ORIGINAL RESEARCH
ORİJİNAL ARAŞTIRMA

The Curative Effect of Swimming Exercise on Electrophysiological Parameters after Sciatic Nerve Injury

ISSN: 2636-848X

DOI: 10.46385/tsbd.1325961

Türk Spor Bilimleri Dergisi
Türk Spor Bil Derg

Cilt 6, Sayı 2
Ekim 2023, 37-50

The Journal of Turkish Sport Sciences
J Turk Sport Sci

Volume 6, Issue 2
October 2023, 37-50

Osman ÖZOCAK [1]
Sevil KESTANE [2]
Huriye Humeyra DURAN [3]
Bekir COKSEVIM [4]

[1] Health Sciences University Physical Therapy and Rehabilitation Hospital
[2] Kırşehir Ahi Evran University, Faculty of Medicine Department of Physiology
[3] Kırşehir Ahi Evran University Faculty of Medecine
[4] Bilecik Şeyh Edebali University Faculty of Medicine Department of Physiology

[1,2] The two authors contributed equally to this work as co-first authors.

Sorumlu Yazar/Corresponding Author:
S. Kestane
e-mail: kestanesevil@gmail.com

Geliş Tarihi: 13.07.2023

Kabul Tarihi: 24.09.2023

Abstract

The sciatic nerve damage can cause symptoms such as loss of muscle strength and pain as a result of trauma to the nerve due to pressure, stretching or cutting, as well as lifelong disability. Despite the increasing knowledge about sciatic nerve regeneration mechanisms, full functional recovery is still insufficient. It is well known that exercise improves overall health. The current study aimed to reveal the therapeutic effects of swimming exercises using electrophysiological methods in rats with experimental sciatic nerve injury. Eighteen male Wistar Albino rats were used in this study. The subjects were randomly divided into three groups (n=6): 1-Control (C), 2-Intact Exercise (IntE), 3-Operated Exercise (OpE) groups. Sciatic nerve injury was performed by inducing experimental damage to the left hind extremity sciatic nerve of rats. Swimming exercise was applied for 45 minutes a day, five days a week (25°C) for four weeks. The level of regeneration was evaluated by taking motor function test-sciatic function index and EMG recording performed at the beginning of the experiment (day 0), after nerve damage (day 3), and at the end of the experiment (day 35). It was determined that sciatic function index, amplitude of motor and sensory nerves, peak-peak amplitude, percentage of compound muscle action potential, motor and sensory nerve conduction velocities, which decreased after sciatic nerve injury, increased with swimming exercise ($p<0.05$). Positive and significant effects were observed in terms of nerve regeneration, motor functional recovery, and electrophysiological parameters in the swimming exercise groups. This study showed that swimming exercise has effective results in the level of recovery after sciatic nerve injury.

Keywords: Exercise, electromyography (EMG), recovery, sciatic function index (SFI), sciatic nerve injury

Yüzme Egzersizinin Siyatik Sinir Hasarı Sonrasında Elektrofizyolojik Parametreler Üzerindeki İyileştirici Etkisi

Özet

Siyatik sinir hasarı, sinirin basınç, gerilme veya kesilme gibi nedenlerle travmaya uğraması sonucu kas gücü kaybı ve ağrı gibi semptomlara neden olduğu gibi hatta yaşam boyu sakatlığa da neden olabilmektedir. Rejenerasyon mekanizmaları hakkındaki bilgilerin artmasına rağmen tam fonksiyonel iyileşme hala yetersizdir. Egzersizin genel sağlığı iyileştirerek fiziksel ve psikolojik refahı arttırdığı iyi bilinmektedir. Bu çalışmada yüzme egzersizlerinin, deneysel siyatik sinir yaralanması olan sıçanlarda elektrofizyolojik yöntemler kullanılarak terapötik etkilerinin ortaya çıkarılması amaçlandı. Bu çalışmada 18 adet erkek Wistar Albino rat kullanıldı. Denekler rastgele üç gruba (n=6) ayrıldı: 1-Kontrol (K) grubu, 2-İntak Egzersiz (IntE) grubu, 3-Opere Egzersiz (OpE) grubu. Siyatik sinir hasarı, sıçanların sol arka ekstremite siyatik sinirinde deneysel hasar oluşturularak gerçekleştirildi. Dört hafta süresince, haftada beş gün, günde 45 dakika yüzme egzersizi uygulandı (25°C). Rejenerasyon düzeyi, deney başında (0. gün), sinir hasarı sonrası (3. gün) ve deney sonunda (35. gün) yapılan motor fonksiyon testi-siyatik fonksiyon indeksi ve EMG kaydı alınarak değerlendirildi. Siyatik sinir hasarı sonrası azalan siyatik fonksiyon indeksi, motor ve duyu sinirlerinin amplitüdü, peak-peak amplitüdü, bileşik kas aksiyon potansiyeli yüzdesi, motor ve duyusal sinir iletim hızlarının yüzme egzersizi ile arttığı belirlendi ($p<0,05$). Yüzme Egzersizi yapılan gruplarda sinir rejenerasyonu, motor fonksiyonel iyileşme ve elektrofizyolojik parametreler açısından olumlu ve anlamlı etkiler gözlendi. Bu çalışma yüzme egzersizinin siyatik sinir hasarı sonrasında toparlanma düzeyinde etkili sonuçlar olduğunu gösterdi.

