Research Article

http://dx.doi.org/10.17784/mtprehabjournal.2014.12.214

The effects of electroacupuncture on rat sciatic nerve recovery.

Os efeitos da eletroacupuntura na recuperação do nervo isquiático de ratos.

Rodrigo Antonio Carvalho Andraus[1,2], Mariana Fukuda Sasaki[1], Murilo Paiola da Silva[3], João Paulo Manfré dos Santos[4], Karen Barros Parron Fernandes[5], Rodrigo Franco de Oliveira[5], Vanessa Vilela Monte-Raso[1], Cláudio Henrique Barbieri[6]

Laboratório de Bioengenharia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto (SP), Brazil.

Abstract

Introduction: Acupuncture is an ancient form of treatment used initially in China and it is a currently common practice in the West among health professionals in various clinical conditions, causing local or remote biological responses. **Objective**: This experiment aims to evaluate the influence of electroacupuncture on recovery of peripheral nerve through functional gait analysis and nociception after crush injury in the sciatic nerve in rats. **Methods**: 30 Wistar rats were included, weighting 198g on average, which were divided into three groups. Functional analysis was performed by the method of Sciatic Functional Index (SFI) by shooting the rear footprints performed on a mat with controlled rate in the following days: preoperative; days 7, 14 and 21 post-operation. In the same days the animals were evaluated for nociception using von Frey filaments. **Results**: There was an improvement of the SFI in both treated groups compared with the control group. In the preoperative week there was no statistical difference ($p > 0.05$). In the 1st postoperative week there was statistical difference ($p < 0.05$) when comparing the three groups. The 2nd week yielded the same characteristics of the first week ($p < 0.05$). In the 3rd week there was a reversal of the improvement in groups, and the G2 achieved better rates ($p < 0.05$) than G3 and G1, but G3 was better than G1. In the analysis of nociception there was no statistical difference ($p > 0.05$). **Conclusion**: The electroacupuncture in both treated groups accelerated and improved nerve recovery of the animals according to the SFI.

Keywords: Therapy acupuncture; Functional assessment of gait; Electroacupuncture; Nociceptivety; Physiotherapy.

Submission date 03 September 2014; Acceptance date 09 December 2014; Publication date 17 December 2014

1. Laboratório de Bioengenharia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto (SP), Brazil.

2. PhD and Master program in Ciências da Reabilitação, Universidade Estadual de Londrina (UEL), Universidade do Norte do Paraná (UNOPAR), Londrina (PR), Brazil.

3. Medical Student, Universidade Anhembi Morumbi, São Paulo (SP), Brazil.

4. PhD program in Ciências da Reabilitação, Universidade Estadual de Londrina (UEL), Universidade do Norte do Paraná (UNOPAR), Londrina (PR), Brazil.

5. Professor of PhD and master program in Ciências da Reabilitação, Universidade Estadual de Londrina (UEL), Universidade do Norte do Paraná (UNOPAR), Londrina (PR), Brazil.

6. Professor of the Departamento de Biomecânica, Medicina e Reabilitação do Aparelho Locomotor, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo (USP), Ribeirão Preto (SP), Brazil.

Corresponding Author:
Rodrigo Antonio Carvalho Andraus. Rua Marselha, 491, Jardim Piza, Zip Code: 86041-140, Londrina (PR), Brazil. Phone: (43) 33717990. E-mail: rodrigoandraus@gmail.com
The authors declare no conflicts of interest.

Financial support: Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP.

Resumo

Introdução: A acupuntura é uma forma milenar de tratamento utilizada inicialmente na China e atualmente é uma prática corrente no Ocidente entre os profissionais de saúde nas várias afecções clínicas, causando respostas biológicas locais ou à distância. **Objetivo**: Este experimento tem como objetivo avaliar a influência da eletroacupuntura na recuperação do nervo periférico através da análise funcional da marcha e nocicepção, após a lesão por esmagamento no nervo isquiático de ratos. **Método**: Foram utilizados 30 ratos Wistar de peso médio inicial 198g, divididos em três grupos. A análise funcional foi realizada pelo método do Índice Funcional do Isquiático (SFI) através da filmagem das pegadas traseiras realizadas numa esteira com velocidade controlada nos dias: pré-operatório; 7° dia pós-operação; 14° dia pós-operação e 21° dia pós-operação. Neste mesmo período os animais foram submetidos à avaliação da nocicepção utilizando os filamentos de Von Frey. **Resultados**: Através dos resultados foi constatado que houve melhora do SFI em ambos os grupos tratados quando comparados com o grupo controle. Na semana pré-operatória não houve diferença estatística ($p>0,05$). Na 1ª semana pós-operatória houve diferença estatística ($p<0,05$) quando comparados os três grupos. Na 2ª semana obtiveram-se as mesmas características da primeira semana ($p<0,05$). Na 3ª semana houve inversão da melhora nos grupos, sendo que o G2 obteve melhores índices ($p<0,05$) que G3 e G1, porém G3 foi melhor que G1. Na análise da nocicepção não houve diferença estatística ($p>0,05$). **Conclusão**: A eletroacupuntura em ambos os grupos tratados acelerou e melhorou a recuperação nervosa dos animais de acordo com o SFI.

