JIVS JOURNAL OF ISTANBUL VETERINARY SCIENCES

Evaluation of the efficiency of TENS therapy to the regeneration in N. ischiadicus injured rats

Ebru Eravcı Yalın[1], Yalçın Devecioğlu[1]

1. Department of Surgery, Veterinary Faculty, Istanbul University-Cerrahpasa, Turkey.
Eravcı Yalın, E. ORCID: 0000-0002-0941-6745; Devecioğlu, Y. ORCID: 0000-0002-8175-2321

Research article

Volume: 8, Issue: 3
December, 2024
Pages: 335-342

Article History

Received: 24.12.2024
Accepted: 30.12.2024
Available online: 31.12.2024

ABSTRACT

Transcutaneous electrical nerve stimulation (TENS) is one of the electrotherapy methods, used for physical therapy, to relieve neuropathic pain in the nervous system lesion. However, the effect on peripheral nerve regeneration has been unknown. This work aims to examine peripheral nervous system diseases in which fullness is preserved, the efficiency of TENS to hasten healing, and the suitability of magnetic resonance imaging to diagnose peripheral nervous system diseases. Also, electrophysiologic findings of functional nerve recuperation will be considered after comparing with histopathologic and magnetic resonance imaging. 72 Sprague Dawley rats were randomly assigned to four groups. Group 1; normal without crush lesion, Group 2; control group with crush lesion, Group 3; stimulated group on lesion area, Group 4; stimulated group on gastrocnemius muscle. The animals were sacrificed post-operatively 21. day and 45. day after the electrophysiological, assessment and walking trace analysis, magnetic resonance imaging and nerve samples were obtained for histologic analysis. According to this study, low-frequency TENS leads to delayed regeneration after a crush lesion of the sciatic nerve in rats.

Keywords: rat, TENS, EMG, nerve degeneration, electrophysiology

DOI: https://doi.org/10.30704/http-www-jivs-net.1602816

To cite this article: Eravcı Yalın, E., Devecioğlu, Y. (2024). Evaluation of the efficiency of TENS therapy to the regeneration in N. ischiadicus injured rats. ***Journal of Istanbul Veterinary Sciences, 8*(3), 335-342.** **Abbreviated Title*: J. İstanbul vet. sci.***

Introduction

Traumatic peripheral nerve and root injuries are common in companion animals. These injuries frequently occur as a result of motor vehicle accidents, leading to fractures of the humerus, pelvis, and proximal femur, as well as iliosacral and sacrocaudal fractures and dislocations. Additionally, bite wounds and gunshot injuries are also significant causes of peripheral nerve damage. Iatrogenic nerve injury, arising from incorrectly administered intramuscular injections or surgical procedures, is another common cause. Peripheral nerve damage can manifest as compression, contusion, stretching, avulsion, or complete transaction (Dewey, 2003; Forterre et al., 2007; Rodkey & Sharp, 2003).

In recent studies focused on nerve healing have shed light on the pathophysiological mechanisms and molecular changes associated with peripheral nerve injuries. Despite all the research on nerve healing, adequate nerve recovery has not been achieved to the desired extent following severe injuries. The primary goal in the treatment of peripheral nerve injuries is to restore nerve integrity, thereby re-establishing the transmission of signals and the full functional recovery of the target organs innervated by the nerve. For successful nerve regeneration, processes such as axonal sprouting, axonal growth, target organ reinnervation, and reintegration of the regenerated fibers with the central nervous system need to be completed (Wolthers et al., 2005).

Electrical stimulation has positive effects on regeneration in nerve compression injuries has been proven. Transcutaneous electrical nerve stimulation (TENS) is a widely used electrotherapy modality in physical rehabilitation, primarily aimed at alleviating neuropathic pain associated with nerve lesions. There is currently no consensus regarding the effectiveness of TENS in improving nerve regeneration, as its efficacy is influenced by various factors such as the type, frequency, intensity, and method of application (Alarcon et al., 2022). While some studies suggest that TENS may accelerate reinnervation, other research indicates that it could potentially delay regeneration

*Corresponding Author: Ebru Eravcı Yalın
E mail: ebrueravci@gmail.com

(Baptista et al., 2008). The effects of TENS on peripheral nerve regeneration have yet to reach a consensus in the literature.

The purpose of our study is to evaluate the efficacy of TENS in promoting the healing of peripheral nerve injuries where the integrity of the nerve has not been compromised.

