CORRELATION BETWEEN DIFFERENT METHODS OF GAIT FUNCTIONAL EVALUATION IN RATS WITH ISCHIATIC NERVE CRUSHING INJURIES

ANDRÉA LICRE PESSINA GASPARINI[1], CLÁUDIO HENRIQUE BARBIERI[2], NILTON MAZZER [3]

SUMMARY

A study of the correlation between different methods of functional evaluation of the sciatic nerve of rats after a crushing injury was carried out. Twenty five Wistar rats weighing 250 g in average were submitted to a controlled crushing injury of the nerve and evaluated by two conventional methods for obtaining the sciatic functional index (SFI), based on manual and computer-based measurement of parameters on animals´ footprints, and by a new method developed by the authors, based on walking track movie recording and the measurement of the same parameters, on the 1st, 7th, 14th and 21st postoperative days, a comparison being established with the values obtained preoperatively and between the different methods in all periods. The results showed that the method of recording the walking track allows for a better visualization of the injured paw and that this method positively correlates with the conventional methods, as early as the first postoperative week on, provided that the same SFI formula is used.

Keywords: *Evaluation; Sciatic nerve; Regeneration*

Citation: *Gasparini ALP, Barbieri CH, Mazzer N. Correlation between different methods of gait functional evaluation in rats with ischiatic nerve crushing injuries. Acta Ortop Bras. [serial on the Internet]. 2007; 15(5): 285-289. Available from URL: http://www.scielo.br/aob.*

INTRODUCTION

Functional evaluation of peripheral nerve injury in animals is a challenging experimental factor. After nervous injury, morphological and functional recovery is seldom full and perfect, despite of the use of modern and sophisticated reconstruction techniques(1,2). In experimental environments, injuries recovery is studied basically by histology and morphometry techniques(3,4). Although these processes are useful, it is important to follow-up an injury functional recovery. Experimental studies represent an important research source for understanding injuries and peripheral nerves regeneration(5,6). Expert literature is abundant in experimental studies focusing injury(7,8). The use of therapeutic resources is a highlight on regeneration(9,10). A significant focus is driven to the study of functional recovery evaluation, which is always difficult in animals, for understandable reasons(11,12,13).

De Medinaceli et al.(14), developed a quantitative, reproducible method of the functional status of rats' sciatic nerves by analyzing characteristic data from animals' footprints. This method was modified in 1984, with the addition of information technology resources, making it more practical, faster and user-friendly, keeping the measurement of the so-called Sciatic Functional Index by means of the formula:

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

Other researchers modified the existent methods by mathematic formulas, excluding parameters of significant statistical variance(15,16).

$$SFI = \left[\left(\frac{NPL - EPL}{EPL}\right) + \left(\frac{ETS - NTS}{NTS}\right) + \left(\frac{EIS - NIS}{NIS}\right)\right] \times 73$$

Bain et al.(5) conducted an experimental study in rats intending to develop and test independent and more reliable equations for functional evaluation of sciatic, tibial and peroneal nerves regeneration, by using the following mathematic formulas:

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

$$TFI = -37.2 \times \left(\frac{EPL - NPL}{NPL}\right) + 104.4 \times \left(\frac{ETS - NTS}{NTS}\right) + 45.6 \times \left(\frac{EIT - NIT}{NIT}\right) - 8.8$$

$$PFI = 174.9.3 \times \left(\frac{EPL - NPL}{NPL}\right) + 80.3 \times \left(\frac{ETS - NTS}{NTS}\right) - 13.4$$

Despite those modifications, limiting factors for using the method still existed, making it difficult to apply, such as, for example, the need of hardware with specific software for addressing walking track, and even how to obtain paw prints.

Different techniques have been developed for properly capturing an animal's footprint. Real-time image capturing by means of videotape recording, was an advance in the experimental field of functional evaluation of gait in rats with peripheral nervous injuries(3,13). Visualization, recorded with no footprint failures, enables an easier marking of required measures for obtaining the Sciatic Functional Index (SFI).