Palavras-chave: Terapia por acupuntura; Avaliação funcional da marcha; Eletroacupuntura; Nociceptividade; Fisioterapia.

INTRODUCTION

Peripheral nerves are extensions of the central nervous system responsible for the integration of the activities of the extremities, in their motor and sensory functions. They are susceptible to the same types of traumas that affect other tissues such as crushing, compression, tearing, among others; thus interrupting the continuity of the nerve structure by some type of trauma, results in the interruption of the transmission of nerve impulses in the disruption of their functional activity and consequent sensory loss. In neurological, the onset of the injury recovery process follows a pattern in which there is early inflammation, nonspecific and complicated lesions, vasodilatation and extravasation of fluids from blood vessels, platelet aggregation, accumulation of neutrophils and macrophages, release of proteases and other lysosomal enzymes and the stimulation of fibroblasts, formation of mediators such as interleukins, prostaglandins, histamine and serotonin, as well as tissue edema. This process causes the inflammatory response that results in the repair or cell death and these changes result in systemic reactions.[1,2]

To guarantee that the axon will not suffer with this process it is required the presence and interaction of growth factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), the β transforming growth factor (TGF-β), glial derived neurotrophic factor of the cells (GDNF) and specific receptors, cell adhesion molecules (CAMS).[3-7]

Acupuncture is an ancient form of treatment used initially in China and is a currently common practice in the West among health professionals in various medical conditions. The general theory of acupuncture is based on the premise that there are forms of life energy (Qi) flowing through the body by a network of "meridians" that connect several specific points of the skin - the acupoints - and are essential to health, on the other hand, changes in flow are responsible for diseases. Acupuncture may correct these imbalances flow by inserting needles into specific points that have different characteristics of adjacent areas that are not acupoints: high conductance, impedance and low resistance, high capacitance, high electrical potential.[8-11]

Many studies in animals and humans have shown that acupuncture can cause multiple local or remote biological responses.[12] For a better understanding of its therapeutic effects, some experimental studies have attempted to decipher the anti-inflammatory mechanism of action of acupuncture and its relationship with neurophysiology.[8,11] Among the most studied points there are the BP-6 points (Sanyinjiao) and E-36 point (Zusanli), related with parasympathetic effect to regulate the synthesis of pro-inflammatory and anti-inflammatory cytokine, thus containing the inflammatory process by the hypothalamus-pituitary-adrenal axis.[8]

Although peripheral nerve regeneration capacity has been recognized for over a century, nowadays it is still a major challenge for clinicians and researchers to improve the complications resulting from injuries to peripheral nerves due to the time spent in recovery. Although acupuncture is used as a therapeutic resource in different pathogens - such as addiction, pulmonary dysfunction, neurological problems post-rehabilitation, pain, inflammation, variation in blood supply - the reports are few conclusive and little is known about the performance for tissue repair.[9,12] Based on the comments above, the objective of this

study was to evaluate the effects of eletroacupunture on the nerve repair through functional gait analysis and nociceptive evaluation.

METHODS

The experiment was conducted at the Bioengineering Laboratory of School of Medicine of Ribeirao Preto. The study was approved by the Ethics Committee on Animal Experiments (CETEA) of the School of Medicine of Ribeirao Preto (FMRP) of the University of Sao Paulo (USP).

Experimental group

Thirty adult male Wistar rats were used, with an initial body weight of 180 ± 230 grams. The experimental groups were randomly divided into three groups of 10 rats each, classified as follows: Group 1 (control group, exposed to injury, but without therapeutic interventions); Group 2 (group subjected to injury and treated with electroacupuncture with specific points B50, B57, B60, VB34) and Group 3 (group subjected to injury and treated with electroacupuncture for cerclage).

