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# Methods and techniques to evaluate sciatic nerve recovery in rats

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## ABSTRACT

Quantitative approaches for the evaluation of functional outcome are required in order to develop strategies to improve the regeneration of peripheral nerves. Most of these studies use rats as animal experimental model, as they are inexpensive and easy to work with. In such studies, regeneration can be assessed by numerous methods, including histomorphometry, electrophysiology and gait analysis. Because the sciatic nerve represents the most used one in experimental approaches, testing procedures for the assessment of its function after injuries have been developed. To evaluate the degree of functional loss, a footprint analysis method has been introduced and modified over subsequent years. The method uses indices of hind limb performance showing high degree of correlation with functional recovery. Data support a characterization of the sciatic nerve crush injury that will allow the study of peripheral nerve regeneration in the presence of neuroprotective agents in posttraumatic nerve repair.

**Keywords:** rat sciatic nerve, functional nerve recovery, walking track analysis, gait analysis, nerve morphometry

## 1. INTRODUCTION

Nerve injury can be one of the most devastating types of injuries, from causing pain to disability and loss of work. Therefore, methods to improve the nerve repair process are of great interest. The evaluation of damaged peripheral nerves and their recovery has been the subject of multiple animal studies. The regeneration of nerves after nerve ligation with or without repair has been examined, as well as nerve crush injuries with comparisons to severed nerve responses.

The sciatic nerve is a major nerve of the lower limb supplying great portion of the innervation to the hind limb. Injury of the nerve may appear in different cases, like pelvic fracture, femoral fracture, or agent injection in its anatomical region. Since the sciatic nerve provides motor function to the caudal thigh muscles, if injured, desensitization to the caudal and lateral side of the lower leg will appear [1]. The sciatic nerve is divided into the peroneal and tibial nerves. The peroneal nerve provides sensation and motor function to the lower leg, hock, and paw. The tibial nerve is the branch of the sciatic nerve that passes through the popliteal fossa and supplies motor innervation to the posterior (divided further into deep and superficial) compartment of the leg; it also supplies the sensory component to the plantar surface of the paw [2]. The level of injury defines the symptoms of sciatic nerve damage. Signs of peroneal or tibial nerve damage may be apparent in the case of a low-level lesion. On the other hand, signs of peroneal as well as tibial nerve damage will be seen, and marked gait abnormalities will be faced when reference is made to an upper-level lesion.

Various types of repairs and drug enhancements have also been tested. In the biological sciences, a number of animal models have been developed in order to study peripheral nerve regeneration. However, rats are used extensively because of their small size and availability of numerous identical subjects at low cost [3]. An additional advantage is that it is easy to operate and well studied by many scientists. The sciatic nerve shows an equivalent capacity for regeneration in rats and subhuman primates [4]. The lesioned sciatic nerve of the rat is a well-established animal model (complete nerve transection - neurotmesis model) used to study various aspects of recovery after traumatic injuries. For this reason, it is widely used for

the evaluation of motor and sensory nerve function at the same time [5, 6]. However, evaluation of neural recovery may be difficult.

Very often, the degree of nerve regeneration has been experimentally evaluated and quantified through three types of measures [7]:

- Histological [8-10] or morphological [10, 11] analysis to assess axon regeneration. The number of sprouting axons or their area can be measured. The various forms of analysis have to be performed on sacrificed specimens and may not be indicative of a clinical response6. They are expensive, usually requiring special stains and, possibly, even electron microscopy [12].

- Electrophysiological studies performed using electromyography8 to show muscle reinnervation, evoked potentials [13, 14] to show sensory return, or ENoG (a neurological test first described in 1979) nerve conduction studies [9] to show action potentials crossing the injured area or

- Different functional assessment techniques such as walking track analysis [5, 15], external postural thrust [16], and ankle stance angle [17].

For the success of any experimental study, the selection of the appropriate assessment parameter to measure neural regeneration will be critical. It has been assumed that the abovementioned classes are highly correlated to each other. Different nerve studies have reported the usage of more than one outcome measure, but they have not reported any correlation analysis [18].

Traditional methods of assessing nerve recovery (histomorphometry and electrophysiology) do not necessarily correlate with a return of motor and sensory functions [4]. Poor histological results or poor function haven’t been connected to poor electrical results [15].

