneal functional index (PFI) [9] obtained from the same videos using a mirror placed below the track.

MATERIAL AND METHODS

Ankle motion analysis in normal animals

Ankle motion in rats (female Sprague-Dawley rats, 250 g) was analyzed to determine animal-to-animal and day-to-day variability. The minimum number of trials needed to obtain consistent measurements was determined from the range in results for an increasing number of trails. The mean results for this number of trails were compared for the analysis of three and six normal animals to determine the minimum number of animals needed for consistent measurements. Animal-to-animal variability was determined by comparing means for this number of trails and animals (one-way analysis of variance). Day-to-day variability was determined from comparing results for the same animals on two different days (paired analysis). Interobserver variability was determined for the same trials by two independent observers. Coefficient of repeatability was calculated [21]. Linear correlations (Pearson) between animal speed and ankle motion were investigated.

Sciatic, tibial, and peroneal nerve crush injury

Deficit and recovery of ankle motion after crush injury were analyzed in 12 rats assigned to one of four experimental groups: sham operation or sciatic, tibial, or peroneal crush (three per group).

To apply nerve crush injury, the sciatic nerve was exposed through a 1-cm incision in the buttock, with blunt spreading and retraction of the gluteus maximus muscle. The sciatic, tibial, or peroneal nerve was crushed for 5 s using smooth-tipped forceps. The fascia of the gluteus maximus muscle was closed using a continuous

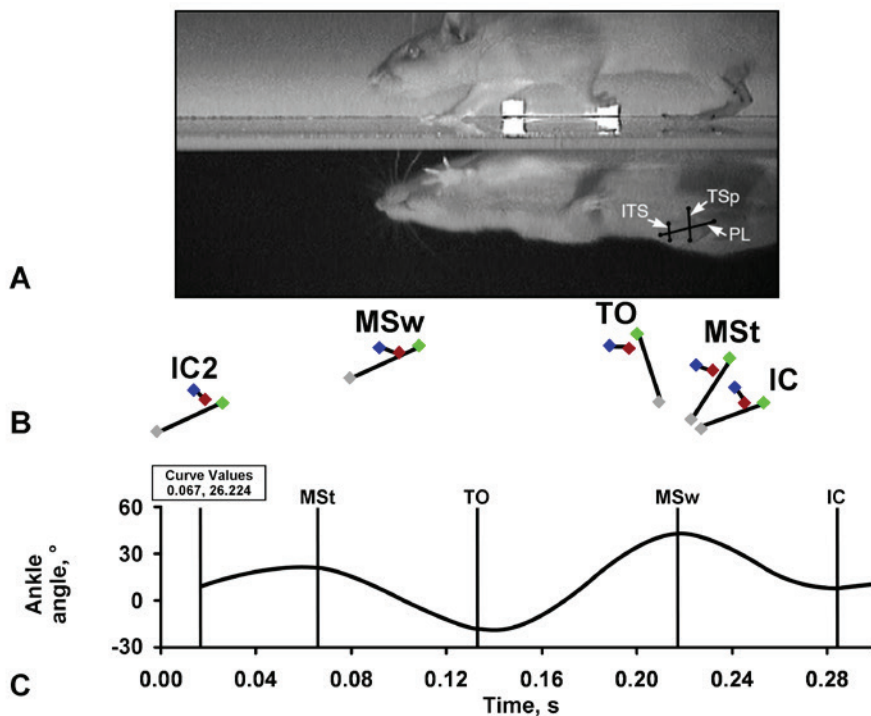


Figure 1

(A) Example of a frame from a digital video showing the rat in the transparent runway with markers on the tibia, lateral malleolus, calcaneus, and fifth metatarsal to create a two-dimensional ankle model. It also shows the mirror that was placed below the transparent track at a 45° angle to analyze the footprints for the print length (PL), toe-spread (TSp), and intermediate toe-spread (ITS) in the same trial. (B) Colored stick figures show the position of the ankle model at the different moments of initial contact (IC), mid-stance (MSt), toe off (TO), mid-swing (MSw), and a second IC. (C) Results for the change in ankle angle after automatic tracking of the markers and filtering (Butterworth filter set to 6 Hz) presented in a report by the Vicon Peak analysis system. A line (in this figure positioned at MSt) can be scrolled along the curve to obtain the value of the ankle angle at any time in the step cycle, as shown in the “Curve Values” box.