Surgical procedure

The animals of all groups (G1, G2 and G3) were identified and weighed to calculate the dose of anesthetic (Ketamine and Xylazine 1: 1), 0.13ml for each 100g of body weight. Shaving was performed on the lateral side of the right thigh. The sciatic nerve was approached through a longitudinal skin incision straight, going from the greater trochanter to the knee. The sciatic nerve was crushed by a clamp made for this purpose by Monte-Raso et al.[13] capable of producing a static load of 5 kg (Figure 1). After crushing, the sciatic nerve was carefully detached from the accessory and left in its original path. The muscles and skin were sutured with simple stitches of monofilament nylon 4/0 (Ethicon®).

Acupuncture

The acupuncture points were located by a high sensitivity ohmmeter, for identification of eletropermeable areas, the lower resistors (acupuncture points). A pen-type locator was used (Point Scanner - VMV Biotherapy/Lautz) which LED, by moving the tip gently, turns on when passing on the acupuncture point. After its location, the points were marked with a pen. The selected points were: B50 (Huagmen), B60 (Kunlun), B57 (Changshan) and VB 34 (Yanglingquan).

Acupuncture was performed with acupuncture needles with stainless steel cables of 2 cm in length and 0.275 mm in diameter, with insertion depth of 3 mm. After bilateral insertion needles were electric stimulated by the Accurate Pulse 195 - VMV Biotherapy/Lautz (Figure 2) with 6V power supply, output voltage of 60 V, with a frequency of 2 Hz over a period of 20 minutes, as used in another study.[11]

Functional gait assessment

Medinaceli et al.[14] introduced a quantitative method, reliable and reproducible of the sciatic nerve functional condition, the sciatic functional index (SFI), which used three parameters of each hind paw - experimental (E) and normal (N) - in addition to the distance between the rear legs (TOF), obtaining a value between –100 (negative one hundred), representing respectively the full dysfunction, and 0 (zero), representing normality.[15,16]

The functional gait assessment is based on the calculation of the Sciatic Functional Index (SFI). For this, after the identification of rats and preceding the surgical procedure, the rats were placed in the treadmill for learning for three days before the surgical procedure. After learning, the preoperative impression of the footprints of the hind legs was recorded, in order to measure the normal SFI.

Although the study is based on the conventional method of De Medinacelie et al.[14], the captation of footprints was performed by shooting a camera connected directly to the computer, allowing the capture of images

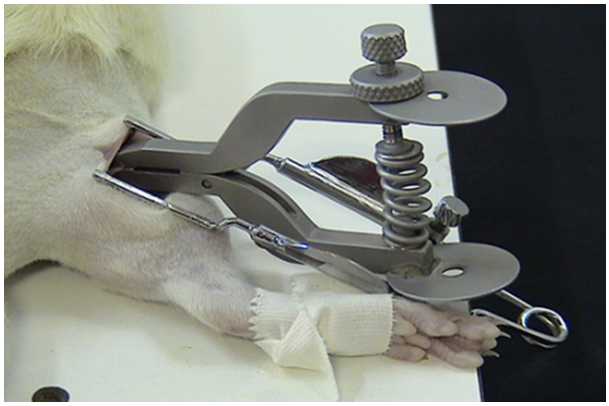

Figure 1. Crushing the sciatic nerve.

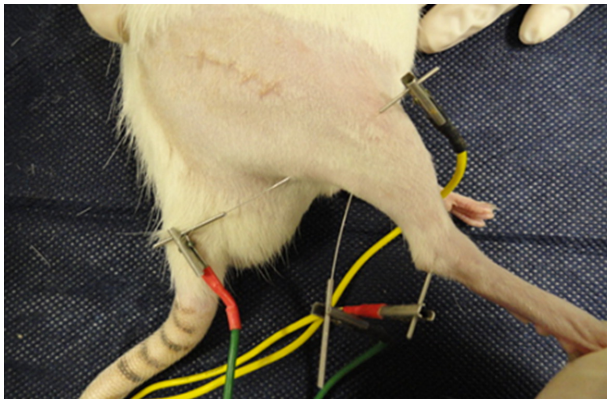

Figure 2. Treatment with electroacupuncture.

