TREADMILL WITH CONTROLLED SPEED FOR RECORDING GAIT

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ABSTRACT

Histological and electrophysiological methods for evaluation of peripheral nerve regeneration do not faithfully reproduce the functional index of limbs, even in controlled experimental conditions. Some methods of functional evaluation have been proposed, but their correlation to histological and electrophysiological data is not completely established, requiring more investigations with improvement of collection, management and processing of obtained data. In this study we developed a treadmill with controlled speed for recording footprint of rats submitted to different kinds of sciatic, fibular and tibial nerve lesions. The footprints were obtained on a walking track by means of a webcam connected to the treadmill and a computer with capacity to record the gait that will be submitted to functional evaluation. The developed treadmill allows the collection and filming of gaits and it has been tested in current experimental studies in rats with sciatic nerve lesion submitted to different kinds of treatment. The treadmill also enables researchers to record gait with constant, controlled and pre-established speed, with the possibility of direct visualization through the treadmill wall. In this manner, some variables that could damage the results of the research are resolved.

Keywords: Crush syndrome. Sciatic nerve. Rats.

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INTRODUCTION

Histological and electrophysiological methods of evaluation of peripheral nerve regeneration do not provide data on the true functional state of the tested limb, even under controlled experimental conditions. Some functional evaluation methods have been proposed[1-4] but the correlation between their results and those of histological and electrophysiological methods has not yet been perfectly established, demanding further investigations. These, on the other hand, require refinement of the methods of obtainment, storage and processing of the data obtained.

Functional evaluation of nerve regeneration

De Medinaceli et al.[1] developed a reliable and reproducible quantitative method for the functional condition of rat sciatic nerve for evaluation of the degree of injury and of recovery, called the Sciatic Functional Index, or SFI. The method is based on the measure of pre-established parameters in the rear footprints of the animals, obtained during gait execution on strips of unused x-ray film. The animals were trained to walk along a bridge that was 43 cm long by 8.7 cm wide, ending in a hutch where they sought shelter. After they were well trained, a strip of x-ray film was placed on the floor of the bridge and the animals' rear paws were dipped in developing fluid. When walking the animals left their rear footprints on the film, which was immersed in setting liquid that fixed them permanently. Four parameters were measured in these footprints (Figure 1) and the comparisons between the injured (experimental) and intact (normal) sides made it possible to establish the SFI, which is a percentile expression of normal. All the parameters were measured the same way in the normal paw (N) and in the injured, or experimental paw (E). To other foot (TOF) distance is the orthogonal measure between the tips of the longest toes of both feet, which represents the length of a step. The print length (PL) is the distance between the two most extreme points of the footprint, in the longitudinal direction, which

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Figure 1 – *Representation of the parameters measured to calculate the Sciatic Functional Index (SFI). N: Normal; E: Experimental (operated); TS: Total spread (1st to 5th toes); IT: Intermediate toes (2nd to 4th); PL: Print length.*

is always higher in the injured paw. The total spread (TS) is the distance between the two most extreme points of the footprint, in the transversal direction, which should coincide with the mark of the two most extreme toes, and the intermediate toe (IT) is the distance between the two intermediate toes. Both measurements are smaller in the injured paw.

The SFI was calculated for normal nerves and nerves submitted to several types of injury, according to the formula:

$$SFI = \left[\frac{(ETOF - NTOF)}{NTOF} + \frac{(NPL - EPL)}{EPL} + \frac{(ETS - NTS)}{NTS} + \frac{(EIT - NIT)}{NIT}\right] * \frac{220}{4}$$

Where:
E: Experimental
N: Normal
TOF: to other foot.
PL: print length.
TS: total spread.
IT: intermediate toes.

The results obtained with this equation were the negative percentile expression of the normal function, in which 0 (zero) corresponds to the normal function, or absence of deficiency, and –100 (minus a hundred) corresponds to total dysfunction. The authors guaranteed that the method was reliable and reproducible, having a correlation with conventional methods, but the calculations, all manual, were very complicated.

De Medinaceli et al.[5] modified the method, adding IT and data processing resources, making it faster, easier and more practical.

Brown et al.[6] conducted a study of the reliability of the SFI method, in which four examiners analyzed the measures in different sequences and on three different occasions, concluding that it is a good noninvasive method.