Materials and Methods

In this experimental study, 72 male Sprague Dawley rats, weighing between 200 and 300 grams, were used. The rats were obtained from the TÜBİTAK Marmara Research Center. During the study, the rats were housed in a room with a 12-hour light/dark cycle, controlled ventilation, and maintained at room temperature. They were provided unlimited standard food (dry pellets) and drinking water. All experimental procedures were performed by the ethical guidelines set by the Istanbul University Animal Ethics Committee.

The rats were divided into four groups, each containing 18 animals (n=18). Group 1 (n=18): Sham group, where a surgical incision was made without inducing nerve injury. Group 2 (n=18): Control group, where crush injury was induced to the sciatic nerve without any further intervention. Group 3 (n=18): Experimental group I, where a crush injury was induced to the sciatic nerve, followed by transcutaneous electrical nerve stimulation (TENS) applied to the injured area. Group 4 (n=18): Experimental group II, where a crush injury was induced to the sciatic nerve, followed by TENS applied to the gastrocnemius muscle.

Surgical procedure

General anesthesia was induced by intramuscular injection of a mixture of 8 mg/0,1 kg Ketamine HCl (Ketalar®, Pfizer) and 1 mg/0,1 kg Xylazine HCl (Rompun®, Bayer). Following anesthesia, the left femur areas of the rats were shaved. The rats were positioned in the right lateral recumbent position, and the surgical area was disinfected with povidone-iodine. The surgical field was covered with sterile drapes, leaving the incision site exposed. An oblique incision was performed on the left femur to open the skin. The biceps femoris muscle was bluntly dissected, and the edges were retracted to expose the sciatic nerve. The sciatic nerve was carefully freed from surrounding tissues, from the sciatic notch to the branching area of the nerve. A surgical clamp was used to induce crush injury to the sciatic nerve. The sciatic nerve compression injury was induced by applying pressure through the single tooth of this clamp for 30 seconds, 10 mm above the branching region (figure 1). This procedure aimed to standardize the sciatic nerve injury . After completing the surgical procedures, the muscle tissue was sutured with continuous 5/0 Vicryl, and the skin was closed using continuous 5/0 silk sutures.

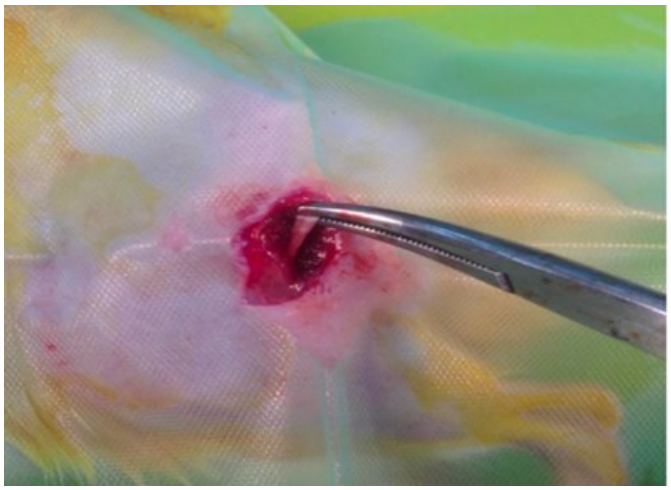

Figure 1. Creation of crush injury in the sciatic nerve

TENS application in experimental groups

In the 3rd experimental group, consisting of 18 rats in Group 3, TENS was applied to the region of the nerve injury. In the 4th experimental group, comprising 18 rats in Group 4, TENS was applied to the gastrocnemius muscle, which is innervated by the injured sciatic nerve. TENS was started 4 days post-surgery in all rats. The electrical stimulation was administered daily for 20 minutes for 15 days, using a 4 Hz frequency and 200 µs pulse duration, via the Physiomed Vetri Combi device.

Over the 45-day follow-up period, general changes were observed in the rats. During the experiment, partial autophagy was observed in the feet of 2 rats in the control group, 2 rats in experimental group 1, and 1 rat in experimental group 2. Since these conditions interfered with the SFI measurements, these rats were excluded from the study. To balance the number of animals across the groups, 1 rat was also excluded from experimental group 2, and the findings were evaluated based on 16 animals in each group. Functional recovery of the sciatic nerve was assessed by gait analysis , and the Sciatic Functional Index (SFI) was calculated for all animals before surgery (2 days prior) and on the 21st and 45th days post-surgery. For the gait analysis, a walking pathway apparatus was prepared with a length of 50 cm, a width of 10 cm, and a side height of 12 cm, ending in a dark room. White sheets of paper, cut to the same size as the corridor, were placed inside the walking path. The rats' left hind paws were pressed onto an ink stamp soaked in black India ink, and the rats were made to walk through the prepared corridor to leave footprints (Figure 2). In general, several sampling attempts were required to