Studies on axon count and degree of myelination could not prove whether the axon reaches the appropriate target organ or not [19]. As a consequence, an inappropriate interpretation of return of function may be the result of estimation beyond the original observation range of the histomorphometric as well as the electrophysiological parameters [15].

If the nature of a research question is about functional outcome, then a functional analysis is best [20]. However, if the research question relates to enhancement of fibre regeneration, then an electrophysiological or morphological analysis is more appropriate. When studies are

designed, there is a need to take in consideration the most interest aspects of nerve regeneration since the experimental results demonstrate no correlation between measures.

## 2. FUNCTIONAL EVALUATION OF SCIATIC NERVE CRUSH RECOVERY

Gutmann and Gutmann were the first who attempted to evaluate the voluntary function after experimental nerve repair [21]. They observed loss of toe spreading after injury of the rabbit peroneal nerve, but they faced serious difficulties in quantifying it. Thirty six years later, Hasegawa [22] measured the loss and recovery of toe spread after nerve crush in the rat. Based on these observations and on the study of rat footprints in neurologic disease [23, 24], de Medinaceli formulated the sciatic functional index (SFI), a unitless index of recovery [25]. Tracks were obtained through the walking of the rat across X-ray film after dipping its foot in photographic developer.

After a variety of unilateral sciatic nerve lesions de Medinaceli measured the distance to other foot or swing of a limb (TOF), the distance between hind limb and the tip of the third toe (print length, PL), the distance between second and fourth toes (intermediate toe spread, IT), and the distance between the first and fifth toes (toe spread, TS). Then, he calculated the SFI by comparing the measurements of the normal and experimental foot. The highest value for each variable on the experimental (E) side is compared with the highest value measured on the normal (N) side, according to the formula (1). Zero percent (0%) represents uninjured function, while -100% represents loss of function resulting when the sciatic nerve trunk is completely transected:

$$\mathrm{SFI} = \left( \frac{\mathrm{ETOF} - \mathrm{NTOF}}{\mathrm{NTOF}} + \frac{\mathrm{NPL} - \mathrm{EPL}}{\mathrm{EPL}} + \frac{\mathrm{ETS} - \mathrm{NTS}}{\mathrm{NTS}} + \frac{\mathrm{EIT} - \mathrm{NIT}}{\mathrm{NIT}} \right) \times 2.2 \times \frac{100}{4} \qquad (1)$$

Formula (1) was derived empirically, and was based on the assumption that all four variables were of equal importance. A weighting factor of 2.2 was included to give an average 100% deficit when the nerve is completely destroyed. The SFI formula was later altered by eliminating consideration of the distance between feet (TOF) and by modifying the weighting of contributing measurements.

Sarikcioglu et al. [18] reported that SFI (presented in functional deficit units) provides a non-invasive method of assessing the functional status of the sciatic nerve in the regeneration

process because in order a rat to walk properly coordinated function involving sensory input, motor response, and cortical integration is required.

The existent methods have been modified by mathematic formulas, where parameters of significant statistical variance have been excluded, in order to become simpler and more reliable. Carlton and Goldberg [26] initially reported results in abstract form only. Bain et al. measured later the tracks of rats with nerve lesions (peroneal, tibial, and sciatic) [27]. Their results were then subjected to multiple linear regression analysis. In this work, it was determined that TOF does not predict function, and the contribution of each remaining factor was weighted to the whole more accurately:

$$\mathrm{SFI} = -38.3\left(\frac{\mathrm{EPL}-\mathrm{NPL}}{\mathrm{NPL}}\right)+109.5\left(\frac{\mathrm{ETS}-\mathrm{NTS}}{\mathrm{NTS}}\right)+13.3\left(\frac{\mathrm{EIT}-\mathrm{NIT}}{\mathrm{NIT}}\right)-8.8 \quad (2)$$

Formula (2) when compared with the de Medinaceli and Carlton equations, gives lower mean values and standard deviations in sham-operated animals (normal rats operated as a control), and it can differentiate peroneal-injured animals from sham controls, which the others could not. Limiting factors for using the method, such as how to obtain paw prints and the necessity for hardware and specific software to take into consideration the walking track, still existed making it difficult to be applied.