Despite of the available literature addressing the use of different methods of functional evaluation, no studies are found based on a correlation between such methods. Thus, we intended to submit animals with sciatic nerve crushing, continuous-load injuries to known functional evaluation methodologies and to adapt videotaping methodology to a software assessing gait function since the first postoperative day, presenting an easier analysis of the footprints, in addition to establish a correlation between those methodologies.

Study conducted at the Department of Biomechanics, Medicine and Locomotive Apparatus Rehabilitation, Ribeirão Preto, University of São Paulo.
Correspondences to*: Rua Aldo Focosi, 301, apto 63 – Jardim Santana - CEP: 14091-310 – Ribeirão Preto – São Paulo - e-mail: andrea.licre@terra.com.br*

1. Physical Therapist, Ph.D. Student of the Post-Graduation Program in Orthopaedics, Traumatologyand Rehabilitation. Department of Biomechanics, Medicine and Locomotive Apparatus Rehabilitation – FMRP – USP
2. Chairman of the Department of Biomechanics, Medicine and Locomotive Apparatus Rehabilitation – FMRP – USP
3. Associate Professor, Department of Biomechanics, Medicine and Locomotive Apparatus Rehabilitation – FMRP – USP

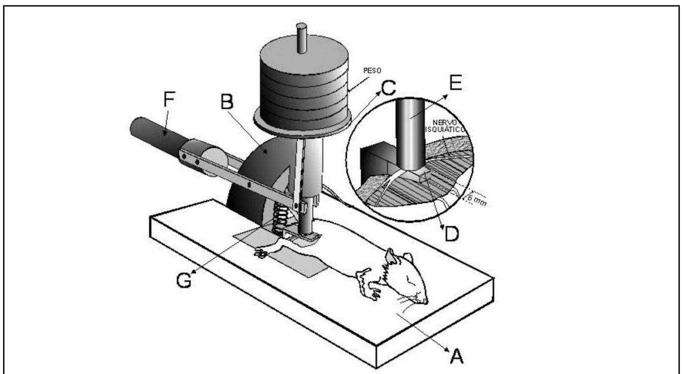
Received in: 16/010/06; approved in: 02/20/07

MATERIALS AND METHODS

For this experiment, 25 male Wistar rats weighting 250 g in average (range: 200 – 300 grams), kept in collective cages containing three animals each, in a temperature-controlled environment, with free access to food (specific standard Laboratory animal food) and water, up to the 21st postoperative day, deadline for experiment completion.

For surgical procedure, the animals were anesthetized by using a blend of 5% Ketamine and 2% Xylazine at 1:4 ratio, applied intraperitoneally at a 0.10 ml/100 g body weight dosage. Subsequently, the animals were weighted. Each animal was identified, and the right lower limb prepared for surgical procedure as usual, including trichotomy, antiseptic solution and site protection with surgical drapes.

The animals were positioned at ventral decubitus, with paws tied together in abduction on a proper support, and the sciatic nerve was addressed through a longitudinal straight lateral skin incision, about 3-cm long. The incision extended from femoral major tubercle to lateral femoral condyle, followed by blunt dissection between ischiotibial and major gluteus muscles, exposing the nerve from its emergence to its distal trifurcation. A domestic-use sterilized cotton wire loop was placed around the nerve for easier handling purposes, and the animals were then transferred to a dead-weight device, especially made for producing a crushing injury with a load of 15,000 g for 10 minutes, involving a 5-mm long segment, just proximal to nerve trifurcation[16]. (Figures 1 and 2).



Source: PACHIONI, C. A. S. Ischiatic nerve crushing injury in rats: a vascularization study. 2006. 92f. Ph.D. Thesis – Medical College, Ribeirão Preto, University of São Paulo, Ribeirão Preto. 2006.