Anahtar Kelimeler: Egzersiz, elektromiyografi (EMG), toparlanma, siyatik fonksiyon indeksi (SFI), siyatik sinir hasarı

To cite this article; Özocak, O., Kestane, S., Duran, H.H., & Çoksevim, B. (2023) The curative effect of swimming exercise on electrophysiological parameters after sciatic nerve injury. *The Journal of Turkish Sport Sciences*, *6*(2), 37-50.

This study was produced from a PhD thesis.

INTRODUCTION

Peripheral nerve injuries, which are an important factor in morbidity, require a long recovery period before and after the operation with the development of neuronal damage. Although the losses caused by peripheral nerve damage are irreversible, it is possible to repair them if the nerve trunk is not damaged (Hu et al., 2007). Peripheral nerve damage causes progressive muscle atrophy and functional loss, with motor, sensory, and autonomic changes in the affected nerve area and there is no easily available successful treatment. The rate of regeneration and functional recovery varies depending on the degree, prolongation, and nature of the damage (Lee and Wolfe, 2000; Mendonça, Barbieri and Mazzer, 2003).

Sciatic nerve injuries include diseases of the peripheral nervous system. Most of these diseases are chronic and the disability rate is high. Exercise acts as a physiological stimulus in humans because it has the capacity to regulate and remodel tissue function (Chatzi et al., 2019). Exercises applied after peripheral nerve injury provide nerve regeneration, axonal regeneration with axonal sprouting and growth by increasing the number and diameter of axons and muscle reinnervation, increase nerve impulse transmission rate and sensorimotor healing, reduce muscle atrophy after denervation, increase neurotrophin levels and help tropism (Asensio-Pinilla, Udina, Jaramillo and Navarro, 2009; Sabatier, Redmon, Schwartz and English, 2008; Udina, Cobianchi, Allodi and Navarro, 2011; Oliveira et al., 2008). Swimming exercises, which allow for early active mobilization and dynamic strength after peripheral nerve damage, are one of the most commonly used rehabilitation methods (Silva et al., 2008).

The type, density, intensity, and duration of exercise, exercise practices contribute to functional recovery by accelerating synaptic elimination, and cause an increase in muscle performance by helping to maintain intramuscular connective tissue density (Udina et al., 2011; López, Modol, Navarro, and Cobianchi, 2015; Teodori et al., 2011).

Purpose of the Research

The purpose of this study was to determine the effect of swimming exercise on motor function test (Sciatic Function Index) and EMG data (motor and sensory nerve conduction), which are indicators of nerve regeneration and reinnervation levels after complete damage and repair of the sciatic nerve in the left hind limb in experimental rats.

METHODS

Experimental Animals

Healthy 3-6-month-old (300±60 g) Male Wistar Albino rats (18 pieces) obtained from the ERU-Experimental Research Application and Research Center (DEKAM) were used. Rats were housed in plastic cages at 23± 2 °C room temperature, 50 ± 10% humidity environment, 12/12 day/night light period, and standard rat feed (20-22% crude protein, 2600-2650 kcal/kg energy, 4-5% crude oil, 5-7% crude cellulose) by feeding ad libitum (Ozocak, 2022). The subjects were randomly divided into three groups (n=6):

Group 1: Control Group (C=6): The group which no application (exercise) was performed.

Group 2: The Intact Exercise group (IntE=6): The group in which only swimming exercises was performed.

Group 3: The Operated Exercise Group (OpE=6): The group in which Sciatic nerve incision and swimming exercises was performed.