A system for management of the sciatic functional index data was described in 1984 [28]. A comparison was conducted between the results obtained with various measurement techniques and the Sciatic Index Management System (SIMS). Reliability and validity of SIMS were excellent, while the data analysis was accelerated, having as a result SIMS to represent a significant advance in the quantification of nerve regeneration data.

The technique of digital video image analysis has been used in order to show variations of footprint parameters, both in walking (dynamic footprint) and in periodic resting (static footprint) [29]. Thus, an algorithm for the calculation of the static sciatic index (SSI) was developed. The SSI on one part uses footprints obtained when the rat is on a static position, while it minimizes bias related to the manner of walking (gait velocity). Using both ITS and TS functions, the static sciatic index is statistically correlated to the SFI. It improves the acquisition of footprints, and has been proved more repeatable and accurate than SFI [30].

Bervar compared the impact of walking and static footprint video analysis, and revealed that the footprint parameters contributed on the functional loss in a different manner for the static approach: the contribution of the toe spread factor (TSF) was still of high significance,

whereas the intermediate toe spread played a marginal role only [29]. The print length factor was not significantly different; therefore is of minimal value.

A modification of the SSI test has been used to obtain images of the plantar surface of the paws by placing animals on a digital scanner connected to a PC [31]. This motivated Bozkurt et al. [32] to develop a simple and convenient video-based technique which would combine advantages of the approach described by Bervar (i.e. higher accuracy, quantification of the degree of functional loss and recovery in a more precise way, repeatability,) with the economical advantages and straightforwardness of the experimental setup described by Grasso et al. [31].

Răducan et al. [33] have published data which are in line with the results reported by Bozkurt et al. [32] supporting the fact that SSI can be measured in both rearing and normal stance without significant differences regarding the nerve regeneration outcome. Taking into account the fact that the calculation of the SSI requires only two footprint parameters (TS and ITS) for a complex functional evaluation in rearing and normal position, Răducan et al. studied PL as well. Comparing the two positions, their results showed that the measurement of PL presents the lowest correlations, whereas the measurement of TS seams to be the most reliable.

In a new method in walking analysis, four different colours had been used, allowing the results of the footprint to more easily be interpreted [34]. The measurement result of print length (PL), inter toe spread (ITS) and toe spread (TS), which belong to the SFI component were modified. The SFI was confirmed with the toe out of angle (TOA) and a new method describing the normal footprint. TOA affects the change of abduction-adduction and internal-external rotation of hind limb. A new method to assess the nerve functional analysis has been developed using the SFI, TOA, and the angles around the meeting point of print length and toe spread in the laboratory. The research was based on six Sprague-Dawley rats. No significant difference has been found between the two hind limbs. The correlation between sciatic functional index, TOA and the angles around the meeting point of PL and TS was good meaning that they can be used as an additional procedure to confirm the SFI and TOA in walking analysis.

## 3. MATERIALS USED TO OBTAIN TRACKS

The degree of nerve regeneration could be determined using functional techniques which include the measurement of hind limb footprint parameters from adult rats. Early studies

employed ink and white paper [25, 35], a special moisture-sensitive paper [36], as well as thickened developer and a radiographic film which consists of a transparent, blue-tinted base coated on both sides with an emulsion [26, 35]. These techniques are easily accessible and simple to perform with minimal discomfort to the rat, but a high error rate accompanies them because of the existence of many non-measurable footprints. The introduction of video imaging techniques to record animal footprints during walking was a critical advance in the field of functional analysis [35] providing higher accuracy, better reproducibility and significantly fewer errors.

In different walking track experiments, the track was lined with various materials, or the rat's hind feet were painted or dipped in various substances, in order to obtain improved prints. According to the first method described in the literature, X-ray film was used on the floor of the corridor and the hind paws were dipped in developer [25]. Prior to Medinaceli's group, various attempts to record the footprints for gait analysis used Vaseline to smear the rat's hind paws and white paper [37]. Hruska et al. originally developed footprint analysis in rats [23] using grease and a piece of grass polygraph chart paper or Vaseline and white paper. Prints produced by such techniques could be susceptible to error in view of the anatomy of the rat plantar surface.

