Original Article

SCIATIC FUNCTIONAL INDEX IN SMASHING INJURIES OF RATS' SCIATIC NERVES. EVALUATION OF METHOD REPRODUCIBILITY AMONG EXAMINERS

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SUMMMARY

The interpersonal reproducibility of the method for evaluating the Sciatic Functional Index (SFI) was measured by a computer-based program developed for this purpose. Twenty Wistar rats were used, and their right sciatic nerve was addressed using general anesthesia. Those nerves were subsequently smashed in a segment of 5 mm proximal to its trifurcation with a special device, with a fixed load of 5 kg for 10 minutes. Animals' footprints were taken at preoperative phase and then in a weekly basis, from the 1st to the 8th week, in a gait track. Prints were digitalized, stored and assessed by predetermined parameter measures, by four examiners, always following the same sequence of parameters marking. Results were submitted to statistical analysis, which evidenced the existence of a high correlation among examiners at preoperative evaluation and on the 3rd, 4th, 5th, 7th, and 8th weeks (equal or higher than 0.82), with an eventual drop on 6th week, but still as significant as the others ($pF<0.01$). On the 1st and 2nd weeks, the correlation index was nearly zero, showing the low reproducibility of the method in that period, in which variation among animals did not differ from the variation among examiners ($p=0.24$ and 0.32, respectively), due to the poor sharpness of footprints.

Keywords: Regeneration; Sciatic Nerve; Rats; Evaluation.

INTRODUCTION

In experimental conditions, the recovery of peripheral nervous injuries is studied mostly through electrophysiology, histology and morphometry techniques. Although electrophysiological and morphological parameters are useful, it is important to know the degree of functional recovery they produce. Functional evaluation in human beings is relatively easy, but, in animals, this is almost impossible when using the same methods, which motivated the search for specific methods in experimental conditions.

Gutmann and Gutmann[1] demonstrated that the inability of spreading posterior foot fingers is a reliable parameter to evaluate the degree of injury, as well as to follow up recovery. Their method, however, was pretty elementary and was not able to measure neither of those. DeMedinaceli et al.[2,3] developed a quantitative, reliable and reproducible method of functional condition of rats' sciatic nerve for evaluating injury and recovery degree, named Sciatic Functional Index, which was subsequently modified by other authors[4,5].

A computer program (software) was developed in our laboratory*, allowing the capture (digitalization with a scanner), storage and analysis of images of rats' footprints, performing calculations according to suggested mathematic formulas and providing the results of functional indexes by DeMedinaceli et al.[2,3], Carlton and Goldberg[4], and Bain et al.[5]. This program was tested in several investigations in our group[6,7,8,9], always showing a strict correlation between morphological and morphometric changes of sciatic nerve and SFI measurements, so that the resulting functional loss caused by injuries produced on rats' sciatic nerves may be monitored by SFI.

Nevertheless, in all investigations, it was clear that predetermined parameters marking on footprints is not always easy, with difficulties being stronger in footprints taken in the first weeks after injury, and being progressively reduced over functional recovery period. In general, printings obtained on the first and second weeks could barely distinguish the footprint, being almost impossible to mark parameters, this fact being responsible for the high degree of variation on initial SFI values, so as to raise doubts on its reliability during that period. Therefore, the present study aimed to evaluate the reproducibility of the method, using a number of investigators

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and a new computer program, with improvements over the previous one, trying to establish the interpersonal correlation of the results.

MATERIALS AND METHODS

Twenty Wistar adult male rats with body weight ranging from 200 to 300 grams (average: 250 g), provided by a single supplier and maintained in collective cages with five animals each, being fed with standard ration and water *ad libitum*. Prior to any other procedure, the animals were trained to walk in a gait belt built for that end, according to a model by DeMedinaceli and cols (Figure 1) and, as soon as they have learned, the preoperative image of the footprint was taken for calculating the normal Sciatic Functional Index (SFI).