***Figure 1-** Equipment used to produce ischiatic nerve crushing injuries in rats (dead weight). Flat Formica® base (A), stainless steel base (B), discs support and plumb discs (C), metal support for peripheral nerve rest (D), crushing shaft (E); system control lever (F); load release spring (G).*

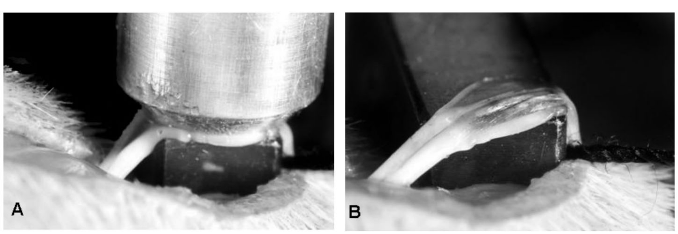


***Figure 2 -** Rat's ischiatic nerve crushing, 5 mm above bifurcation point. 15,000-g load, applied for 10 minutes (A). Rat's ischiatic nerve after crushing (B).*

After the load was applied, it was removed and the nerve was mobilized with the aid of the cotton loop. Surgical wound was closed with tissue suture by planes. The area was washed with 20% iodine alcohol antiseptic solution, and the animals received a single antibiotic dose for infection prophylaxis, being placed back into their original cages.

Each animal's back paws prints were recorded by using three different methods, at preoperative period, and on the 1st, 7th, 14th, and 21st postoperative days.

For assessing footprints and for calculating the Sciatic Functional Index, according to the method by De Medinaceli et al.[14] , the animals were submitted to walk on a 43-cm long vs. 8.7 cm wide vs. 5.5-cm high wooden track, with a dark small shelter at the end. On the walking track, paper strips were applied with the same measurements, previously prepared (bromophenol blue solution (Sigma®) in acetone, allowed to dry and stored in plastic containers in a dry environment). The animals had their paws wet by a water detergent domestic solution, which minimizes footprints blurring. (Figure 3).

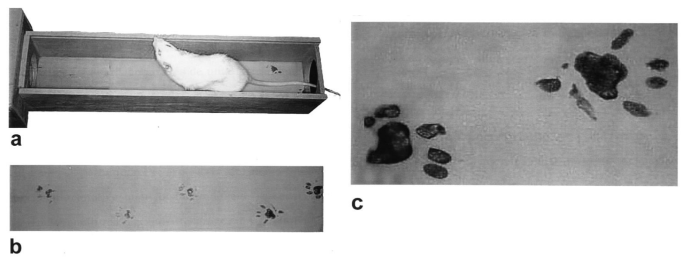


***Figure 3 -** Illustration of rats' gait record.*

Footprints images on paper strips were digitalized on a scanner and stored in a computer with a developed built-in graphical analysis software * routinely used in Laboratory[6,9,17]. That software, built on Windows® platform, allows for capturing, storing and assessing footprints images, providing Sciatic Functional Index calculation by the methods by De Medinaceli et al.[14] Carlton and Goldberg[15] and Bain et al.[5].

Those paper strips containing animals' footprints were manually assessed. Animals' paws measurements were highlighted and, with the aid of a school rule, with 1-cm increments, the values were calculated and stored. Subsequently, these data were included on a worksheet built on Excel application (Windows®), providing Sciatic Functional Index calculation based on the mathematic formula by De Medinaceli.

For the analysis of videotaped walking track prints and calculation of the Sciatic Functional Index, according to the method by Bain et al[5], the animals had to walk on a transparent acrylic track of similar size as the wooden track used with the method described above, but closed at its upper portion in order to prevent rats from escaping. Similarly, the animal positioned on the walking track had to walk all the way through towards a dark small shelter. The walking track was built with lighting on both sides with 120 W fluorescent lamps and gait recording was provided by using a digital video camera SONY DCR – DVD 203, positioned beneath the track. (Figure 4).