Lowdon et al.[7] created a method for the obtainment of footprints on strips of paper impregnated with bromophenol blue, according to De Medinaceli's method, and analyzed according to the method of Bain et al.[2], both of routine use in our laboratory.[8-10] The paper strips impregnated with bromophenol blue take on a yellowish hue when dry, becoming blue when wet. To obtain the footprints, the animals' rear paws were immersed in a solution of domestic detergent, which produces less print blots than the X-ray film. (Figure 2)

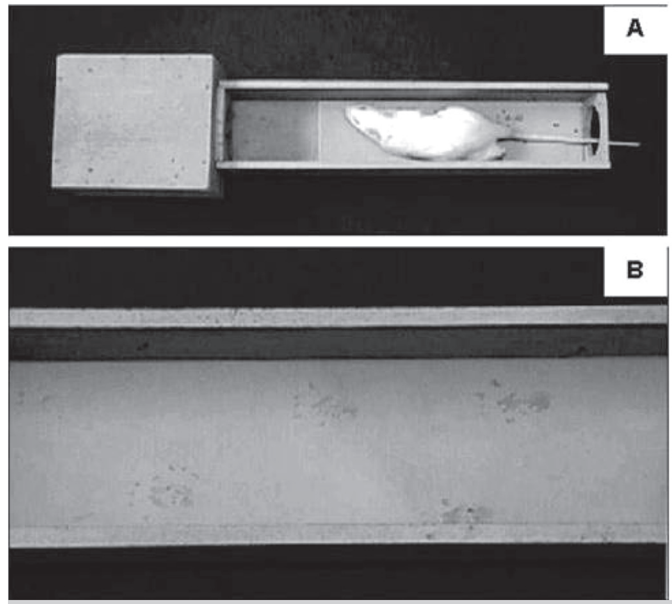

Figure 2 – *Record of prints on the bridge. Overview of the Bridge (A). Prints recorded using the method proposed by Lowdon and collaborators (B).*

Monte-Raso et al.[10] evaluated reproducibility among examiners of the SFI evaluation method, measured by a computer program developed for this purpose. The animals' footprints were obtained according to a method proposed by Lowdon et al.[7] in the preoperative phase and, afterwards, weekly from the 1st to 8th week, were evaluated on a gait track by the measurement of predetermined parameters by four examiners always following the same parameter demarcation sequence using a computer program.[11] The results were submitted to statistical analysis, which showed a high rate of correlation among examiners in the preoperative evaluation and in the 3rd, 4th, 5th, 7th and 8th weeks (0.82 or higher), with casual decrease in the 6th week, but remained significant like the others ($p_F<0.01$). In the 1st and 2nd weeks, the correlation index was close to zero, showing the low reproducibility of the method in this period, in which variability among the animals did not differ from variability among the examiners ($p=0.24$ and 0.32, respectively), due to the poor definition of the footprints.

Gasparini et al.[12] correlated different methods of functional gait assessment of rats with injury caused by crushing of rat sciatic nerve, the SFI (proposed by De Medinaceli) calculated in a manual and computerized manner (also proposed by De Medinaceli), the SFI calculated manually and with the filming method (proposed by Varejão)[13] and the computerized SFI with the filming method. The authors concluded that the analysis methods used guarantee the monitoring of functional recovery, but that the hypothesis of correlation of methods is real for the conditions in which the same mathematical formula is used, regardless of whether it is manual or computerized but, in the case there is combination among mathematical formulas and no correlation among methods, they also concluded that the method of evaluation through filming guarantees the monitoring of its regeneration in the first weeks, eliminating the difficulties existing in the traditional method of De Medinaceli, yet the authors did not control the walking speed of the animals.

In spite of the reliability of the SFI method, in almost all the studies conducted previously in our department, it was obvious that the evaluation produced difficulties in the first weeks after production of the sciatic nerve lesion because the footprints were invariably of poor quality, since the paralysis imposed on the animals' limbs prevented well-marked steps. Therefore, the aim of this study was to improve a method of assessment of functional recovery of sciatic nerves of rats submitted to crushing injury through the obtainment of the image in a more refined manner, by means of filming with more precise control of the animal's walking speed.

MATERIAL AND METHODS

We designated a treadmill (Figure 3) that was built by a company from Ribeirão Preto, specialized in research equipment (Insight®) and the final model of the treadmill appeared in an instruction manual containing the equipment data.