Figure 2. The walking corridor ending in a dark room

obtain clear and distinct footprints. Using the most suitable footprints on the paper strips, the following measurements were taken with the help of a millimeter ruler: the distance between the heel and the toe (print length, PL), the distance between the first and fifth toes (step width, SW), and the distance between the second and fourth toes (mid-step width, MSW). The values obtained from the measurements were placed into the formula developed by Medinacelli and later modified by Bain-Mackinnon-Hunter to calculate the SFI. In values ranging from 0 to -100, an index of 0 indicates normal function, while an index of -100 signifies complete loss of function. The differences in the SFI values between the groups were statistically analyzed.
Magnetic resonance imaging (MRI) of the left leg was performed before the TENS application on the 4th day, and again on the 21st and 45th days, prior to nerve sample collection, for three randomly selected rats from each group.

Electrophysiological measurements were taken under deep anesthesia from the left leg of the rats before surgery and on the 21st and 45th days after surgery. Three electrodes were used: one active, and the others as reference and ground electrodes. For measurement, while the rats were under anesthesia, the area to be measured was shaved and cleaned with alcohol. To prevent direct contact with the surface and maintain a constant temperature, a hot water bottle was placed beneath the rats. The active electrode was placed on the mid-region of the gastrocnemius muscle, the reference electrode on the tendon region, and the ground electrode on the tail. The stimulus electrode was positioned between the L3-L4 vertebrae. After delivering a stimulus with a frequency of 1 Hz and a duration of 0.1 ms, the responses were analyzed using Neurosoft software. In the second stage, electrophysiological measurements were taken by exposing the sciatic nerve through an incision of approximately 1 cm at the mid-thigh level. The stimulation unit, with an inter-electrode distance of 1.1 cm, was placed on the sciatic nerve, and the first stimulus was delivered from the first electrode. Recordings from the gastrocnemius muscle were obtained using a needle electrode and transferred to a computer with the help of an amplifier. Similarly, a stimulus was delivered through the second stimulation electrode, and recordings were made. In both cases, three stimuli were delivered, and the conduction velocities were calculated and averaged. The within group and between group analysis included the evaluation of peak-to-peak amplitude values, latencies, and nerve conduction velocities.

After the electrophysiological assessments, the rats were euthanized under general anesthesia, and pathological samples were collected. Sciatic nerve samples, including the gastrocnemius muscle attached to the nerve, were harvested with the damage site medial to the specimen. The collected tissues were processed through routine histological procedures, and 5-7 µm thick sections were obtained from paraffin blocks. These sections were stained using the hematoxylin-eosin method and examined under a light microscope at 400x magnification. Axons in the observed field were counted, and the presence of edema cells was evaluated.

Results

During the study, partial autophagy (self-cannibalization) in the feet was observed in 2 rats from the control group, 2 rats from experimental group 1, and 1 rat from experimental group 2. Since this condition interfered with the Sciatic Functional Index (SFI) measurements, these rats were excluded from the study. To equalize the group sizes, 1 rat was also excluded from experimental group 2, and the findings were evaluated based on 16 rats per group.

All rats in each group were walked on the gait path before the experiment, and the Sciatic Functional Index (SFI) values were recorded on Day 0. The average pre-experiment SFI value (Day 0) for all groups was found to be -5.50 (± 3.80). On Day 21 and Day 45 post-surgery, no significant differences were observed between the control and TENS groups when comparing the average SFI values. However, when each group was assessed individually, significant differences were found

in the mean SFI values between the pre-experiment (Day 0), post-experiment Day 21 (SFI 21st Day), and Day 45 (SFI 45th Day), indicating nerve regeneration was supported.

Magnetic resonance imaging (MRI) scans taken before the TENS application on Day 4 revealed hyperintense areas in T2 sequences, consistent with inflammation and edema in the surgical site. No significant differences in appearance were observed between Day 21 and Day 45 in either inter-group or intra-group comparisons. Compared to Group 1, there was significant denervation atrophy in the gastrocnemius muscle, consistent with nerve degeneration.

When examining the latency values of all rats, a significant increase was observed post-injury compared to the pre-surgical baseline. However, no significant differences were found in latency values between the control and experimental groups. Regarding peak-to-peak amplitude values, a significant decrease was observed in all groups that underwent surgical procedures. No significant difference in amplitude values was detected between the control and experimental groups. Nerve conduction velocity (NCV) measurements were also obtained., the pre-injury average sciatic nerve conduction velocity in all groups was measured as 46.22 ± 3.25 m/s. In this study, where sciatic nerve injury was performed in the left leg, the average left sciatic nerve conduction velocity in the sham group was 51.3 ± 2.65 m/s. In the control group, where injury was induced, the nerve conduction velocity decreased to 18.6 ± 1.64 m/s. In the TENS-treated Groups 3 and 4, the conduction velocities decreased to 19.4 ± 1.6 m/s and 18.8 ± 1.7 m/s, respectively.