To obtain footprints, Zellem et al. [38] used diluted black poster paint, while they lined the walking pathway with electroencephalogram paper. Johnston et al. proposed block printing paint and office copier paper, implying advantages over the original method of X-ray developer and film such as:

(a) the availability of paper against X-ray film, the easy cutting and better traction provided,

(b) paint is non-toxic and can easily washable,

(c) the rat's feet are not exposed to potentially caustic developer,

(d) print smearing and slippage are kept to a minimum, and

(e) it is easy to visualize the paint of the plantar surface before the rat walks so that the important anatomical structures be imaged [39].

In 1979, the gait analysis was introduced using paper and ink to stain the hind feet [24]. Lowdon et al. [36] described a complex technique where a paper soaked in bromophenol is required; the same technique was chosen in order to both analyse the reliability of the SFI [9]

and to investigate the functional indices for sciatic, peroneal, and posterior tibial nerve lesions in the mouse [44].

The results of Lowdon et al. suggested that the SFI for a group of normal rats is similar whether the measurement is based on paper tracks or on X-ray film tracks. Paws are better gripped on paper and therefore they slip less often [36, 38]. The method where paint is used to image the plantar surface reduces the possibility for the radial diffusion problem (commonly seen with developer) to appear, because the abduction ability of the toes is impaired as a result of both the sciatic lesion and the harmful effects of the developer's surface tension.

When compared with the X-ray and developer method, the paint and paper technique is less expensive, easier to read, and non-toxic. Dyed paper is rapidly and simply prepared taking care to keep it absolutely dry. Modifying the Johnston's procedure, Shen and Zhu made use of white paper and carbon ink [40]. Paper tracks should be of value when the computer assisted reading technique is used [28]. In this case, the total cost of the materials is appreciably lower than that of the X-ray film. A substantial saving could be attained if the number of rats to be run regularly is large.

For walking track analysis, the photographic paper and film developer method has been proposed [41]. Satisfactory results were obtained when Dijkstra et al. used finger paint and white paper in order to overcome disadvantages such as the high cost, slippery surface and the caustic effects possibly caused by the developer [35].

The use of a mirror in order to obtain two views of the rat’s hind paw (plantar and side) has been proposed [42] and a video recording technique for the measurement of SFI was reported [43, 35]. The subjects were filmed from the side, while placed in a 30x15x100 cm runway made by Perspex, and with a mirror below the animal (at an angle of $45^{o}$). Each frame of the video image is selected from a non-hesitant step cycle [35]. Video analysis of standing, an alternative footprint analysis to calculate the functional loss index following an injury to the sciatic nerve of a rat was described by Bervar [29]. In this context, static sciatic index (SSI) was defined as a loss index in static conditions.

## 4. CONCLUSIONS AND FUTURE PERSPECTIVES

Though there are some limitations, the rat is the most commonly used animal model in nerve regeneration research. The severity of experimental injury to the sciatic nerve of the rat can be

quantified using the sciatic functional index of de Medinaceli, who first reported that, although time-consuming, the SFI could be used to evaluate total lower limb function in rats, along with nerve, muscle and joint function. The SFI method is valuable, since it can provide sequential information about nerve injury and recovery, which cannot be determined by an electrophysiological or histological technique.

Selection of appropriate evaluation methods is crucial, when measuring experimental nerve recovery. Assessment of regeneration following peripheral nerve lesions is achieved by various methods. The analysis of gait, showing the recovery of function, the ultimate goal of the repair machinery, is the method having the highest importance.

Walking track analysis is a technique allowing the objective assessment of the functional capacity of the limb in the rat sciatic nerve model. Though walking track analysis contributes decisively to the study of nerve repair and regeneration, it must be cautiously been approached.

Functional tests have different strengths and weaknesses based on the parameters they are designed to examine, the type of outcome data they produce, the technical difficulty and the time required to administer, and the need for expensive or highly specialized equipment. Each assay is designed to test a specific hypothesis and provide data to illustrate differences between groups. Each assay is designed to test a specific hypothesis and provide data to illustrate differences between groups. In choosing the correct functional assay for a specific experiment, the investigator must choose the test that best answers the question and produces the type of data that is suited to that experiment, i.e. quantitative, qualitative, numeric, temporal, percent of normal function, etc.

A useful characterization of the sciatic nerve crush injury is ensured by recent data, further allowing the investigation of peripheral nerve regeneration in the presence of agents with potential neuroprotective effect in post-traumatic nerve repair.

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