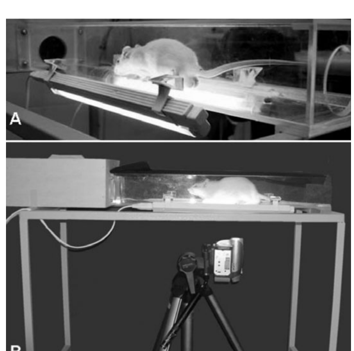


***Figure 4 -** Acrylic walking track developed at the precision laboratory of the Medical College of Ribeirão Preto, University of São Paulo (the structural details of the piece followed the measurements of the walking track optimized by De Medinaceli, Freed, Wyatt (1982,. 43-cm aisle, 5.5cm high and 8.7-cm wide).Preoperative training phase (A)and (B).*

The footprints images obtained from the videotape were addressed by computer by means of the Image J software, and edited for transforming pixels into millimeters, in order to allow for the calculation of Sciatic Functional Index modified by Bain et al.[5] (Figures 5 and 6).

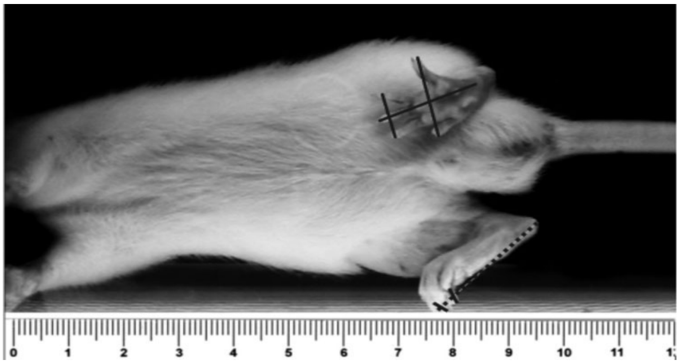
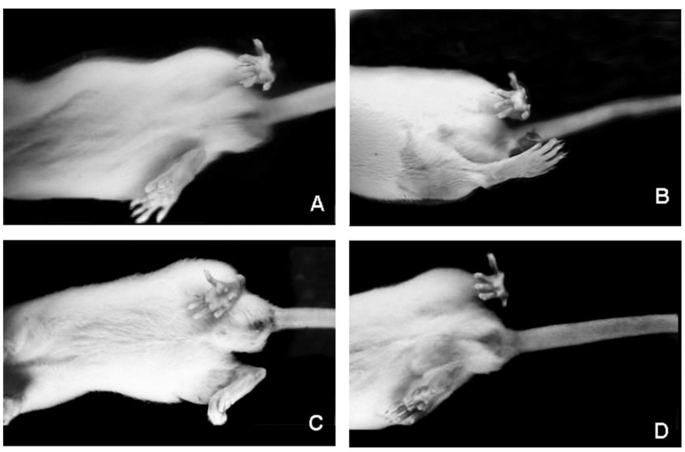


***Figure 5 -** Image of rats' footprints marked by Image J software. (normal E (left) paw marked with solid line; experimental D (right) paw marked with dotted and solid line (toes spread)).*



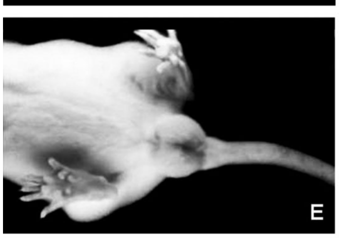


***Figure 6 -** Rat's footprint recorded during gait. Use of digital video camera Sony, model DCR-DVD 203, provided by the Bioengineering Laboratory, Medical College of Ribeirão Preto, USP, Ribeirão Preto. Rat's preoperative walking track (A); 1st postoperative day (B); 7th postoperative day (C); 14th postoperative day (D); 21st postoperative day (E). Injured right paw (experimental) and normal left paw.*

The Sciatic Functional Index, which corresponds to gait functional analysis, was calculated on footprints obtained from the methods described above, using the formula modified by Bain et al.[5] as follows:

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

Where:

N: normal, or non-operated; E: experimental, or operated; PL: print length; TS: *total toe spread*, or distance between first to fifth toe; IT: *intermediate toes spread*, or the distance between the second and fourth toes.