in real time. The motion capture was performed using a transparent treadmill provided with an electric motor controlled by a potentiometer that allows to control the speed of 14m/min. The tests were performed at a speed of 3-4 m/min, filmed by a digital video camera type 1.3 megapixel webcam, positioned horizontally and collimated with a mirror located below the work area and inclined to 45° so that the camera could capture the image of the plants of the legs of the animals that walk on the treadmill developed by Monte-Raso et al.[16] The images were adequated to the ideal size using Adobe Photoshop software (version CS3®.); edited for the use of the software Peripheral Nerves functional analysis (PNFA), modified by Yamasita[17] that allows the identification, image analysis and data storage with the help of the graphical analysis program based on the Equation 1:

$$SFI = 38.3 \times \frac{EPL - NPL}{NPL} + 109.5 \times \frac{ETS - NTS}{NTS} + 13.3 \times \frac{EIT - NIT}{NIT} - 8.8 \quad (1)$$

To calculate the SFI three parameters are used for each paw: the length of the shank (PL) is the distance between the two most extreme points of the footprint in the longitudinal direction, which is always higher in the injured paw due to poor fibular nerve function and consequently leads to functional impairment of the plantiflexors; the total spread fingers (TS) is the distance between the two most extreme points of the footprint in the transverse direction and should coincide with the sign of the two most extreme fingers, and the spread of intermediate fingers (IT) is the distance between the two intermediate fingers, both smaller in the injured paw.[18]

Nociceptive evaluation

The nociceptive test is performed with Semmes-Weinstein Monofilaments, to detect any damage in patients, in general consists of a set of nylon monofilaments, with different diameters, able to promote a pressure of force from 0.05 to 300 g when touched perpendicularly to the skin until they bend.[19]

The tactile test employed here was with the Von Frey filament, and the nociceptive threshold was defined by the filament diameter. The animals were placed in acrylic compartments, on a non-malleable metal screen and acclimated for 30 minutes before testing. The mechanical stimulus was directed perpendicularly to the plantar surface of both hind legs, for comparison between the normal and the deficit. The tension-response to several filaments, which produce different degrees of mechanical stimulation of harmless to harmful, was analysed.

The sessions started with the application of 10g filament. If the stress-response was harmful, the next lower filament was used. However, if the tension-response were innocuous, the immediately above filament was employed, and so on. This evaluation was performed in two periods: pre-operative (no injury) and day 21 postoperative (after the injury and its treatment). A pilot group of six animals was evaluated to determine the number of evaluations needed, and it was observed that during the first and second weeks there was no difference in response.

Statistical analysis

The IBM SPSS statistical package version 20.0 was used. Firstly, the Shapiro-Wilk test ($p > 0.05$) was employed, which showed that the data presented normal distribution. Data are presented as mean and standard deviation, ANOVA of 1 factor was used to comparisons among the groups, with 95% confidence intervals and significance level of 5% ($p < 0.05$).

RESULTS

A total of 30 rats were evaluated, which were distributed in 10 in the control group, 10 in the electroacupuncture group and 10 points in the group electroacupuncture cerclage. The average weights of the animals showed no statistically significant differences ($F = 0.122$, $p = 0.885$) in the preoperative week, 1st, 2nd and 3rd weeks. The data are presented in Table 1.

It was noted that in the SFI of the preoperative week there was no difference among the groups, but in the 1st postoperative week the cerclage group achieved better results when compared to the control group, and the group treated with specific points also achieved better results when compared to the control group . In the second week these results held, plus statistically significant difference between the cerclage group and the group of specific point, with the group cerclage the best results. However, in the third week there was an inversion, with the group treated with specific points showing better results when compared to the cerclage group. Data are presented in Table 2 and Figure 3.

Table 1. Average weight of the animals.

Mean	PO	W1	W2	W3
G1	202	266	312	380
G2	188	232	290	365
G3	198	249	278	350

Subtitle: PO = Postoperative; W1 = 1st week; W2 = 2nd Week; W3 = 3rd week.

Table 2. SFI mean.

Week	W1	W2	W3	P
PO	–4.04	–3.77	–5.87	0.182
S1	–90.64[a.b]	–82.62[a]	–77.32[b]	0.001*
S2	–85.68[a.b]	–74.18[a.c]	–60.14[b.c]	<0.001*
S3	–42.69[a.b]	–15.62[a.c]	–28.96[b.c]	<0.001*

Subtitle: PO = Postoperative; W1 = 1 week; W2 = 2nd Week; w3 3rd week.
* Indicates statistical significant differencel Equal letters indicate differences between groups.