3.0 polyglactin 910 suture. Skin was closed with wound clips. The same surgical procedure was used for the sham operation but without nerve crush.

All rats were filmed 1 week before injury and weekly after injury for 6 weeks. Animals were housed in separate cages with a 12-h light-dark cycle. To prevent contractures, a wire mesh was placed in the cage [22]. The left foot was treated daily with Chew-Guard (Butler Animal Health Supply LLC) to prevent autotomy. All procedures were approved by the Mayo Clinic Institutional Animal Care and Use Committee.

Mean results for ankle motion after sciatic, tibial, and peroneal crush were compared with means in sham-operated animals using the Student’s *t* test.

2D digital motion ankle analysis

Before filming, rats were briefly anesthetized using isoflurane inhalation (IsoFlo, Abbott Animal Health, UK). The left lower limb was shaved. Black dot markers were placed on bony landmarks with a permanent marker. The proximal point of the lower third of the tibia, the lateral malleolus, the calcaneus and the fifth metatarsal were marked (always with the ankle fixed in a 90° angle) to create a 2D biomechanical model of the ankle (Figure 1A) [19]. Black dots were also placed on the undersides of the toe tips for digital footprint analysis (Figure 1A).

Filming was performed in a darkened room by two observers using the Vicon Peak motion analysis system. Animals were placed in a transparent runway (120cm long, 12cm wide, 30cm tall with a 45° angled mirror below the track). Rats were trained to run to a dark box at the end of the runway. Images were acquired with a 60-Hz digital camera (Dinion^{XF}, Bosch Security Systems) placed 1 m from and perpendicular to the runway to prevent optical distortion. Only trails with acquisition of one complete step cycle (from the moment the left foot touches the floor of the runway at the beginning of the stance phase until it touches the floor again at the end of the following swing phase) and a left and right footprint were used (Figure 1B). Trials were selected for gait speed based on a step cycle duration of 0.25-0.50s. Trials in which the rats were galloping (both feet in the air) were excluded.

Trials were processed using software (PeakMotus 8, Vicon Peak) that tracked the marker dots in all frames of the video. Marker placement was manually corrected as needed. The frequency of the step cycle was 2-4 Hz (0.25-0.50 s). Therefore, data were filtered using a Butterworth filter set to 6 Hz. Results were presented as the ankle angle in degrees as a function of gait cycle (Figure 1C). From the continuous curve, ankle angle was recorded at different even times during the gait cycle (Figure 1):

- *Initial contact (IC)*: the moment the left foot touches the ground
- *Mid stance (MSt)*: the moment the right foot in the air crosses the left foot bearing the animal's weight.
- *Toe off (TO)*: the moment the left foot comes off the runway, the moment of maximum plantar flexion
- *Mid-swing (Msw)*: the moment the left foot crosses the right foot in the stance, the moment of maximum dorsiflexion.

Ankle angles were reported in degrees from the neutral position with dorsiflexion being positive and plantar flexion being negative. Gait speed was calculated by dividing the horizontal displacement of the marker on the fifth metatarsal from IC to IC by duration of the step cycle.