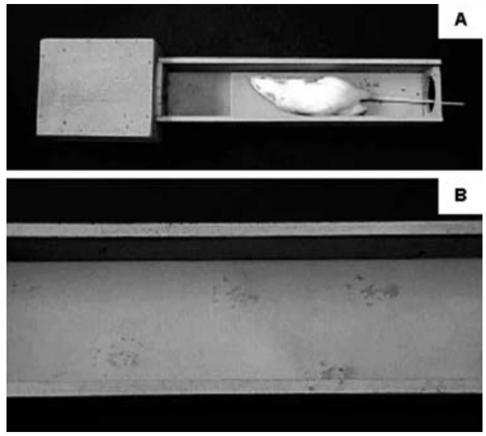

Figure 1 – *Record of footprints on the belt. View of the Belt (A). Recorded footprints (B).*

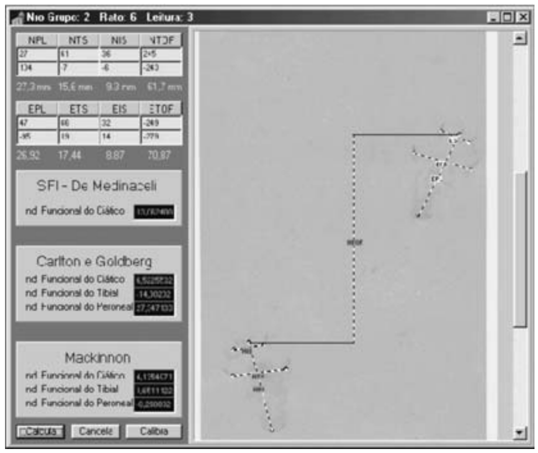

Figure 2 – *Software program screen.*

Surgical Procedure

Rats were anesthetized with a combination of anesthetic drugs (Ketamine 5%, Xylasine 2%, ratio 1:4, 0.13 ml/100 g body weight). Sciatic nerve was addressed through a straight longitudinal skin incision, 3 cm long, at the lateral surface of the thigh, from the major trochanter up to knee. Once exposed, the nerve was submitted to a smashing injury, comprised in a segment of 5 mm long, proximal to its trifurcation, using, for this, tweezers specially built for this purpose, calibrated for a static load of 5,000 g. Then, the nerve was carefully detached from the tweezers and repositioned in its original bed, and surgical wound being closed by planes with isolated stitches of nylon 5/0 (Mononylon, Ethicon®).

Capture and analysis of footprints

Footprints images of the lower feet were captured using paper strips soaked with bromophenol blue in 1% acetone solution and allowed to dry, according to the method proposed by DeMedinaceli and cols. and modified by Lowdon et al.[10]. The piece of paper impregnated with bromophenol blue becomes yellow when dried, but turns immediately and permanently to blue again when in contact with water or any aqueous solution. Instead of water, in this study, animals' low feet were plunged into regular domestic detergent, which precludes dispersion of prints. Then, the animals walked on impregnated paper strips arranged on the belt and left, in average, three prints of each foot. Strips containing footprints were allowed to dry and copied in a high-resolution scanner, and digitalized images were stored and analyzed via computer, using the software previously mentioned[6]. Data concerning each animal were individually identified, so as to allow follow-up over time. The parameters measured for both normal (N) and operated (E) feet were footprint's length (PL, or longitudinal distance between the tip of the longest toe and the heel), total toes spreading (TS, or cross-sectional distance between the first and fifth toes), and intermediate toes spreading (IT, or cross-sectional distance between the second and the fourth toes). Using the specific computer program (Figure 2), key points for each parameter were simply clicked with the mouse at monitor's screen, in a previously determined sequence, and the SFI was then automatically calculated, including the creation of a graph with regeneration curve related to time. The formula used for SFI calculation was the one proposed by Bain et al.[5], as follows:

$$SFI = -38.3 \times \frac{EPL-NPL}{NPL} + 109.5 \times \frac{ETS-NTS}{NTS} + 13.3 \times \frac{EIT-NIT}{NIT} - 8.8$$

Footprints were obtained and analyzed in a weekly basis, from the first to the eighth weeks postoperatively, first by an investigator experienced with the method and, then, by thee examiners to whom the method was carefully taught. Each examiner should make three evaluations of each footprint in alternate days, thereby extracting an average value, which was taken for subsequent calculations and for statistical analysis.