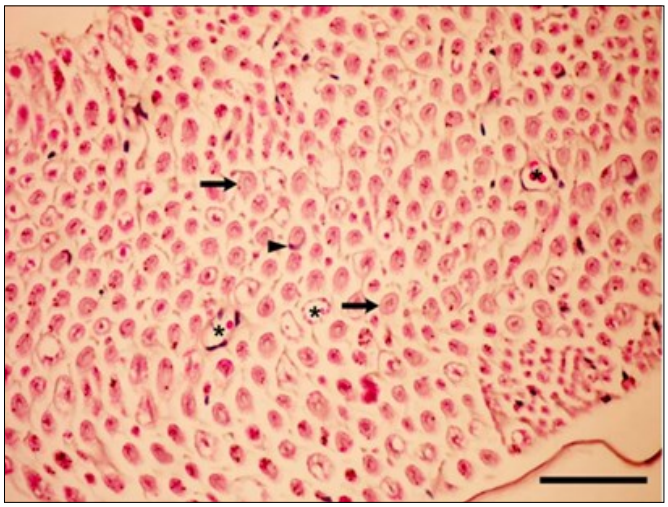

Figure 3. Normal nerve section (sham-operated), myelinated axons (arrows), Schwann cells (arrowheads), and capillary vessels (stars) H&E, Bar = 100 µm.

In Group 1 biopsy samples obtained from the sciatic nerve (n.ischiadicus), the nerve fibers were found to be wrapped in myelin sheaths and arranged in a normal pattern, with the presence of a small number of Schwann cells (Figure 3). No pathological changes were observed, apart from slight artifacts due to routine tissue processing. No pathological changes were observed in tissue samples taken from the gastrocnemius muscle (m. gastrocnemius) either.

In Group 2 biopsy samples taken from the distal portion of the sciatic nerve (n.ischiadicus) injury site on Day 21, the epineurium exhibited an edematous appearance, with a small number of mononuclear inflammatory cell infiltrates. The perineurium was separated from the nerve fibers, and the endoneurium appeared irregularly scattered. Myelinated axons showed largely dissolved myelin sheaths, with evidence of vacuolar degeneration. Additionally, an increase in the number of Schwann cells and a small number of mononuclear inflammatory cell infiltrates were observed (Figure 4). Histopathological examination of gastrocnemius muscle (m.gastrocnemius) samples from the control group on Day 21 revealed no changes except for mild myositis.

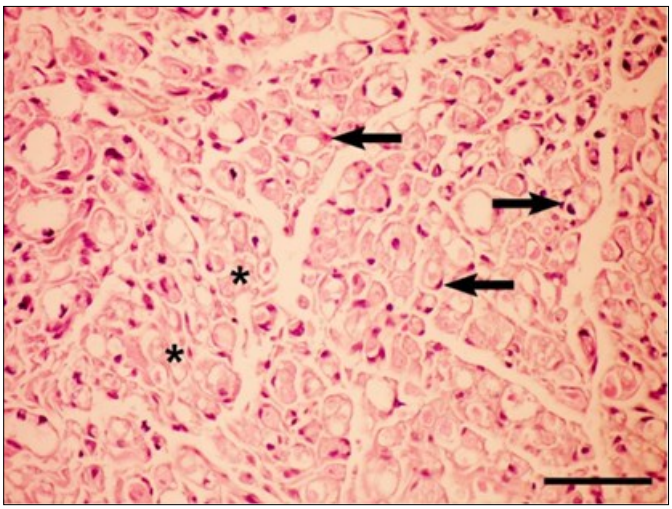

Figure 4. Control Group, Day 21, increase in the number of Schwann cells (arrows) and degeneration in the myelin sheath and axons (stars), H&E, Bar = 50 µm.

In Group 3 and Group 4, similar to the control group, in animals that underwent TENS application to both the sciatic nerve (n.ischiadicus) and gastrocnemius muscle (m.gastrocnemicus), histological sections taken from the distal part of the injury site of the sciatic nerve on day 21 showed edema in the epineurium and infiltration of a few mononuclear inflammatory cells. The endoneurium exhibited an irregular, scattered structure, with myelinated axons having mostly lost their myelin sheaths, and vacuolar degeneration was observed. Additionally, an increase in the number of

Schwann cells and infiltration by a small number of mononuclear inflammatory cells was detected (Figure 5). On day 21, no histopathological differences were observed between the control group and the TENS-treated groups.

