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A new treadmill-type motorized walking belt machine for video recording of the rat's gait and sciatic functional index measurement. A comparative study with other methods

Vanessa Vilela Monte-Raso, Giuliano Barbieri, Nilton Mazzer, Marisa de Cássia Registro Fonseca, Cláudio Henrique Barbieri*

Departamento de Biomecânica, Medicina e Reabilitação do Aparelho Locomotor, Av. dos Bandeirantes s/n, Faculdade de Medicina de Ribeirão Preto – USP, Campus Universitário Bairro Monte Alegre 14049-900, Ribeirão Preto, SP, Brazil

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ABSTRACT

The sciatic functional index (SFI) is a remarkable tool to assess dysfunction and functional recovery of the sciatic nerve of rats. Usually measured on hind foot imprints on paper, a new method is now being proposed, by direct analysis of video recorded foot sole images obtained with a treadmill-type walking belt machine functioning with gait speed control (G1). Results were compared with the SFI measured on imprints on paper (G2) and on video recorded foot sole images obtained with a static see-through runway (G3). The right sciatic nerve of 19 adult female Wistar rats was crushed by the application of a controlled load. Impressions/images obtained both preoperatively and at weekly intervals for eight consecutive postoperative weeks were digitized, stored and analyzed in a computer loaded with specific software, the SFI being automatically calculated after measuring the appropriate parameters. SFI differed significantly between G1 and G2 and G1 and G3 ($p < 0.05$), but not between G2 and G3 ($p > 0.05$) during the first and second postoperative weeks, nonsignificant differences ($p > 0.05$) being observed for any comparison between groups during the third through eighth postoperative weeks. We conclude that the three methods yielded equivalent results from the third week onward, but both video recording methods (G2 and G3) permitted a more adequate early evaluation (first and second weeks), since the SFI parameters were more easily identifiable. Images obtained with the walking belt machine are more uniform and sharper, thus contributing to reduce the influence of biases observed with imprints on paper.

1. Introduction

Most of the knowledge gathered thus far about peripheral nerve lesion, treatment and regeneration has come from animal investigations, which are still crucial to clarify many aspects not yet entirely known. The sciatic nerve of rats is a convenient and reliable model for the study of peripheral nerve regeneration. Morphologic, morphometric and electrophysiological studies are virtually obligatory to evaluate nerve regeneration in experimental investigations, but do not provide any information about the functional state of the nerve.

Functional evaluation of nerve regeneration in experimental investigations on rats used to be virtually impossible until De Medinaceli et al. (1982, 1984) developed the sciatic functional index (SFI) method to assess hind foot function following a sciatic nerve injury and repair. In fact, the SFI is a negative indicator of the degree of nerve dysfunction varying from zero to −100, with zero corresponding to normal function and −100 indicating complete dysfunction and has been shown to correlate directly with the histomorphometric parameters of the sciatic nerve of rats (Bain et al., 1989; Maeda et al., 1999; Oliveira et al., 2001). The original De Medinaceli SFI method was later modified by Carlton and Golberg (1986), who removed one of the parameters initially proposed (distance to the other foot) and introduced the tibial and peroneal functional indexes (TFI and PFI, respectively), and by Bain et al. (1989), who modified the formulae again by adding more accurate correction factors based on linear regression of the indexes obtained.

The method originally described by De Medinaceli and coworkers was entirely manual (De Medinaceli et al., 1982), but was quickly transformed into a computer-aided method (De Medinaceli et al., 1984) in a Pascal base. We ourselves transposed De Medinaceli's software into a Windows base and improved it by introducing tools to automatically calculate the SFI, TFI and PFI according to all formulae previously described, using it in a number of investigations (Oliveira et al., 2001; Mendonça et al., 2003; De Sá et al., 2003; Monte Raso et al., 2005).

* Corresponding author. Tel.: +55 16 36330336; fax: +55 16 36330336.
E-mail address: chbarbie@fmrp.usp.br (C.H. Barbieri).

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Our own experience with the application of the method has shown that the imprints obtained on paper are very difficult to interpret and the key points of all parameters used in the formulae are almost impossible to recognize during the first 2 or 3 weeks following lesion of the sciatic nerve, thus contributing to a reduced precision of the measurements. Actually, an investigation on the reproducibility of the method among four observers confirmed this suspicion, also showing a high rate of reproducibility from the third week onward, exactly because the imprints became much more interpretable after this period (Monte-Raso et al., 2008).

A method of video recording of the animals foot sole from below was presented by Varejão et al. (2003), who developed a new parameter, the so-called toe-out-angle (TOA), applied together with the SFI measurement in an investigation on the regeneration of the rat's sciatic nerve in a model of segmentary resection and interposition of a biodegradable nerve guide. Probably developed as a replacement of, or complement to, the SFI, both methods were successful in demonstrating the recovery of the sciatic nerve and correlated with each other. The TOA was used in conjunction with other methods in further investigations, but the authors recognized that the SFI was still the gold standard for the assessment of the regeneration of the rat's sciatic nerve (Varejão et al., 2004a,b; Luís et al., 2007). However, the question of gait speed effective control had not been addressed.