For statistical treatment of data, we used the Pearson's correlation coefficient (r), which quantifies the degree in which two variables (x and y) are correlated, provided the correlation is linear. This coefficient has no measurement unit, and ranges from -1 to 1. If r=0, no linear correlation exists between x and y, and the variables are not correlated. In order to assure results, we used the Spearman's rank correlation coefficient, r_s.

RESULTS

In the three respective methods, at first, the animals did not apply load on the injured paw, thus dragging it; keeping toes at full adduction, as a result of a serious sciatic nerve dysfunction. Over the experiment, the animals gradually recovered their ability to apply load on the injured paw and to open outer toes first, followed by intermediate toes.

Correlation between different gait functional evaluation methods manual postoperative SFI and computed postoperative SFI

A total of 125 walking tracks, each one with at least three footprints were measured by manual and computed methods by means of white paper sheets prepared with bromophenol. The same number of tracks was used, as image pictures, for assessing the videotape.

In the correlation established between Sciatic Functional Index (SFI) at manual and computed postoperative analysis, the Pearson's correlation coefficient was r = 0.54, thus determining a positive correlation between both, because as the sciatic functional index increases on the computed method, so increases the manual sciatic functional index. Upon data distribution, the Spearman's rank correlation coefficient was also applied. The p-value result of r (0.0108) was lower than the significance level ($p \leq 0.05$), thus ruling out the H_0 hypothesis (no correlation), assuring correlation between studied methods (Figure 7).

Manual postoperative SFI and postoperative SFI with videotaping

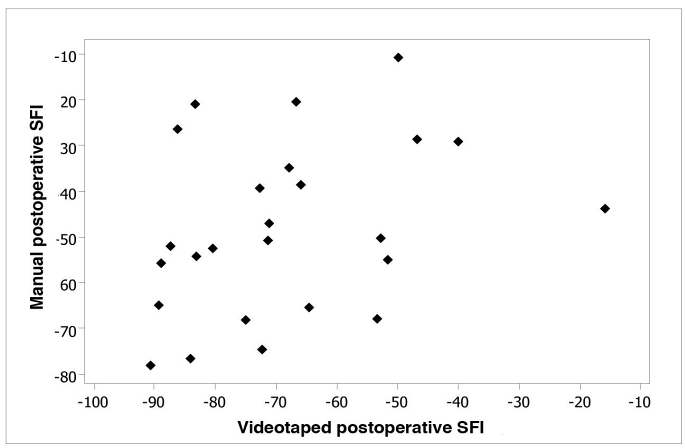


***Figure 7 -** Dispersion diagram for Manual postoperative SFI versus Computed postoperative SFI.*

Data concerned to the correlation established between the Sciatic Functional Index (SFI) at manual postoperative analysis and at the postoperative analysis with videotaping result in a Pearson's correlation coefficient of r = 0.32, determining a positive correlation between both, because as the sciatic functional index increases on the videotape method, so increases the manual sciatic functional index. Attention should be given to an atypical point on the graph that impacts values distribution, which attributes a curve shift towards that point. In this case, applying the Spearman's rank correlation coefficient was crucial, revealing a value of $r_{s\,=}$ 0.40. The statistical hypotheses test considering a p-value < 0.05 (fixed significance level) for checking the reliability of values recorded for Pearson and Spearman, assured correlation (p-value of r = 0.0558; p-value of $r_s = 0.0245$). (Figure 8).