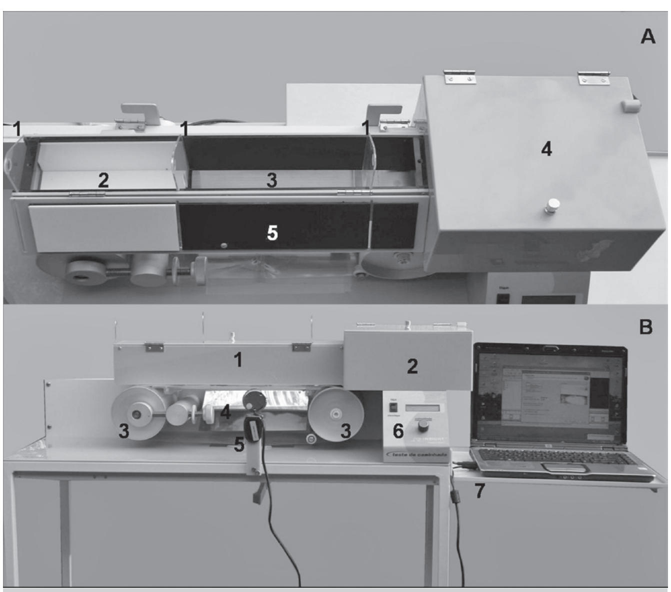

Figure 3 – *Treadmill for gait capture by a filming method with speed control. (A): View of the work area: 1 – Drop gate to restrain the animals, 2 - waiting area, 3 - work area, 4 - "animal shelter" hutch, 5 – roof in antireflective matte acrylic. (B): Overview of the treadmill: 1 – gait corridor "waiting and work areas", 2 - "animal shelter" hutch, 3 - cylinders, 4 - mirror, 5 - support for webcam, 6 - motor, potentiometer, display and on-off switch, 7 - support for portable computer.*

Treadmill with speed control

The treadmill was made of 4 mm gray, 4 mm crystal and 4 mm matte black acrylic, with a metallic framework coated with white epoxy paint. Its height is 1090 millimeters, its width 400 millimeters and its length 850 millimeters, with a weight of 20 kilograms, supply voltage of 110/220 manual volts, with adjustable level feet, and speed adjustment from 0 to 14 meters per minute. The treadmill was developed with polycarbonate, which due to its transparency allowed the filming of the animals' gait, using a 1.3 mega pixel webcam coupled to a portable computer.

Then we carried out a pilot study to test the operation of the treadmill, whose methodology is reported below.

Animals and experimental group

The experimental work was developed in the Bioengineering Laboratory of Faculdade de Medicina de Ribeirão Preto. Ten adult male Wistar rats were used for the study, with body mass ranging between 250 and 300 grams, supplied by the Central Biotery of the Prefecture of the Campus of USP de Ribeirão Preto and kept in collective cages with five animals in each one. The animals were allowed unrestricted access to standard food and water.

A functional assessment was executed on the normal animals, without injury for two days running, using the new treadmill with a filming method with controlled speed, followed by a functional assessment at two different times, using software developed in our laboratory and modified recently.[11]

Functional gait analysis

Functional gait analysis was performed through the obtainment of images by means of video using a treadmill with controlled speed. The filming was based on other studies[13,14] with some modifications.

Footprints by the new proposed filming method with controlled gait speed.

The Insight® group initially developed a treadmill made of acrylic for better footprint visualization, endowed with a speed controlling motor, based on the wooden bridge proposed by De Medinaceli. The gait was recorded with a positioned webcam equipped with a USB cable that connected it directly to a portable computer. (Figure 3)

After learning to walk on the treadmill, the footprints of the rats' normal gait were filmed for two days in a row.

The images of the footprints obtained in the filming sessions were adapted to the ideal size by means of Photoshop Adobe software in version CS3® and edited for transformation of the ideal size for use of the analysis program, in order to allow the calculation of the Functional Sciatic Index.

These images were introduced into the computer program, which allows the identification, analysis of the images according to the parameters selected previously and data storage with the aid of the graphic analysis program especially developed for this purpose,[15] in the Bioengineering Laboratory of FMRP. This program was modified recently[11] and is routinely used in our laboratory.[8-10,16,17] Once the footprint was installed in the program, the parameters analyzed were measured by simply clicking the mouse cursor on the points corresponding to each parameter, in the predetermined sequence. The parameters measured were print length (PL), TS, or total spread of toes, from the 1st to the 5th, and IT or intermediate toes from 2nd to 4th, as suggested by Bain et al.[2] Once the parameters are recorded, the program automatically calculates the Functional Sciatic Index (FSI) value, which will also automatically store it in a file, in order to permit the analysis of the regeneration curves over time.

Statistical Analysis

The data were analyzed using the method of Kolmogorov and Smirnov, adopting a significance level of 5% ($p<0.05$), GraphPad InStat software, version 3.06.