Statistical analysis

For the statistical analysis of data, the random effects model was used[11]; at first, we aimed to validate the evaluation method, by comparing the variation of values obtained from the rats for each examiner to the variation seen for each rat among investigators by means of F test with a significance level below 1% ($p<0.01$), in each week of the total follow-up period, of eight weeks (nine evaluations, including pre-operative). If the values for a same rat were homogeneous for the four investigators, then the method was considered efficient to evaluate injury recovery. To complement this study, an intra-class correlation coefficient was

* Selli, M. Development of a computer-based method for functional assessment of peripheral nerves injuries through gait analysis: an experimental model in rats. Mastership essay. FMRP-USP, Ribeirão Preto, December 15th, 1998.

calculated, which measures homogeneous results reproducibility degree; the closer it is to the unit, the more efficient a method is.

RESULTS

General

Animals tolerated anesthesia and the surgical procedure well. At first, the animals could not support load on operated foot, presenting footdrop and toes in full adduction, as a result of a severe sciatic nerve dysfunction. They gradually recovered the ability to apply load to the operated limb and to spread toes.

Time	Pf	r
initial	<0.01	0.84
1st week	0.24	0.06
2nd week	0.32	0.04
3rd week	<0.01	0.88
4th week	<0.01	0.88
5th week	<0.01	0.82
6th week	<0.01	0.74
7th week	<0.01	0.82
8th week	<0.01	0.89

***Table 1 –** Test's P values (*pf*), intra-class correlation coefficient (r).*

Gait Functional analysis (SFI)

One hundred eighty (9 x 20) footprints were assessed, totaling 720 (4 x 180) evaluations. At preoperative evaluation, the average SFI value measured by all four investigators on the 20 animals was -5.72 (range: -6.84 to 3.45). Differences among investigators were not significant at preoperative period, but measurements were highly significantly correlated to each other (<0.01) and the intra-class correlation coefficient (r) was 0.84, showing a high reproducibility of the method for intact animals (Table 1).

On the first week postoperatively, the average SFI value was -78.88 (range: -93.91 to -61.55), through -77.9 (range: -96.22 to -62.9) on the second week, -33.72 (range: -35.47 to -31.89) on the third week, -25.5 (range: -26.68 to -24.88) on the fourth week, -21.86 (range: -22.4 to -21.22) on the fifth week, -19.79 (range: -22.8 to -19.19) on the sixth week, -13.7 (range: -14.36 to -13.09) on the seventh week, and -11.46 (range: -11.86 to -10.94) on the eighth week.

The differences among values individually measured by investigators were significant for the first and second weeks postoperatively, and the measurements did not correlate to each other (=0.24 and =0.32, respectively), but no longer in none of subsequent weeks (<0.01) (Table 1).

The intra-class correlation coefficient was 0.06 and 0.04 on the first two weeks, showing a very weak correlation among values measured by investigators. But, son subsequent weeks, it ranged from 0.74 to 0.89, evidencing a higher homogeneity among measurements (Figure 3).

