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Is the Sciatic Functional Index always reliable and reproducible?

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Abstract

The Sciatic Functional Index (SFI) is a quite useful tool for the evaluation of functional recovery of the sciatic nerve of rats in a number of experimental injuries and treatments. Although it is an objective method, it depends on the examiner's ability to adequately recognize and mark the previously established footprint key points, which is an entirely subjective step, thus potentially interfering with the calculations according to the mathematical formulae proposed by different authors. Thus, an interpersonal evaluation of the reproducibility of an SFI computer-aided method was carried out here to study data variability. A severe crush injury was produced on a 5 mm-long segment of the right sciatic nerve of 20 Wistar rats (a 5000 g load directly applied for 10 min) and the SFI was measured by four different examiners (an experienced one and three newcomers) preoperatively and at weekly intervals from the 1st to the 8th postoperative week. Three measurements were made for each print and the average was calculated and used for statistical analysis. The results showed that interpersonal correlation was high (0.82) in the 3rd, 4th, 5th, 7th and 8th weeks, with an unexpected but significant ($p < 0.01$) drop in the 6th week. There was virtually no interpersonal correlation (correlation index close to 0) on the 1st and 2nd weeks, a period during which the variability between animals and examiners ($p = 0.24$ and 0.32, respectively) was similar, certainly due to a poor definition of the footprints. The authors conclude that the SFI method studied here is only reliable from the 3rd week on after a severe lesion of the sciatic nerve of rats.

Keywords: Sciatic nerve of rats; Crush injury; Functional evaluation; Sciatic functional index; Multiple examiners; Interpersonal reproducibility

1. Introduction

Under experimental conditions, the recovery of peripheral nerve injuries is mainly studied by electrophysiology, histology and morphometry. Although the electrophysiological and morphological parameters are useful, it is important to determine the degree of functional recovery they imply. Functional evaluation is relatively easy in humans but in animals it is practically impossible to use the same methods, a fact that has led to a search for specific methods to be used under experimental conditions.

Gutmann and Gutmann (1942) demonstrated that the loss of the ability to spread the toes of the hind leg is a reliable parameter for the evaluation of the extent of injury to the sciatic nerve and for the monitoring of recovery; however, the method proposed by these investigators was quite rudimentary and did not permit to quantify any parameter. A reliable and reproducible quantitative method for the assessment of functional condition, including the evaluation of the extent of injury and of the degree of recovery of the sciatic nerve of the rat, the so-called Sciatic Functional Index (SFI), was introduced in the early 1980s, first as an entirely manual version (De Medinaceli et al., 1982) and later as a computer-aided method (De Medinaceli et al., 1984). The so-called De Medinaceli's SFI method is based on the measurement of well defined key points and parameters of the animals' foot prints and on a mathematical formula, as follows:

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

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where: E: experimental; N: normal; TOF: to-other-foot, or the orthogonal distance between sequential footprints; PL: print length from heel to longest toe; TS: total spread, or the transverse distance between the 1st and the 5th toes; IT: intermediate toes, or the transverse distance between the 2nd and 4th toes.

De Medinaceli's method was soon adopted and later modified by some investigators, who removed the parameter "to-other-foot" from the original formula and introduced correction factors, so as to improve its performance (Carlton and Golberg, 1986; Bain et al., 1989). Carlton and Golberg (1986) also introduced the Tibial Functional Index (TFI) and the Peroneal Functional Index (PFI), later modified by Bain et al. (1989).

The SFI method was used in our own laboratory in a number of investigations using the sciatic nerve of rats as a model (Oliveira et al., 2001; Mendonça et al., 2003; De Sá et al., 2003; Monte-Raso et al., 2005), with the assistance of a computer software specifically developed for that purpose, which permits the capture (digitation with a scanner), storage and analysis of rats' hind foot imprints, with calculations performed according to the mathematical formulae proposed, and providing the results of the sciatic functional indices of De Medinaceli et al., Carlton and Goldberg and Bain et al. The results obtained in these investigations contributed to the demonstration of a close correlation between the SFI figures and the morphometric changes of the sciatic nerve following lesion and/or treatment and to the conclusion that the functional loss resulting from injury and the influence of various methods of treatment can be monitored by the SFI alone.