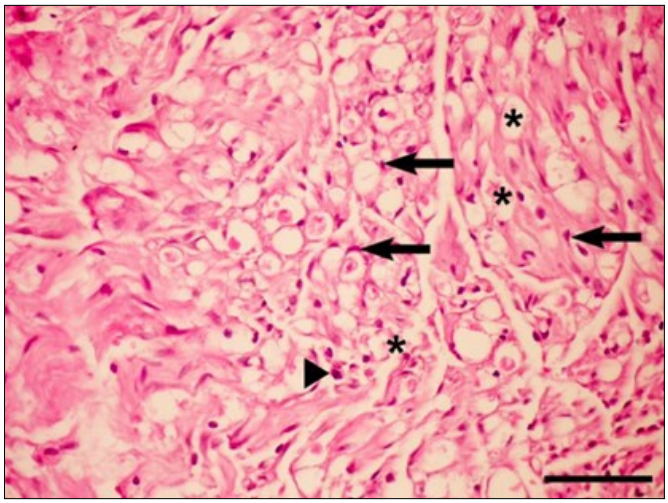

Figure 5. In the experimental group on day 21, an increase in the number of Schwann cells (arrow), mononuclear cells between nerve cells (head of arrow), and degeneration in the myelin sheath and axons (star).

In biopsy samples taken from the distal portion of the sciatic nerve (n.ischiadicus) injury site of control group rats on day 45, there was significant Schwann cell proliferation and the presence of mononuclear inflammatory cell infiltrates. It was observed that the degenerative axonal structures were progressively disappearing. Histopathological examination of gastrocnemius muscle (m.gastrocnemius) samples from the control group on day 45 revealed mild degenerative changes (Figure 6).

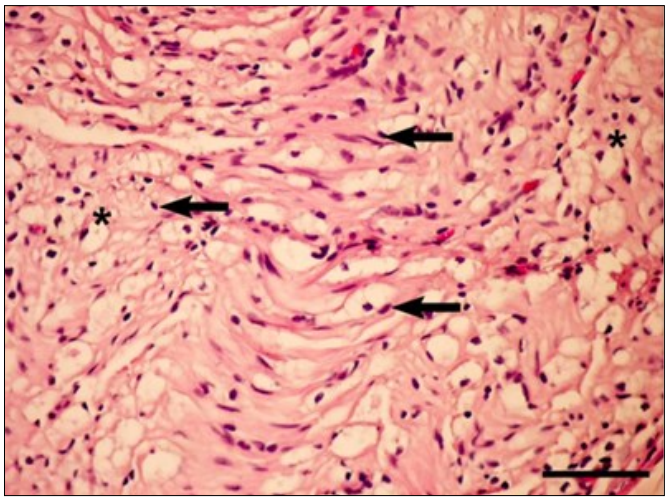

Figure 6. Control group, Day 45, significant increase in the number of Schwann cells (arrows), minimal degeneration in myelin sheath and axons (stars), H&E, Bar = 50 µm.

Similar to the control group, in animals treated with TENS on both the sciatic nerve (n.ischiadicus) and gastrocnemius muscle (m.gastrocnemius), sections taken from the distal portion of the sciatic nerve injury site on Day 45 showed significant axonal and myelin degeneration. However, no Schwann cell proliferation was observed compared to the control group on Day 45 (Figure 7). When histopathological comparisons were made between the control group and the TENS-treated groups on Day 45, it was found that regeneration in the control group was much more pronounced than in the TENS groups. However, no differences were observed between the n.ischiadicus and m.gastrocnemius groups. In the examination of gastrocnemius muscle (m.gastrocnemius) samples from animals treated with TENS on both the sciatic nerve and gastrocnemius muscle on Day 45, atrophic changes in muscle cells were observed, along with hyalinization and activation of connective tissue between muscle fiber.