SFI, TFI, and PFI

Footprint analysis was performed in the same trials using the motion analysis software. Measurements were taken from footprint images in the frame before heel rise by manually identifying all toes. The most posterior point of the heel still in

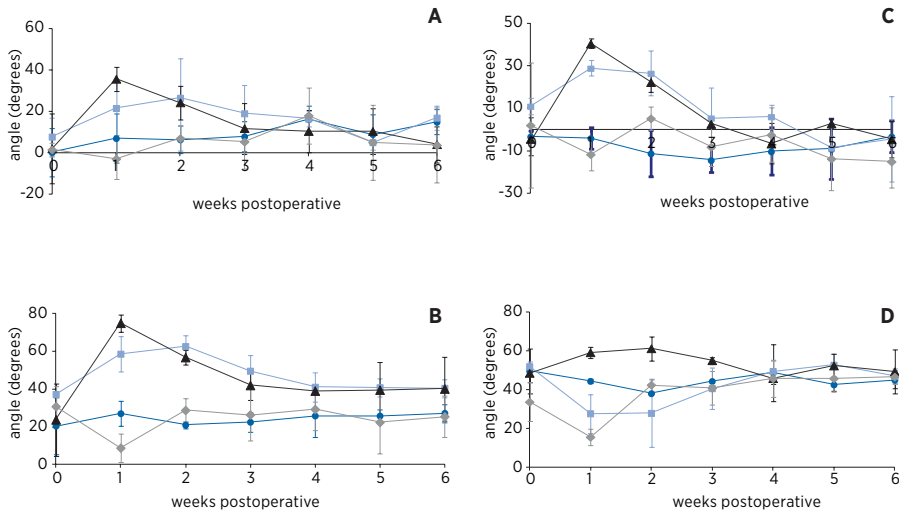


Figure 2

Recovery of the ankle angle over time at (A) initial contact, (B) mid-stance, (C) toe off, and (D) mid-swing after sham operation (O) or sciatic (■), tibial (Δ), or peroneal (◆) nerve crush injury (three animals per group).

contact with the runway was determined from both the next frame (showing heel rise) and the side view of the ankle. Measurements for print length (from tip of third toe to heel), toe spread (from tip of first toe to tip of the fifth toe) and intermediate toe spread (tip of the second toe to tip of the fourth toe) were digitally acquired (Figure 1A). Scores for the SFI, TFI, and PFI were calculated using the following formulas (modified by Bain et al [9], used with permission):

$$\text{SFI} = -38.3 (\text{EPL-NPL})/(\text{NPL}) + 109.5 (\text{ETS-NTS})/(\text{NTS}) + 13.3(\text{EIT-NIT})/(\text{NIT}) - 8.8$$

$$\text{TFI} = -37.2 (\text{EPL-NPL})/(\text{NPL}) + 104.4 (\text{ETS-NTS})/(\text{NTS}) + 45.6(\text{EIT-NIT})/(\text{NIT}) - 8.8$$

$$\text{PFI} = 174.9 (\text{EPL-NPL})/(\text{NPL}) + 80.3 (\text{ETS-NTS})/(\text{NTS}) - 13.4$$

where EPL = experimental print length, NPL = normal print length, ETS = experimental toe spread, NTS = normal toe spread, EIT = experimental intermediate toe spread, NIT = normal intermediate toe spread.

Table 1

Ankle motion in normal rats

Measurement	Mean±SD (n=6)	Mean±SD (n=3)	P value		
			Animal-to-animal variability*	Day-to-day variability†	CR (n=3)
Ankle angle, °					
IC	2.8 ± 9.0	1.2 ± 11.2	0.02	0.50	19.6
MSt	32.6 ± 17.0	31.2 ± 8.7	0.45	0.78	5.4
TO	3.9 ± 8.3	0.4 ± 6.1	0.28	0.05	5.8
MSw	50.9 ± 7.7	53.7 ± 5.8	0.18	0.17	5.5
Gait speed, cm/s	62.9 ± 18.4	57.9 ± 3.3	0.52	0.21	

CR, coefficient of repeatability; IC, initial contact; MSt, mid stance; MSw, mid swing; TO, toe off.

*Difference in values for different animals ($n=3$) filmed on the same day.

†Difference in values for the same animals filmed on different days (3 rats, 4 or 10 trials/rat).

RESULTS

Ankle motion analysis in normal animals

Ankle angle in normal animals approximated to a sinusoidal form (Figure 1C). The maximum angle of plantar flexion was reached at TO and the maximum angle of dorsiflexion at MSw. Analysis of the range in results for an increasing number of trials showed that after four trials the range did not increase. We therefore obtained four trials per rat for analysis of ankle motion. Filming and selection of four trials on average took 5 min and tracking of markers about 5 min per trial; therefore, a total of 25 min were needed for analysis of one animal.