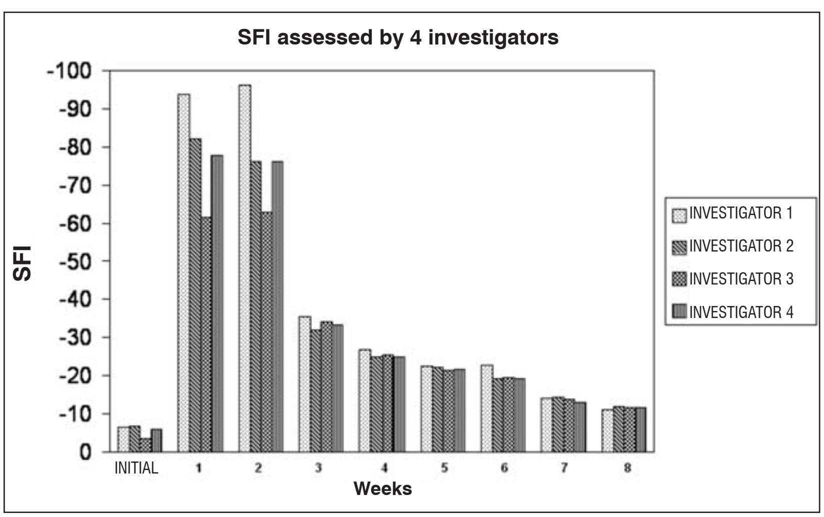

***Figure 3 –** Weekly evaluation of SFI by the four investigators.*

DISCUSSION

Peripheral nerves regeneration seen in experimental studies presents controversial aspects, because it is not always possible to establish a clear correlation between results achieved by different methods in a same investigation, or between results achieved by a same method employed in different investigations or conducted by different authors. Most of peripheral nerves regeneration studies employ histomorphometric and electrophysiological methods, which, although essential, do not anticipate any information about the corresponding functional recovery, which would be of best interests. Functional evaluations are very difficult in animals, for obvious reasons, but De Medinaceli et al.[2,3], introduced the SFI method in rats, modified by other authors[4,5] which enables a satisfactory evaluation of injured sciatic nerve recovery over time. Studies previously conducted by our group showed a clear correlation between morphologic regeneration – evaluated by nerves morphometry, and functional recovery, measured by SFI[6,7,8,9,12].

Despite the obstacles, the SFI method still seems to be the most versatile one, because its application is easy and cheap compared to other proposed methods, and these are probably the reasons for its wider application. Brown et al.[13], conducted a study on SFI method reliability in which four investigators analyzed measurements in different sequences and in three different occasions, concluding that this is a good non-invasive method. Basso et al.[14], developed a qualitative evaluation scale (BBB) to assess recovery after spinal cord injury; animals carrying those injuries were allowed to walk in an arena, some parameters being used to qualify the degree of injury and motion recovery, but it is not a quantitative method, as SFI. However, a high correlation has previously been shown between SFI and BBB method [15]. Varejão et al.[16], developed a quantitative kinematical method for evaluating nervous regeneration, based on the measurement of animal's foot angle (TOA, or *toe-out-*

angle), using as reference the calcaneus and the fifth and third toes, as assessed through computer-based analysis. According to the authors, this method had a good correlation with SFI, but is difficult to apply. Bervar[17] compared an evaluation method using video-analysis with SFI and found a positive correlation between each other, as well, yet concluding that the video-analysis method offers the advantage of making digital images available.

Although SFI method reliability, in all our previous studies, the difficult evaluation on the first weeks after sciatic nerve injury production was clear, because footprints were invariably of bad quality, since palsy imposed to animal's limb precluded a well marked step. With the subsequent nerve regeneration and animal's functional recovery, footprints became clearer, enabling a more adequate marking of footprints' key points. So, difficulties seen during the early weeks after injury production raised doubts on method's fidelity in that phase, which is the purpose of our investigation.

The methodology employed here has followed the same approach as previous studies, with an injury model by sciatic nerve smashing, which presents the advantage of preserving at least partially the nerve framework and not requiring specific training or repair techniques and materials, as in the case of section followed by suture [2,3,6,7,8,9]. Injury by smashing was produced in all animals with tweezers specially made for that purpose, regularly calibrated with a load cell; thus, its application was controlled and the injury produced was more homogeneous, similarly to other previous studies, with the use of an assay universal machine, and quite different from the methods described by other authors, such as hemostatic or watchmaker-type tweezers[18]. Furthermore, injuries were produced always by the same investigator, for being most experienced with the method. Among the other three investigators, one knew how to deal with the method, but the remaining two did not, despite being largely experienced in computers. For these, the method was exhaustively explained and the test routine was demonstrated many times, until they felt confident to perform it. Besides, the tests performed by them were checked by the most experienced investigator, yet without bias, even when one of them made a mistake changing his measurements average, on the sixth week, causing a decrease of the inter-investigators correlation index.