The idea was raised that the SFI method could be further improved by analyzing the SFI parameters directly on the foot sole of the animals, whose gait speed could also be effectively controlled. A new investigation was then planned by our group specifically with the purpose of introducing a new method. A treadmill-type motorized walking belt machine functioning with gait speed control was devised and built to permit indirect video recording of the animals foot soles from below. In this paper we present our first experience with the use of the above mentioned machine, as compared with the conventional paper imprint analysis and with a method of direct recording from below with a digital camera under a see-through walking track, similar to that used by Varejão and co-workers.

2. Material and methods

The experiment was approved by the Ethics Committee on Experimental Use of Animals of our institution. Twenty adult male Wistar rats weighing 268 g on average (range: 249–281 g) were used and housed in collective cages with five animals each, with free access to water and specific rat chow throughout the experiment.

2.1. Operative procedure

Under general anesthesia with a single intraperitoneal injection of a 1:4 combination of 5% ketamine (0.1 ml/100 g body weight) and 2% xylazine (0.07 ml/100 g body weight) and after routine preparation of the operative field on the lateral aspect of the right thigh (hair trimming from lumbar spine to knee and disinfection with 20% iodine ethyl alcohol solution), the right sciatic nerve was exposed through a 3 cm long posterolateral longitudinal straight incision on the lateral aspect of the thigh, followed by blunt dissection between the gluteus maximus and quadriceps muscles. The crush injury was inflicted with an adjustable spring forceps specially constructed for this purpose (Monte-Raso et al., 2009) and calibrated for a static load of 5000 g with a precise load cell for loads of up to 50 kg; calibration was checked after every 10 consecutive applications. The 5000 g load was applied for 10 min, thus causing a severe crush injury circumscribed to a 5 mm-long intermediate segment 5 mm proximal to the trifurcation of the nerve. The surgical wound was covered with wet gauze during the entire crushing procedure to prevent drying, particularly of the exposed nerve. At the end of the programmed 10 min, the adjustable spring forceps was carefully removed to avoid any extra damage to the nerve, which was returned to its original bed. Immediate macroscopic examination showed that the nerve was obviously crushed, with the diameter greatly decreased in the direction of the load applied and increased in the orthogonal direction. The wound was closed by planes with isolated stitches of unabsorbable suture (5/0 Mononylon, Ethicon®) and the animal was allowed to recover from anesthesia without restraint. The surgical procedure was identical for all animals.

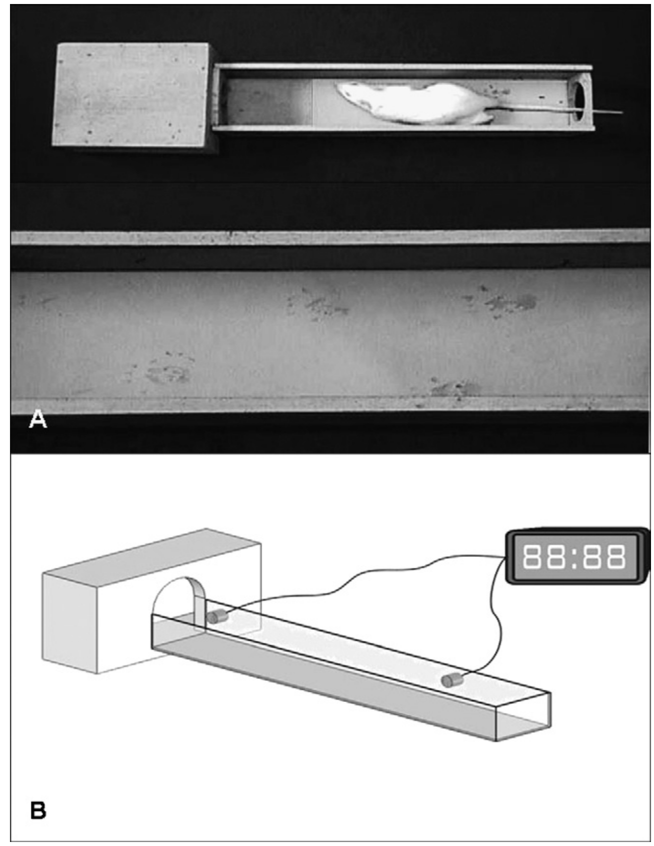

Fig. 1. Imprints obtained on paper impregnated with bromophenol blue (A). Schematic drawing of the electronic chronometer and sensor adapted onto the runway (B).

2.2. Foot print recording and analysis

All 20 animals were assessed by the three SFI methods used in this investigation, namely the conventional hind foot imprints (Group 1) and two types of video recorded images (Groups 2 and 3).

The hind foot imprints of Group 1 were obtained on a wooden walking track (43 cm × 8.7 cm × 5.5 cm) provided with a dark shelter at the end for the rats to hide and adapted with a digital chronometer turned on by an electronic sensor at the track entrance and turned off by another one at the shelter door, to permit measuring the time required for the rat to cross the distance between the two points (Fig. 1). Imprints were obtained by making the animals walk on paper strips previously impregnated with bromophenol blue and left to dry before use (Lowdon et al., 1988), also used in our previous investigations (Mendonça et al., 2003; De Sá et al., 2003; Monte Raso et al., 2005; Monte-Raso et al., 2008). The paper so prepared becomes yellow, but turns immediately and permanently blue when in contact with water or any water-based solution