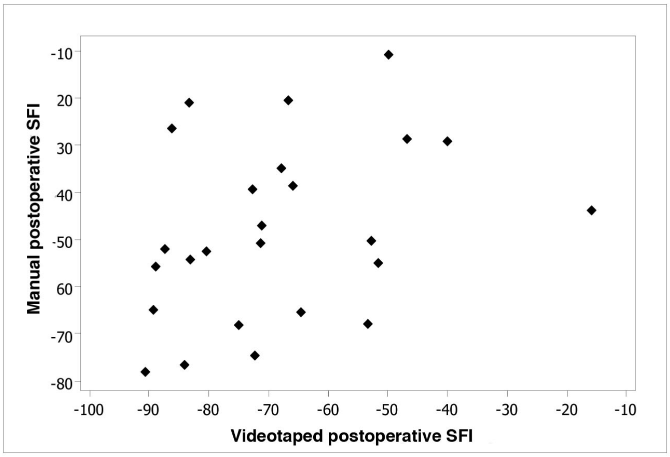


Figure 8 *- Dispersion diagram for Manual postoperative SFI versus Videotaped postoperative SFI.*

Computed postoperative SFI and postoperative SFI with videotaping

The analysis of the correlation established between Sciatic Functional Index (SFI) on computed postoperative and post-operative SFI with videotaping called our attention, because the Pearson's correlation coefficient was $r = 0.36$ determining a positive correlation between both. The Spearman's rank correlation revealed a value of $r_s = 0,29$. These data alone characterize a positive correlation between both, that is, a linear distribution of the values obtained for Sciatic Functional Index. However, when values are distributed on the graph, there is no linearity, but a heterogeneous distribution of data. Thus, the reliability of r and r_s values with the statistical hypotheses test considering a p-value < 0,05 was confirmed, where the absence of correlation between methods was detected (p-value r= 0.1310; p-value $r_s = 0.1877$). (Figure 9).

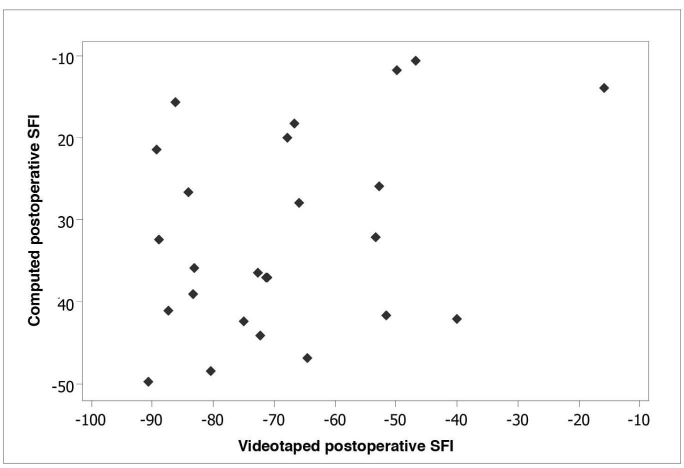


Figure 9 *- Dispersion diagram for Computed postoperative SFI versus Videotaped postoperative SFI.*

DISCUSSION

Peripheral nerves injuries are always a reason for concerns in terms of functional recovery, because it is not always full[1,2], sometimes leaving permanent sequels, which has been encouraging a large number of scientific investigations about the uncountable variables involved on nervous regeneration process. A large portion of those investigations are experimental by nature, conducted on animals, usually submitted to invasive evaluation methods, such as electroneuromyography, which does not anticipate information about an animal's functional status, or terminal methods, requiring animals' sacrifice and removal of the nerve for histomorphometric studies[4,11]. On the other hand, functional evaluation in animals is always an issue, for understandable reasons, being very difficult, for example, to quantify sensitiveness, or even motricity.

Gutmann and Gutmann[18] noticed that, in rabbits, the ability to spread back paw's toes depended on peroneal nerve's functional integrity. This was the starting point for the development of a new functional evaluation method in rats submitted to sciatic nerve crushing injury and analysis of back paws prints, named Sciatic Functional Index, or SFI [14], which was subsequently modified[5,15].