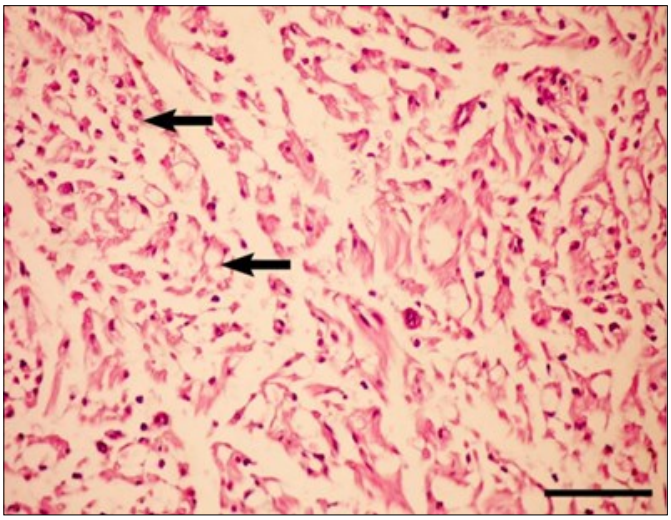

Figure 7. In the experimental group, Day 45, severe degeneration in myelin sheath and axons (arrows), H&E, Bar = 50 µm.

Discussion

The rat sciatic nerve is the most widely used model for examining functional, histological, and electrophysiological changes after peripheral nerve injury, and for evaluating the effectiveness of different surgical and medical treatment approaches (Varejao et al., 2004). The long course of the rat sciatic nerve, its location in the middle femur area that allows easy dissection, makes it indispensable in nerve research. Another reason for the preference of this nerve in experimental models is that it is a mixed, polyfascicular type nerve, containing axons of various sizes and types, providing a comprehensive research opportunity. This allows for the simultaneous evaluation of both sensory and motor functions (Martin et al., 2006).

In peripheral nerve research, the crush injury model is commonly used for axonotmesis damage, particularly with the rat sciatic nerve. The crush injury model is an

ideal model for studying the cellular and molecular mechanisms of peripheral nerve regeneration and for investigating the effects of various factors on the regeneration process. There are several defined techniques for inducing crush injuries in peripheral nerves in experimental models. The primary challenge in these types of studies is the lack of standardization of the injury extent. Even when a fixed pressure is applied, some nerve fibers may remain unaffected and retain their continuity, which can lead to errors, particularly in electrophysiological measurements. To minimize this issue, Luis and colleagues designed a clamp that applies a pressure of 54 newtons in their experimental models (Luis et al., 2007). Varejao et al. demonstrated in their studies that crush injury with this pressure results in complete functional deficit, with normal values returning no earlier than the 7th week (Varejao et al., 2004). In this study, the use of a clamp was employed to induce similar and consistent nerve damage in the majority of the sciatic nerves. The injury was standardized by applying a single-tooth compression with the clamp. Following the compression injury, a complete loss of function was observed in all subjects. During the subsequent follow-up period, the degree of regeneration was found to be consistent within the respective groups.

In sciatic nerve regeneration, gait analysis is one of the most commonly used methods to assess functional recovery. The Sciatic Functional Index (SFI) was defined by De Medinacelli in 1982 and has since been modified by various researchers. The index is based on measurements taken from the paw prints of the animals, which provides insight into the functional recovery of the nerve. This simple, non-invasive technique is the most frequently used evaluation method because it can be repeated at different time points on the same animal, and it assesses coordinated movement, which results from both sensory and motor recovery (Shen & Zhu, 1995). Kanaya et al. proposed that the SFI is the best method for evaluating nerve regeneration, as the final stage of nerve regeneration is stepping. In this study, the modified SFI formula by Bain et al. was used to make measurements (Kanaya et al., 1996).

One of the main disadvantages of SFI measurements is that the results can vary depending on the technique used and the researcher's precision, increasing the risk of error or inaccurate measurements. Before collecting paw prints, animals need to undergo a training period. Another potential source of error is the unwanted development of contractures in the affected limb. Even if reinnervation occurs in the muscles, joint movement loss and contractures can prevent proper stepping. Hare et al. reported in their study that, even after a year following autologous nerve graft repair, there was no significant improvement in the SFI, likely due to contractures (Hare et al., 1992). In the current study, no joint contractures that would interfere with paw print measurements were encountered. However, varying degrees of autophagia were observed in 10 animals, and four animals with severe autophagia were excluded from the study. The incidence of autophagia is closely related to the type of nerve injury, with more frequent occurrences in complete nerve transections and less so in crush injuries (Martins, 2006). Although different degrees of autophagia were observed in the animals, no behavioral changes suggesting hyperalgesia were noted.

When assessing the sequential measurements of each group, no significant differences were found in the sham group, as expected. In contrast, in the other three groups with crush injuries, significant differences were observed between their sequential measurements, supporting nerve regeneration. When comparing groups by weeks, the sham group was significantly different from the other three groups, with values closer to normal. The control group (Group 2) and the TENS-treated groups (Groups 3 and 4) did not show significant differences. Furthermore, no significant difference was observed between the TENS groups, which applied TENS to different areas. These results suggest that TENS treatment, both at the injury site and in the muscles innervated by the damaged nerve, does not significantly influence functional recovery.