Mean results for different ankle angles were not significantly different for the analysis of three or six animals ($P > 0.05$). We therefore determined animal-to-animal, day-to-day, and interobserver variability of ankle motion in three animals. Results were not significantly different from animal to animal and day to day (Table 1). Interobserver variability, for the coefficient of repeatability, was small. There was no significant correlation between the speed of the animal and the different ankle angles (IC, $P=0.51$; MSt, $P>0.99$; TS, $P=0.50$; MSw, $P=0.16$).

Deficit and recovery of ankle motion after sciatic, tibial or peroneal nerve crush injury

During follow-up, no autotomy or foot ulcers occurred, and ankle motion was not limited by contractures. The mean weight of the animals increased from 250 ± 9 to 268 ± 10 g after 6 weeks.

Table 2

Deficit in ankle motion 1 week after nerve crush injury

Crush injury	Ankle angle, degrees (°)*				SFI/TFI/PFI scores
	IC	MSt	TO	MSw	
Sham operation	7.1 ± 12.0	27.0 ± 6.6	-4.0 ± 5.2	44.5 ± 1.2	SFI, -3.19 ± 9.11 TFI, 11.3 ± 8.5 PFI, 2.3 ± 19.3
Sciatic nerve	21.6 ± 13.0	58.5 ± 9.5 [†]	28.8 ± 3.6 [†]	27.8 ± 9.9 [†]	SFI, -78.40 ± 3.29 [‡]
Tibial nerve	35.7 ± 5.9 [†]	74.9 ± 4.5 [†]	40.4 ± 2.2 [†]	59.2 ± 3.0 [†]	TFI, -79.5 ± 1.9 [‡]
Peroneal nerve	-2.8 ± 9.7	8.8 ± 7.4 [†]	-11.6 ± 7.6	15.7 ± 4.3 [†]	PFI, -54.6 ± 1.6 [§]

IC, initial contact; MSt, mid stance; MSw, mid swing; PFI, peroneal functional index; SFI, sciatic functional index; TFI, tibial functional index; TO, toe off.

*Data are presented as mean ± SD.

[†]Results were significantly different from those of sham-operated animals ($P < 0.05$).

[‡]Results were significantly different from those of sham-operated animals ($P < 0.001$).

[§]Results were significantly different from those of sham-operated animals ($P = 0.007$).

Gait speed significantly decreased after sciatic crush (37.2 ± 2.7 cm/s) compared with sham-operated animals (57.7 ± 10.5 cm/s). Gait speed did not change after tibial (57.4 ± 11.4 cm/s) and peroneal crush (54.1 ± 6.8 cm/s). Ankle angles significantly changed compared with angles in sham-operated animals (Table 2). After sciatic crush, the maximum angle of plantar flexion and dorsiflexion were decreased ($P < 0.001$ and $P = 0.04$, respectively), and the angle at MSt showed a decreased plantar flexion ($P = 0.009$). After tibial crush, there was decreased plantar flexion for the angles at TO and MSt (both $P < 0.001$). The angle at MSw showed increased dorsiflexion ($P = 0.01$). After peroneal crush, the maximum angle of dorsiflexion at MSw was decreased ($P < 0.001$). All other angles showed increased plantar flexion (but only significantly for the angle at MSt, $P = 0.03$).

All angles recovered to normal (sham-operated) values within 2-4 weeks after sciatic, tibial, or peroneal crush injury (Figure 2) except for the angle at MSt (Figure 2B). This angle reached a plateau 4 weeks after sciatic and tibial crush and was still significantly different from the angle in sham-operated animals 6 weeks after sciatic crush ($P = 0.03$) (after tibial crush, $P = 0.27$).