Experimental Sciatic Nerve Injury

The sciatic nerves of the left hind limbs of rats were damaged with a complete incision. The nerve injury was performed under an operating microscope. Rats were anesthetized with a combination of intraperitoneal (i.p.) 50 mg/kg Ketamine and 10 mg/kg Xylazine. After cleaning the area to be operated

on with povidone-iodine, the incision was made obliquely on the left hind limb from 2-3 mm distal of the hip joint fold. After reaching the biceps femoris muscle and opening it by blunt dissection along the posterior border of the femur, the sciatic nerve was revealed. The sciatic nerve was cut with a full layer of fine-tipped dissection scissors. The proximal and distal nerve endings of the sciatic nerve were brought end-to-end and repaired with four primary epineural sutures using a 10/0 Ethylon suture. The biceps muscle and the skin were repaired with a suture. The rats were left to recover in a warm environment by dressing with povidone-iodine after the nerve damage (Ozocak, 2022; Celebi, 2013). Penicillin streptomycin group antibiotic was given to prevent infection for 4 days after nerve damage, and meloxicam type analgesic was given to reduce pain.

Swimming Exercises

Exercise application consists of adaptation and swimming exercise protocols; swimming adaptation (10 minutes/day) was applied for 1 week before swimming exercise. Adaptation to swimming exercise and swimming exercise protocols were applied in a pool (Morris water tank) with a water temperature of 25 °C and a length of 80 cm and a water depth of 60 cm.

Adaptation swimming exercises were performed for 1 week in the OpE group before sciatic nerve damage and in the intact group the first week to ensure the rat's adaptation to training. In group OpE, the sciatic nerve damage was created after the adaptation period.

In order to prevent infections after sciatic nerve damage in the OpE group, swimming exercise protocol was applied starting from the 7th day following the healing of scar tissue and wound (second week after the adaptation protocol), and in the intact exercise group, swimming exercise protocol was applied from the first week after the adaptation protocol. The swimming exercise protocol was applied 5 days for a week, 45 minutes a day, during 4 weeks. The rats were rested for 2 days each week at the end of the 5-day swimming exercise protocol. Subjects were allowed to keep their heads under water for less than 5 seconds. To prevent the risk of drowning, if the animals remained under water for more than 5 seconds, intervention was made and they were allowed to surface (Kuzay et al., 2022; Ozocak, 2022).

Methods Used in Data Acquisition

To determine the effectiveness of exercise, motor function tests, and electrophysiological (EMG) data recordings were performed before the experiment (Day 0), on the 3rd day after nerve damage, and at the end on the 35th day (Figure 1).

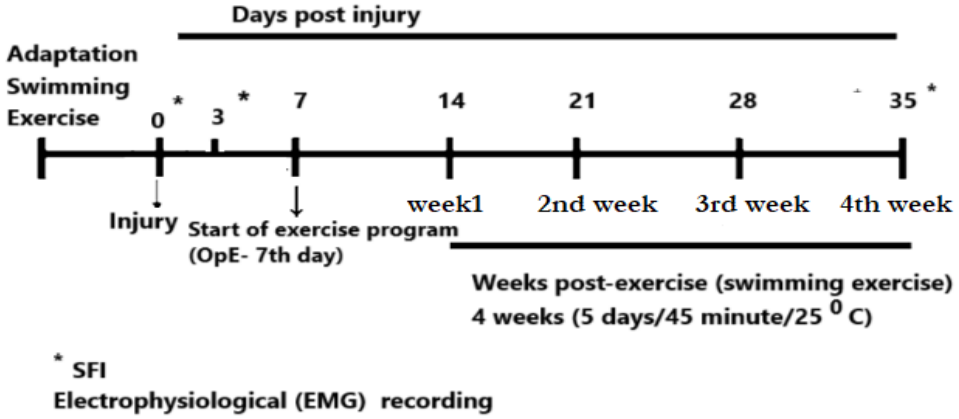

Figure 1. Timeline of the experimental procedures

SFI-38.3{EPL-NPL/NPL}+109.5{ETS-NTS/NTS}+13.3{EIT-NIT/NIT}-8.8.

EPL: The distance from the heel to the third toe on the foot, where the experimental application was performed

NPL: The distance from the heel to the third toe in a normal foot, without experimental application

ETS: The distance from the first and the fifth toe on the experimental applicated foot.