Electrophysiological tests are frequently used to evaluate peripheral nerve regeneration. These tests are based on the measurement of action potentials generated by nerve fibers stimulating muscle fibers, which are then amplified and analyzed. The action potentials obtained are compound muscle action potentials (CMAPs), and various parameters can be measured from these potentials for evaluation. Although electrophysiological tests provide information about the axons passing through the nerve repair site, they do not indicate whether these axons are able to make sufficient distal connections. In this study, electrophysiological measurements were taken at baseline, as well as on days 21 and 45 post-surgery, using electromyography (EMG) on all groups of rats. The electrophysiological measurements included the latency, amplitude, and peak-to-peak amplitude of the CMAPs. The latency refers to the time between the stimulus and the beginning of the muscle contraction, and it is considered an important indicator of myelination (Baykal et al., 2002). In a study by Chen et al. in 2007, an increase in latency values was reported following nerve damage. Similarly, this study showed a significant increase in latency values post-injury.

However, no significant difference in latency was observed between the control and experimental groups.

The amplitude and peak-to-peak amplitude of the CMAPs reflect the total depolarization waves of active muscle fibers that can reach the electrode. Therefore, amplitude is directly related to the number of active neurons. Studies by Baykal et al. in 2002 and Wolthers et al. in 2005 demonstrated a significant decrease in amplitude after injury. In accordance with the literature, results of this showed a significant decrease in amplitude following injury in the control group and TENS groups. The peak-to-peak amplitude results also showed a similar change. These EMG measurements demonstrated that there was no change in electrophysiological parameters between rats treated with or without TENS.

Nerve conduction velocity (NCV) is another important parameter that provides information about the condition of nerve fibers. It measures the speed at which a stimulus travels between two electrodes and is widely used to assess the structural integrity of nerve fibers. In 2004, Varejao et al. reported an NCV of approximately 45.6±3.2 m/s for the intact limbs of rats. Similarly, studies by Arnaoutoglou et al. (2006) and Sayyed et al. (2006) reported average NCVs of 48.02±1.92 m/s and 50.39±2.17 m/s for the intact limbs of rats. In our study, the pre-injury average sciatic nerve conduction velocity was measured at 46.22±3.25 m/s for all groups, which is consistent with the literature. In the sham group, the average conduction velocity for the left sciatic nerve was 51.3±2.65 m/s. In the control group, where injury was inflicted on the left sciatic nerve, the NCV dropped to 18.6±1.64 m/s. In the TENS-treated groups (Groups 3 and 4), the NCV decreased to 19.4±1.6 m/s and 18.8±1.7 m/s, respectively. According to Varejao et al., Cragg and Thomas (1964) reported in their long-term follow-up studies after crush injuries that regenerated fibers never fully returned to normal in electrophysiological measurements.

In the light microscopic examination of the sciatic nerve sections obtained from the experimental control group on the 21st and 45th days, which were stained with hematoxylin-eosin, it was observed that although a significant portion of the myelinated nerve fibers maintained their normal structure, there was a notable decrease in the axon diameters and myelin sheath thickness compared to the intact group. In the light microscopic examination of the sciatic nerve sections with TENS application after nerve crush injury, it was striking that the cell debris related to degeneration could not be cleared from the region. A reduction in the number of myelinated axons was present. This situation was interpreted as an indication that nerve healing was delayed.

Transcutaneous Electrical Nerve Stimulation (TENS) is an electrotherapy method that involves placing superficial electrodes on the skin for pain relief (Akyüz, 2001; Bockstahler, Levine, Millis, 2004). The exact effect of peripheral nerves is not fully understood. In this study, we observed that low-frequency TENS application had a negative impact on the histological healing of the nerve. However, this result was not consistent with the functional and electrophysiological recovery of the nerve. In the light microscopic examination of the sciatic nerve sections obtained from the experimental control group on the 21st and 45th days, which were stained with hematoxylin-eosin, it was observed that although a significant portion of the myelinated nerve fibers maintained their normal structure, there was a notable decrease in the axon diameters and myelin sheath thickness compared to the intact group. In the light microscopic examination of the sciatic nerve sections with TENS application after nerve crush injury, it was striking that the cell debris related to degeneration could not be cleared from the region. A reduction in the number of myelinated axons was present. This situation was interpreted as an indication that nerve healing was delayed.

In conclusion, as the use of TENS becomes more widespread in veterinary medicine, we recommend that it be applied only after the type and localization of the existing neurological damage have been accurately determined to avoid potentially adverse effects on nerve healing.