Since then, functional evaluation by SFI was shown to be a warranted reproducibility method with a strong correlation with the degree of morphologic regeneration[6]. SFI is, in fact, a negative indicator of nerve dysfunction degree, ranging from–100 to zero, -100 meaning full dysfunction, and zero, normal function. This method is based on fixed parameters measurable from footprints and their input on simple mathematic formulas, though subjected to errors.

Varejão et al.[13], suggested a new evaluation method by direct inspection by means of videotaping rats' footprints, advocating that this method would assure good evaluation, even in early weeks after injury, when difficulties in obtaining data are reported. However, in another experimental study, based on the measurement of an angle of the animal's paw (TOA – toe-out-angle), using the calcaneus and the third and fifth toes as reference points, and assessed by means of computed analyses[2], that group concluded that this method has a good correlation with the SFI by De Medinaceli[14]. However, the method described by Varejão et al.[13] is more difficult to apply and largely depends on investigator's subjectivity.

Despite the existence of studies in which the SFI methods described by De Medinaceli et al.[14] and Varejão et al.[13] have been used, it seemed to us that the functional differences between both were not clear enough, which motivated us to develop the present investigation, not only for comparing both methods, but also to correlate them, applying the methodology described by the previous and modified by Bain et al.[5] to the latter, thus creating a third new method (walking track videotaping plus parameters measurement), and then comparing to conventional methods that originated it, performed manually and with the aid of a computer. The controlled sciatic nerve crushing injury model in rats was regarded as optimal for this investigation, because this kind of injury can be produced easier than section followed by microsurgical repair. Additionally, in a crushing injury, neural structure is partially preserved, favoring nerve regeneration within a shorter term. Crushing was provided for 10 minutes, with a 15,000-g load, in order to achieve a severe injury, especially on thicker fibers, particularly assuring motor dysfunction. Rats' back paws images were printed on paper soaked with bromophenol blue, and animals' gait was videotaped and subsequently digitalized and evaluated by the computer imaging software Image J, which allows for an easy visualization of the footprints and for the application of the method by De Medinaceli et al.[14], as early as the first weeks after injury. Collected data were applied to the formula described by Bain et al.[5], which was modified from the one by De Medinaceli et al[19] and excluding the TOF (distance *to-other-foot*) parameter, thus eliminating the risk of results variations imposed by gait speed and step distance.

Our results demonstrate correlation between the SFI formulas by De Medinaceli et al.[14] and by Bain et al.[5] , in spite of the modification introduced in the latter (TOF parameter exclusion). From the analysis in which the correlation coefficient seeks to establish between manual SFI data and videotape SFI data, the distribution profile of achieved data shows to be different, with values being generated at significance threshold point, generating a distribution with positions tending to disorganization, almost without linearity, , but the statistical test assures positive linearity, establishing a correlation between each other. We thought, thus, that the linearity unbalance factor was the use of the original De Medinaceli et al.[14] formula correlated to the modified formula by Bain et al. [5]. An exception made to literature considering the TOF measurement as causative of potential variables in finding the SFI [5,15]. In the attempt to correlate computed SFI values and videotape SFI values, a disorganized distribution of data is found, which is not supported by statistical test, thus characterizing lack of correlation between data.

CONCLUSION

Functional evaluation methods described in this experimental study correspond to tools that assure regeneration and functional recovery follow-up, where the hypothesis of correlation between methods is real for conditions in which the same mathematic calculus is used, regardless of being manual or computed, with identification of the same measurement parameters. In cases where a combination of mathematic formulas exist targeting SFI, and the parameters measured are different from each other, there is no correlation between methods, reinforcing the use of individual formulas for separated experiments. Upon the need of accurate records, even in the first weeks, we conclude that the functional evaluation method with videotaping is the best one for regeneration follow-up, with immediate footprint data, mitigating difficulties faced with the traditional method by De Medinaceli.