However, in the present authors' experience, the footprints obtained in the 1st and 2nd week after injury barely permitted the definition of the key points for measuring the parameters to be introduced in the formulae. The key points only began to clearly appear during the 3rd week. As a consequence, there used to be a wide variation in the SFI values from the 1st to the 3rd week, to the point that the SFI method seemed unreliable and irreproducible during this period. Thus, it was the objective of the present investigation to assess the reliability and reproducibility of the method from the 1st to the 8th week using four evaluators (one experienced with the method and three newcomers) and a new and improved computer software in an attempt to establish the interpersonal correlation of the results.

2. Methods

The experiment was approved by the Ethics Committee on Experimental Use of Animals of the Ribeirão Preto School of Medicine. Twenty adult male Wistar rats weighing 200–300 g (mean: 255.75 ± 32.22 g) were obtained from a single supplier and kept in collective cages, five to a cage, with free access to specific rat chow and water. Before any other procedure, the animals were trained to walk on a walking track constructed for this purpose according to the model of De Medinaceli et al. (Fig. 1). As soon as they had learned to do it, a preoperative footprint was obtained for the calculation of the normal SFI.

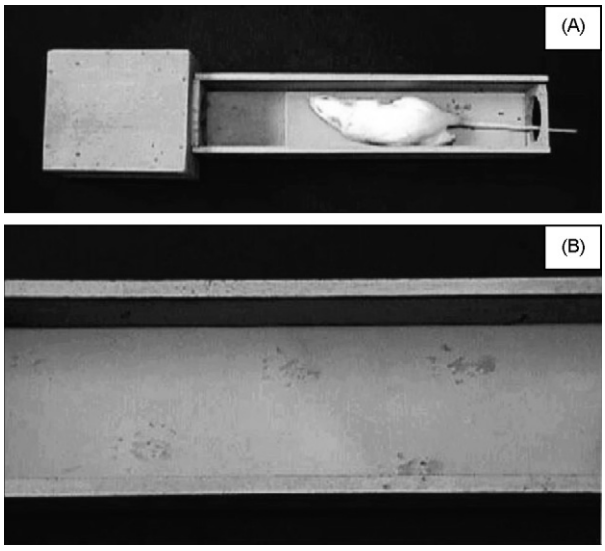

Fig. 1. Recording of the footprints on the walking track. General view of the walking track (A). Recorded paw prints (B).

2.1. Surgical procedure

The rats were anesthetized with a single intraperitoneal injection of a 1:4 combination of 5% ketamine and 2% xylazine (0.13 ml/100 g body weight). After routine preparation of the operative field (hair trimming, 20% iodine ethyl alcohol solution) the entire right sciatic nerve was exposed through a 3 cm long posterolateral longitudinal straight incision on the lateral aspect of the right thigh, followed by blunt dissection between the gluteus maximus and quadriceps muscles, and submitted to a crush injury circumscribed to a 5 mm long segment proximal to its trifurcation, using a spring forceps especially constructed for this purpose and calibrated for a static load of 5000 g; calibration was checked with the load cell after every 10 consecutive applications. The nerve was then carefully detached from the forceps and returned to its original bed and the surgical wound was closed plane by plane with separate 5/0 nylon sutures (Mononylon®, Ethicon).

2.2. Footprint recording and analysis

Hind paw prints were recorded on previously prepared paper strips impregnated with a 1% bromphenol blue acetone solution and left to dry, according to the modification of De Medinaceli's method by Lowdon et al. (1988). Paper impregnated with bromophenol blue becomes yellow after drying but immediately and permanently returns to blue when in contact with water or any aqueous solution. In the present study, the hind paws of the animals were immersed in common domestic detergent instead of water, since this prevents dispersion and blurring of the print. The animals walked on the impregnated paper strips placed on the walking track, leaving three footprints of each paw. The strips containing the footprints were allowed to dry and copied with a high resolution scanner and the digitized images were stored and analyzed in the computer using a new version of the software mentioned earlier (Oliveira et al., 2001). The data for each