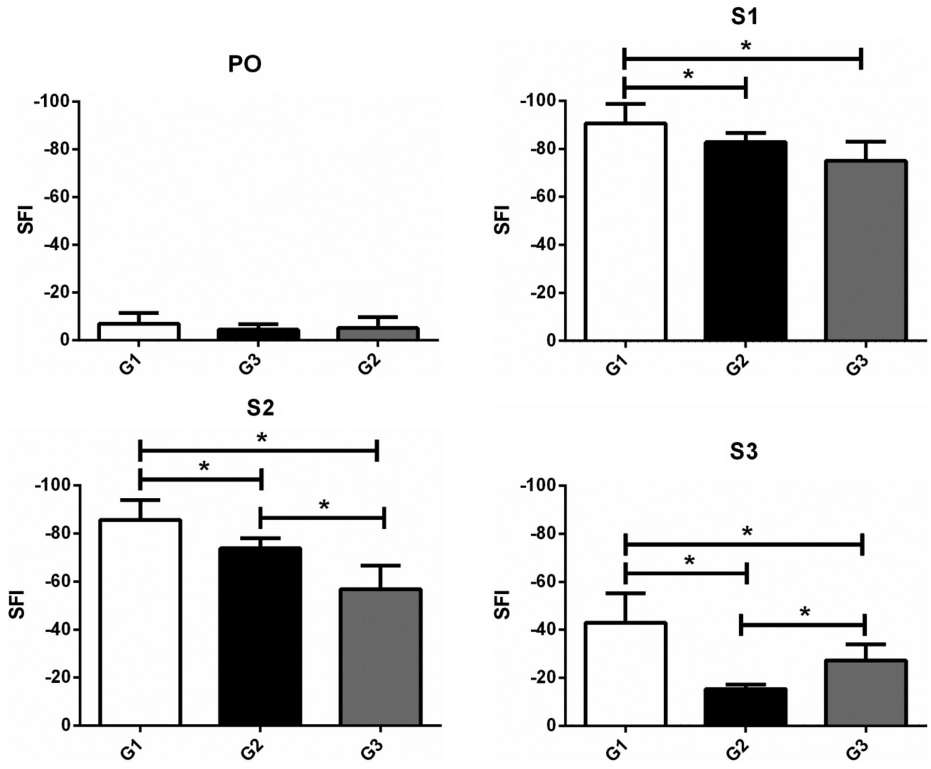

Figure 3. Average of SFI. Subtitle: PO = Postoperative; W1 = 1 week; W2 = 2nd Week; W3 = 3rd week.

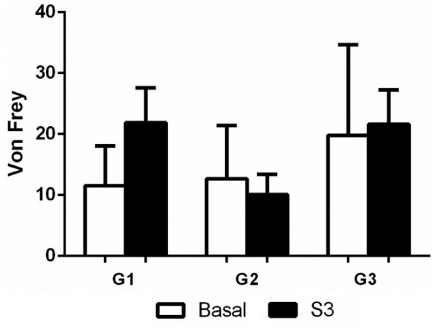

Figure 4. Average Von Frey test. Subtitle: G1 = control group; G2 = Group subject to injury and treated with electroacupuncture with specific points B50; B57; B60; VB34; G3 = Group subject to injury and treated with electroacupuncture for cerclage.

In the analysis of the Von Frey test, statistically significant differences were not observed (p> 0.05). In group 1 (control), the mean values were 11.5g (± 6.570) and 21.87g (± 5.693) at week 3. In group 2 (specific points), the mean values were 12.66g (± 8.742) and 10.09g (± 3.330) at week 3. Finally, group 3 (cerclage) obtained the mean values of 19.83g (± 14.849) and 21.6g (± 5.680) at week 3. Data are presented in Figure 4.

DISCUSSION

This study aimed to assess whether the intervention with electroacupuncture promotes improvement in nerve recovery in functional task. Following what was proposed by Yin et al.[20] the points of insertion site have been described, enabling the reliability of the survey, allowing comparison of different ways of treatment. Associated with this, we used the locator of points which proves to be an efficient method.[11]

Since no statistically significant differences were observed among groups in different time points, we can consider that the weight factor did not influence the results of the SFI and the Von Frey test.