Acknowledgments

This study derived from the first author's PhD thesis titled “Evaluation of the Efficiancy of TENS treatment, to the Regeneration in N. Ischiadicus Injurid Rats by using ENMG, MRG, Walking Track Analysis and Hystopathologic Examination.”

The study was supported by the Scientific Research Projects Commission with the decision number 2007/11030.

References

Arnaoutoglou, C. M., Sakellariou, A., Vekris, M., Mitsionis, G. I., Korompilias, A., Ioakim, E., Harhantis, A., & Beris, A. (2006). Maximum intraoperative elongation of the rat sciatic nerve with tissue expander: Functional, neurophysiological, and histological assessment. *Microsurgery, 26*(4), 253-261.

Baykal, S., Boz, C., Çakır, E., Baytan, Ş. H., Karakuş, M., & Kuzeyli, K. (2002). The effects of pentoxifylline in experimental nerve injury. *Turkish Journal of Medical Sciences, 32*, 207-210.

Baptista, A. F., Gomes, J. R. S., Oliveira, J. T., Santos, S. M. G., Vannier Santos, M. A., & Martinez, A. M. B. (2008). High- and low-frequency transcutaneous electrical nerve stimulation delay sciatic nerve regeneration after crush lesion in the mouse. *Journal of the Peripheral Nervous System 13*, 71-80.

Dewey, C. W. (2003). *A practical guide to canine and feline neurology* (pp. 397-401). Iowa State Press.

Forterre, F., Tomek, A., & Rytz, U. (2007). Iatrogenic sciatic nerve injury in eighteen dogs and nine cats (1997–2006). *Veterinary Surgery, 36*, 464-471.

Hare, G. M., Evans, P. J., Mackinnon, S. E., Best, T. J., Brain, J. R., Szalai, J. P., & Hunter, D. A. (1992). Walking track analysis: A long-term assessment of peripheral nerve recovery. Plastic *and Reconstructive Surgery, 89*, 251-258.

Kanaya, F., Firrell, J. C., & Breidenbach, W. C. (1996). Sciatic function index, nerve conduction tests, muscle contraction, and axon morphometry as indicators of regeneration. *Plastic and Reconstructive Surgery, 98*(7), 1264-1271.

Luis, A. L., Amado, S., Geuna, S., Rodrigues, J. M., Simões, M. J., Santos, J. D., Fregnan, F., Raimondo, S., Veloso, A. P., Ferreira, A. J., Armada-da-Silva, P. A., Varejão, A. S., & Maurício, A. C. (2007). Long-term functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non -serrated clamp. *Journal of Neuroscience Methods, 163*(1), 92-104.

Martins, R. S., Siqueira, M. G., da Silva, C. F, & Plese, J. P. (2006). Correlation between parameters of electrophysiological, histomorphometric and sciatic functional index evaluations after rat sciatic nerve repair. *Arq Neuropsiquiatr, 64*(3B), 750–756.

Rodkey, W. G., & Sharp, N. J. (2003). *Surgery of the peripheral nervous system.* In:Slatter D: Textbook of Small Animal Surgery, Philadelphia, WB Saunders. 1218-1226.

Shen, N., & Zhu, J. (1995). Application of sciatic functional index in nerve functional assessment. *Microsurgery, 16*, 552-555.

Varejao A. S., Melo-Pinto P., Meek M. F., Filipe V. M., & Blulas Cruz J. (2004). Methods for experimental functional assessment of rat sciatic nerve regeneration. *Neurological Research, 26*, 186-194.

Varejao A. S, Cabrita A. M., Meek M. F., Bulas-Cruz J., Melo-Pinto P., Raimondo S., Geuna S., & Giacobini-Robecchi M. G. (2004). Functional and morphological assessment ofa standardized rat sciatic nerve crush injury with a non-serrated clamp. *Journal Neurotrauma, 21*(11), 1652–1670.

Wolthers, M., Moldovan, M., Binderup, T., Schmalbruch H., & Krarup C. (2005). Comparative electrophysiological, functional and histological studies of nerve lesions in rats. *Microsurgery, 25* (6), 508–519.

Alarcon, J. B., Chuhuaicura, P. B., K. A., Sluka, Vance Carol, G. T., Fazan, V. P. S., Mbioch, K. A. G., Fuentes, R. E., & Dias, F. J. (2022). Transcutaneous electrical nerve stimulation in nerve regeneration: A systematic review of in vivo animal model studies neuromodulation: *Technology at the Neural Interface, 25*(8), 1248-1258.