Within the severity of the investigation, results achieved were highly reliable and confirmed the suspect that the bad quality of footprints during the first two weeks made an adequate evaluation almost impossible. Such fact was evidenced by the absence of an intra-class correlation in that phase, with rates near to zero (0.06 and 0.04), much below those observed from the third week on (above 0.82). At the preoperative period, as well as from the third postoperative week on, with a better footprint quality, evaluations became easier and measurements values more homogenous. Additionally, the variability among rats was not different from the variability among investigators for the first two weeks ($p=0.24$ and 0.32, respectively).

CONCLUSION

The authors conclude that the experimental method of functional evaluation by the Sciatic Functional Index in rats is fully reproducible and reliable when applied after the second week of an injury produced by nerve smashing.

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REFERENCES

1. Gutmann E, Gutmann L. Factors affecting recovery of sensory function after nerve lesion. J Neurol Psychiatry. 1942; 5:117-29.
2. De Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condution of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol. 1982; 77:634-43.
3. De Medinaceli L, Derenzo, E, Wyatt, RJ. Rat sciatic funcional index data management system with digited input. Comp Biom Res. 1984; 17:185-92.
4. Carlton JM, Golberg NH. Quantitative integrated muscle function following reinervation. Surg Fórum. 1986; 37:611-12.
5. Bain JR, Mackinnon SE, Hunter RT. Functional evaluation of complete sciatic peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg. 1989; 83:129-38.
6. Oliveira EF, Mazzer N, Barbieri CH, Selli M. Correlation between funcional index and morphometry to evaluate recovery of the rat sciatic nerve following crush injury: experimental study. J Reconstr Microsurg. 2001; 17:69-75.
7. Mendonça AC, Barbieri CH, Mazzer N.Directly applied low intensity direct electric current enhances peripheral nerve regeneration in rats. J Neurosci Methods. 2003; 129:183-90.
8. De Sá JM, Mazzer N, Barbieri CH, Barreira AA .The end-to-side peripheral nerve repair functional and morphometric study using the peroneal nerve of rats. J Neurosci Methods. 2003; 136:45-53.
9. Monte RasoVV, Barbieri CH, Mazzer N, Fasan VS. Can therapeutic ultrasound influence the regeneration of peripheral nerves? J Neurosci Methods. 2005; 142: 185-92.
10. Lowdon IMR, Seaber AV, Urbaniak JR. An improved method of recording rat tracks for mesurament of the sciatic functional index of De Medinaceli. J Neurosci Methods. 1988; 24:279-81.
11. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames, Iowa :Iowa State University Press; 1980. 507p.
12. Oliveira EF, Mazzer N, Barbieri CH, Del Bel EA .The use of a muscle graft to repair a segmentary nerve defect an a experimental study using the sciatic nerve of rats as model. J Neurosci Methods. 2003; 133: 19-26.
13. Brown CJ, Evane PJ, Mackinnon SE, Bain JR, Makino AP, Hunter DA, Hare G. Inter-and intraobserver reability of walking-track analysis used to assess sciatic nerve function in rats.Microsurgery 1991;12:76-79.
14. BassoDM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma. 1995; 12:1-21.
15. Souza AS, Da Silva CA, Del Bel EA.Methodological evaluation to enalyze functional recovery after sciatic nerve injury. J Neurotrauma. 2004; 21:627-35.
16. Varejão ASP, Cabrita AM, Geuna S, Pinto PM, Filipi VM, Gramsbergen A, Meek MF. Toe out angle: a functional index for the evaluation of sciatic nerve recovery in the rat model. Exp Neurol. 2003; 183:695-99.
17. Bervar M. Video analysis of standing- an alternative footprint analysis to assess functional loss following injury to the rat sciatic nerve. J Neurosci Methods. 2000;102:109-16.
18. Bridge PM, Ball DJ, Mackinnon SE, Nakao Y, Brandt K, Hunter DA, Hertl C. Nerve crush injuries: A model for axonotmesis. Exp Neurol. 1994; 127:284-90.