It was observed in this study that the groups treated with electroacupuncture had better SFI, showing better performance in peripheral nerve recovery. In the first week of treatment there was no statistically significant differences among groups, in the first and second weeks the cerclage group had better SFI, however at the end of the third week the group of specific points showed the best results, it should be highlited that the cerclage group still had better results than the control group. It is believed that the electroacupunture has accelerated the nerve recovery, in both methods, as specific points or around the lesion, with its anti-inflammatory properties.

Thus, it can be said that there was morphological recovery, since according to Oliveira et al.[21] there is a correlation between the morphological recovery analysis and the SFI, and that only the functional assessment provides sufficient information on the injured nerve recovery without requiring the sacrifice of the animal.

Functional recovery after peripheral nerve injury is associated with a better performance when using electrical stimulation, in order to minimize or prevent muscle atrophy and thereby promote the recovery of peripheral nerve injury.[22]

Other studies have shown that acupuncture promotes nerve recovery, as the one of Chen et al.[23] who showed increased axon density of the sciatic nerve after acupuncture. There is evidence in the skin retail revitalization on the back of Wistar rats, however at different points of the ones used in this study, with the VG-14 (Dazhui), VG-2 (Yaoshu) and F-13 (Zhangmen), and they identified significant improvement after 8 days.[24]

Note that the functional gait analysis is probably the most important criterion of evaluation of the recovery of an injured nerve, since the return of motor function features a good evaluation criterion because it is less dependent on the subjectivity and capable of direct measurement in animals.[25]

No difference was observed in the sensory evaluations conducted by the Von Frey test, probably due to the overlap of neighboring areas of innervation that makes the sensory evaluation an inaccurate method.[25]

Stand out as study limitations the lack of a calculation of sample size, lack of analysis by intention to treat the loss of rats during the 3-week evaluation. Thus we suggest studies to control these variables.

CONCLUSION

The electroacunpucture is an efficient method for the recovery of the sciatic nerve, being the cerclage method the most effective in the first two weeks after injury and the specific points method more efficient in the totally of the treatment.

REFERENCES

1. Dodd J, Jessell TM. Axon guidance and the patterning of neuronal projections in vertebrates. Science. 1988 Nov;242(4879):692-9. http://dx.doi.org/10.1126/science.3055291. PMid:3055291.
2. Andraus RAC, Barbieri CH, Mazzer N. A irradiação local com o laser de baixa potência acelera a regeneração do nervo fibular de ratos. Acta Ortop Bras. 2010; 18(3):152-7. http://dx.doi.org/10.1590/S1413-78522010000300007.
3. Mendonça AC, Barbieri CH, Mazzer N. Directly applied low intensity direct electric current enhances peripheral nerve regeneration in rats. J Neurosci Methods. 2003 Oct;129(2):183-90. http://dx.doi.org/10.1016/S0165-0270(03)00207-3. PMid:14511820.
4. Raso VVM, Barbieri CH, Mazzer N, Fasan VS. Can therapeutic ultrasound influence the regeneration of peripheral nerves? J Neurosci Methods. 2005 Mar;142(2):185-92. http://dx.doi.org/10.1016/j.jneumeth.2004.08.016. PMid:15698658.
5. Siqueira R. Lesões nervosas periféricas: uma revisão. Rev. Neurociência. 2007; 15(3):226-33.
6. Endo C, Barbieri CH, Mazzer N, Fasan VS. Low-power laser therapy accelerates peripheral nerves regeneration. Acta Ortop Bras. 2008; 16(5):305-10.
7. Jiang M, Zhuge X, Yang Y, Gu X, Ding F. The promotion of peripheral nerve regeneration by chitooligosaccharides in the rat nerve crush injury model. Neurosci Lett. 2009 May;454(3):239-43. http://dx.doi.org/10.1016/j.neulet.2009.03.042. PMid:19429091.
8. Yanaguizawa T, Yamamura Y, Silveira VLF. Efeito da eletroacupuntura nos pontos E-36 (Zusanli) e BP-6 (Sanyinjiao), E-40 (Fenglong) e R-7 (Fuliu) sobre a resposta inflamatória aguda na pata de ratos injetados com carragenina. Rev. Paul. Acupunt. 2000; 6(1):2-6.
9. Vickers A, Wilson P, Kleijnen J. Acupuncture. Qual Saf Health Care. 2002 Mar;11(1):92-7. http://dx.doi.org/10.1136/qhc.11.1.92. PMid:12078381.
10. Ahn AC, Martinsen OG. Electrical characterization of acupuncture points: technical issues and challenges. J Altern Complement Med. 2007 Oct;13(8):817-24. http://dx.doi.org/10.1089/acm.2007.7193. PMid:17983337.
11. Okubo R,Sanada LS, Matheus JPC,Garcia SB, Volpon JB, Shimano AC. Can electroacupuncture enhance biomechanical and histological analysis after experimental muscular contusion? Adv Stud Biol. 2009;1:105-118.
12. Lee JA, Jeong HJ, Park HJ, Jeon S, Hong SU. Acupuncture accelerates wound healing in burn-injured mice. Burns. 2011 Feb;37(1):117-25. http://dx.doi.org/10.1016/j.burns.2010.07.005. PMid:20692770.
13. Monte-Raso VV, Moro CA, Mazzer N, Fonseca MCR, Fazan VP, Barbieri G, et al. Uma nova pinça regulável para a produção de lesões por esmagamento do nervo ciático do rato. Acta Ortop Bras. 2009; 17(4):236-8. http://dx.doi.org/10.1590/S1413-78522009000400009.
14. 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 Sep;77(3):634-43. http://dx.doi.org/10.1016/0014-4886(82)90234-5. PMid:7117467.
15. Brown CJ, Evans PJ, Mackinnon SE, Bain JR, Makino AP, Hunter DA, et al. Inter- and intraobserver reliability of walking-track analysis used to assess sciatic nerve function in rats. Microsurgery. 1991; 12(2):76-9. http://dx.doi.org/10.1002/micr.1920120204. PMid:2011070.
16. Monte-Raso VV, Fonseca MCR, Mazzer N, Barbieri G, Rosa RC, Zamarioli A, et al. Esteira com velocidade controlada para captação da marcha. Acta Ortop Bras. 2010; 18(1):49-53. http://dx.doi.org/10.1590/S1413-78522010000100010.
17. Yamasita AC, Mazzer N, Barbieri CH. Desenvolvimento de um software flexível no estudo de regeneração nervosa periférica. Acta Ortop Bras. 2008; 16(3):177-9. http://dx.doi.org/10.1590/S1413-78522008000300011.
18. Costa J, Camargo VM, André ES. Desenvolvimento de um método de baixo custo para avaliação da marcha em ratos. Fisioter Mov. 2008; 21(2):115-23.
19. Randall LO, Selitto JJ. A method for measurement of analgesic activity on inflamed tissue. Arch Int