SFI, TFI, and PFI

Scores from the SFI, TFI, and PFI were significantly decreased compared with the scores for these indices in sham-operated animals after sciatic (-78.4 ± 3.3), tibial (-79.5 ± 1.9), and peroneal crush injury (-54.6 ± 1.6) (Table 2). Scores returned to normal 2 weeks after tibial and peroneal crush and 3 weeks after sciatic crush.

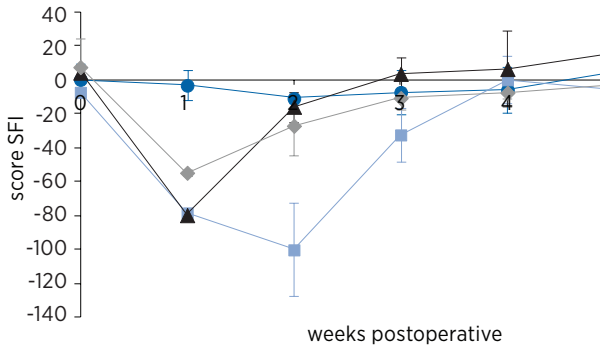


Figure 3

Recovery of sciatic, tibial, and peroneal functional index (SFI, TFI, PFI) scores over time after sham operation (O, SFI score) or sciatic (■, SFI), tibial (Δ, TFI), or peroneal (◆, PFI) nerve crush injury (three animals per group). (SFI could only be determined in two of three animals 1 week after sciatic crush injury because of exorotation of the foot in one animal).

DISCUSSION

Functional analysis is the most important evaluation method for translating experimental treatments for nerve repair into patients. We investigated the use of a 2D digital video motion analysis to assess function in the rat sciatic nerve model. This method was found to be reliable. Results for different ankle angles were not significantly different from animal to animal or day to day except for angle at IC. Interobserver variability for the analysis of the same trials was relatively small. The system was easy to use; analysis of one animal took about 25 min and could be performed by personnel not specifically trained in gait analysis. We took significant measures to reduce variability. Santos et al [16] reported that animal-to-animal variability might be caused by difference in speed. We controlled this by training the animals in the runway before filming and by filming the animals at the same time of day (with a 12-h light cycle, animals were found to be more active during the morning than later in the day). Trials were also selected based on a total duration of step cycle of 0.25–0.50 s. No significant correlations between speed and different ankle angles were found. Future technical refinements may further reduce variability. For instance, tattooed markers may reduce day-to-day variability. More accurate marker placement (e.g. by visualizing bony landmarks with radiography) may improve animal-to-animal variability. Higher camera recording speed may further reduce variability. The maximum angle at TO sometimes occurred between frames (Figure 1C). Finally, other factors such as weight of the animal and laboratory settings may influence results. Therefore, an age-matched control group should be included.

2D digital video ankle motion analysis was found to be a more sensitive method to assess function in the rat sciatic nerve model than the SFI. At the end of the experiment (6 weeks after sciatic nerve crush injury), the angle at MSt was still significantly different from that in sham-operated animals, whereas the SFI had returned to normal at 4 weeks after injury. This sensitivity to detect subtle deficit after nerve crush injury demonstrates that it can be used in longitudinal studies to assess subtle differences in recovery after experimental treatments. In addition, ankle motion analysis is not limited by exorotation, contractures, or autotomy, and it evaluates function of more proximally located muscles, which are reinnervated earlier than distal foot muscles.

The results after separate sciatic, tibial and peroneal nerve crush injury showed that 2D digital video ankle motion analysis can be used to detect subtle differences in plantar flexion and dorsiflexion. After sciatic crush, both the angle of maximum plantar flexion, and after peroneal crush only the angle of maximum dorsiflexion, confirming results by Yu et al. [18]. In addition, in our study, the maximum angle of dorsiflexion was increased after tibial crush, as was the maximum angle of plantar flexion after peroneal crush, as a result of the unopposed function of antagonistic muscles.

2D digital video ankle motion analysis is a reliable and sensitive method to assess function in the rat sciatic nerve model. It opens opportunities to evaluate subtle differences in peripheral nerve regeneration in experimental models with different paradigms.

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