ACNOWLEDGEMENTS

The authors acknowledge Professor José Batista Volpon and the FMRP - USP Bioengineering Laboratory staff, Professor Amilton Antunes Barreira, from the Department of Neurology and Psychiatry for being so kind to approve our use of the laboratory equipment, and Mr. Renato Meirelles e Silva and Mrs. Maria Cristina Lopes Schiavoni for their technical assistance. We also acknowledge Rodrigo Okubo, student of the Physical Therapy Course at USP Ribeirão Preto, for contributing with his technical knowledge on image recording and edition.

REFERENCES

1- Sunderland S. Nerve and Nerve Injury. 2ed. London: Churchill Livingstone, 1985.
2- Varejão AS, Cabrita AM, Patrício JA, Bulas-Cruz J, Gabriel RC, Melo-Pinto P, et al. Functional assessment of peripheral nerve recovery in the rat: gait kinematics. Microsurgery. 2001; 21:383-8.
3- Dijkstra JR, Meek MF, Robinson PH Gramsbergen A. Methods to evaluate functional nerve recovery in adult rats: walking track analysis, video analysis and the withdrawal reflex. J Neurosci Methods. 2000; 96: 89-96.
4- de Medinaceli L. Interpreting nerve morphometry data after experimental traumatic lesions. J Neurosci Methods. 1995; 58:29-37.
5- Bain JR, Mackinnon SE, Hunter DA. 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 functional index and morphometry to evaluate recovery of the rat sciatic nerve following crush injury: experimental study. J Reconstr Microsurg. 2001;17:69-75.
7- Akassoglou K, Akpinar P, Murray S, Strickland S. Fibrin is a regulator of Schwann cell migration after sciatic nerve injury in mice. Neurosci Lett. 2003; 338:185-8.
8- Szabo RM. Nerve compression syndromes: diagnosis and treatment. England: Slack; 1989.
9- Mendonça AC, Barbieri CH, Mazzer N. Directly low intensity direct electric current enhances peripheral nerve regeneration in rats. J Neurosci Methods. 2003; 129:183-90.
10- Walker JL, Evans JM, Resig P, Guarnieri S, Meade P, Sisken BS. Enhancement of functional recovery following a crush lesion to the rat sciatic nerve by exposure to pulsed electromagnetic fields. Exp Neurol. 1994; 125:302-5.
11- Chen LE, Seaber AV, Urbaniak JR. The influence of magnitude and duration of crusch load on functional recovery of the peripheral nerve. J Reconstr Microsurg. 1993; 9:299-306.
12- Pola R, Aprahamian TR, Bosh-Marcé M, Curry C, Gaetani E, Flex A et al. Age-dependent VEGF expression and intraneural neovascularization during regeneration of peripheral nerves. Neurobiol Aging. 2004; 25:1361-8.
13- Varejão AS, Cabrita AM, Meek MF, Bulas-Cruz J, Filipe VM, Gabriel RC, et al. Ankle kinematics to evaluate functional recovery in crusched rat sciatic nerve. Muscle Nerve. 2003; 27:706-14.
14- de Medinaceli L, Freed WJ, Wyatt RJ. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol. 1982; 77:634-43.
15- Carlton JM, Goldberg NH. Quantitating integrated muscle function following reinnervation. Surg Forum. 1986; p. 611-2.
16- Chen LE, Seaber V, Glisson RR, Davies H, Murrell GA, Anthony DC et al. The functional recovery of peripheral nerves following defined acute crush injuries. J Orthop Res. 1992; 10: 657-64.
17- 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. 2004; 15: 45-53.
18- Gutmann E, Gutmann L. Factors affecting recovery of sensory function after nerve lesion. J Neurol Psychiatr. 1942; 5:117-29.
19- de Medinaceli L, Derenzo E, Wyatt RJ. Rat sciatic functional index data management system with digited input. Comp. Biomechanical research. 1984; 17: 185-192.