Pharmacodyn Ther. 1957 Sep;111(4):409-19. PMid:13471093.

20. Yin CS, Jeong HS, Park HJ, Baik Y, Yoon MH, Choi CB, et al. A proposed transpositional acupoint system in a mouse and rat model. Res Vet Sci. 2008 Apr;84(2):159-65. http://dx.doi.org/10.1016/j.rvsc.2007.04.004. PMid:17559895.

21. 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 Jan;17(1):69-75. http://dx.doi.org/10.1055/s-2001-12691. PMid:11316287.

22. Cavalcante EVV, Silva LGM, Montenegro EJN, Pontes NT Fo. Efeito da eletroestimulação no musculo desnervado de animais: revisão sistemática. Fisioter Mov. 2012; 25(3):669-78. http://dx.doi.org/10.1590/S0103-51502012000300022.

23. Chen YS, Yao CH, Chen TH, Lin JG, Hsieh CL, Lin CC, et al. Effect of acupuncture stimulation on peripheral nerve regeneration using silicone rubber chambers. Am J Chin Med. 2001; 29(3-4):377-85. http://dx.doi.org/10.1142/S0192415X01000393. PMid:11789580.

24. Tabosa A, Uema D, Orlandi DM, Freitas RRA, Rogerio T, Uamamura Y. Efeito da eletroestimulação por eletroacupuntura dos pontos VG-14 Dazhui), VG-2 (Yaoshu) e F-13 (Zhangmen) na vitalização de retalho de pele do dorso de ratos Wistar. In: Anais do II Congresso da World Medical Association of Acupuncture; 2003; São Paulo. São Paulo: World Medical Association of Acupuncture; 2003.

25. Varejão ASP, Cabrita AM, Patrício JÁ, Bulas-Cruz J, Gabriel RC, Melo-Pinto P, et al. Functional assessment of peripheral nerve recovery in the rat: gait kinematics. Microsurgery. 2001; 21(8):383-8. http://dx.doi.org/10.1002/micr.21803. PMid:11757066.