RESULTS

A total of ten animals were submitted to gait on the treadmill with speed control and their gaits filmed. Twenty footprints were therefore analyzed and measured. (Table 1)

Table 1 – *FSI values in the first and second evaluation*

Rat	Gait 1	Gait 2
R1	–0.916	–3.473
R2	–0.763	–3.457
R3	–1.658	–2.785
R4	–3.683	–2.412
R5	–2.635	–0.026
R6	–1.151	–2.566
R7	–4.546	–4.069
R8	–3.211	–3.285
R9	–1.781	–3.065
R10	–2.389	–0.970
Mean Value	**–2.273**	**–2.611**
Standard Deviation	**1.255**	**1.234**

In the first gait assessment the mean values of FSI were -2.7 (variation from -0.7625 to -4.5458) $\pm$ 1.2, and in the second assessment the mean values were -2.6 (variation from -0.0257 to -4.0693) $\pm$ 1.2. (Table1) No significant statistical difference was observed in the comparison of these data ($p=0.5618$). (Figure 4)

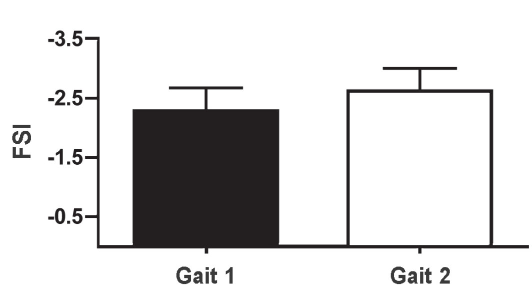

Figure 4 – *Behavior of FSI in the first evaluation and in the second evaluation.*

DISCUSSION

A lot of the knowledge accumulated about degeneration, treatment and regeneration of the peripheral nerves has been produced by experimental studies on small animals, particularly on rats, whose spontaneous regeneration speed favors short lasting studies. The rat sciatic nerve is a reliable model to study different types of lesion and treatment methods, and the crushing lesion is one of the preferred types, as it causes rupture of the nerve fibers without rupture of most of the nerve sustention structures, which facilitates the subsequent regeneration of the lesion.[18-20]

Functional evaluations are very difficult in animals, for obvious reasons, but De Medinaceli et al.[1] introduced the rat functional sciatic index (FSI) method, modified by Bain et al.[2], which makes it possible to satisfactorily evaluate the recovery of the injured sciatic nerve over time. Studies previously conducted by our group showed the existence of correlation between regeneration, evaluated by nerve morphometry, and functional recovery, measured by the FSI.[8-10,16]

In our laboratory, the obtainment of footprints was performed with a basis on the method proposed by Lowdon et al.[7], who described an alternative method to that of X-ray film strips for recording footprints, proposing the use of paper strips, in the same dimensions used with the films and treated with a solution of 0.5% anhydro-sulfone of bromophenol blue in acetone.

Once dry, the paper strips thus treated acquired an orange hue and needed to be stored in a dry place, as humidity made them gradually turn bluish. The preparation of the paper strips is simple and fast, and their functioning is adequate if the drying and storage are satisfactory. The chief advantage of this method is its low cost, besides the reduced risk of the animal slipping during gait, and the image quality superior to that of the X-ray.

Despite the reliability of the FSI method in all our previous studies, the evaluation was obviously difficult in the first weeks after the production of the sciatic nerve injury, because the footprints were invariably of poor quality, since the paralysis imposed on the animal's limb prevented well-marked steps. With the subsequent regeneration of the nerve and the functional recovery of the animal, the footprints became clearer, allowing a more adequate demarcation in the key points of the footprints. The difficulties observed then in the first weeks after production of the lesion gave rise to doubt regarding the fidelity of the method in this phase, which was what led us to investigate the reliability of the analysis method.[18]

In our department,[12] the footprint capture method has recently evolved to obtainment by means of filming on a treadmill made in acrylic based on the studies of Varejão et al.[13]

These observations gave rise to the idea of building a treadmill with controlled speed equipped with a webcam connected directly to a portable computer allowing the filming of the animals' gait, and eliminating some variables observed in previous studies by our group. Speed control allows constant gait without the animal stopping, which could provide an untrustworthy measurement as the animal's weight could favor the spreading of the toes, permitting a false impression of functional improvement. Moreover, webcam use allowed an immediate visual analysis of gait, enabling the researcher to select the most suitable one during its execution.

Altogether ten animals were trained and submitted to gait on the treadmill with speed control. The animals' gaits were filmed in two distinct situations, on two different days, and no statistical difference was observed in the comparison of data.

CONCLUSION

The treadmill with speed control designed, built and tested in experimental investigation proved to be easier to use and more practical than the others used in the previous experiments, as speed control and webcam use decreased some variables found in earlier studies.

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