NTS: The distance between the first and fifth toe in the normal foot, without experimental practice

EIT: The distance between the second and the fourth toe in the experimental applicated foot

NIT: The distance between the second and the fourth toe in the normal foot without experimental application

In the values obtained between zero and -100, zero: indicates a normal function, while -100: indicates a complete loss of function. In the obtained SFI values, it was statistically evaluated whether there was a difference between the groups.

Motor Function Tests (Walking Path Analysis)

A walking path assembly with 8.2 x 42 x12 cm dimensions was prepared to evaluate motor function. Both hind legs of the rats were pressed against a blue ink-impregnated stamp, footprints were taken and walking path analyses were performed by running on a strip of prepared paper strips. Using the most appropriate footprints on the hind limbs of each exercised and control (C) rats, the distance-pressure length between the heel and the tip of the finger (print length, PL), the distance-step with between the first and fifth fingers (toe spread, TS), the distance-mid step with between the second and fourth fingers (intermediate toe spread, IT) was measured with the help of a millimetric ruler (digital caliper). Measurements were made by gait analysis and the values obtained were placed in the formula with the help of the multiple linear regression formula modified by Bain et al. and the Sciatic Function Indices (SFI) were calculated. For all three measurements, a factor was created to separate the difference between the ercised and control (C) feet by the normal values (Bain, Mackinnon and Hunter, 1989; Mohammadi, Vahabzadeh and Amini, 2014).

Electrophysiological (EMG) Evaluation

Non-invasive spontaneous and stimulated EMG recordings were taken from the left (damaged) hind limbs of rats using a 4-channel Biopack MP36 System Device and a BSL Stimulus System in the Department of Physiology Laboratory of the ERU Faculty of Medicine.

The records were obtained with the help of BSL 3.7. Pro software programs recorded on the computer and used for analysis. Motor and sensory nerve transmission rates, Action Potential (Compound Muscle Action Potential (CAP) and Sensory Nerve Action Potential (SNAP)) data were obtained by applying the spontaneous activity and stimulation characters through stimulation and recording electrodes placed on the left hind limbs of rats.

Motor nerve conduction speed and CMAP data were obtained by bipolar stimulation electrodes placed proximally to the sciatic nerve incision in the left hind legs of rats ; an active recording electrode placed on the gastrocnemius muscle based on the muscle-tendon principle, a reference recording electrode placed on the gastrocnemius tendon, a ground electrode placed on the tail (Ozocak, 2022; Ashoura et al., 2015; Farzamfarı et al., 2019; Sakar, 2010).

Sensory nerve conduction velocity and SNAP data were obtained via bipolar stimulation electrodes placed distally of the hind leg in the direction of orthodromic conduction and recording electrodes placed proximally of the sciatic nerve, ground electrode placed on the tail. For EMG recordings, a monophasic, square wave current (electric current with a current frequency of 1 Hz and a current duration of 1 ms) was applied as a single (single) warning with a BSL Stimulator System (0-100 Volts) for stimulation purposes. Each warning was given at 5-sec intervals (Ozocak, 2022; Dai et al., 2014; Yang et al., 2015; Pollari, et al., 2018). The current intensity was gradually increased until the supramaximal response amplitude was achieved after the threshold values of rats (Widick, Tanabe, Fortune and Zealear, 1994).

Action Potentials (CMAP and SNAP) data from EMG recordings were analyzed using BioPac Pro 3.7 (BioPac Systems, Inc., USA) software. Parameters related to the stimulus-response relationship, such as delay time (latency), response time, total time, amplitude, peak-peak amplitude (p-p), percentage CMAP values, and motor-sensory nerve conduction rates (Figure 2), which are measurement data of the action

potential, were calculated (Iijima, Ajiki, Murayama and Takeshita, 2016; Rodinskii, Serdyuchenko and Demchenko, 2013).

In this study, % CMAP values, which are also indicators of the regeneration of the operated sciatic nerve and the regeneration index, were obtained by calculating the percentage of the difference between the CMAP amplitude value in the damaged left hind limb and the CMAP amplitude value in the intact right hind limb, were calculated.

$$\% \text{ CMAP} = 100 \times \text{CMAP (left)} / \text{CMAP (right)}$$

Motor and sensory nerve conduction velocities (MNCV and SNCV) were calculated as m/second (m/s) by dividing the distance between the excitation and recording electrodes by the latent period. Experimental applications and data acquisition, intracardiac blood collection, and euthanasia with cervical dislocation were performed on the 35th day after completion in each group (Ozocak, 2022; Sakar, 2010).

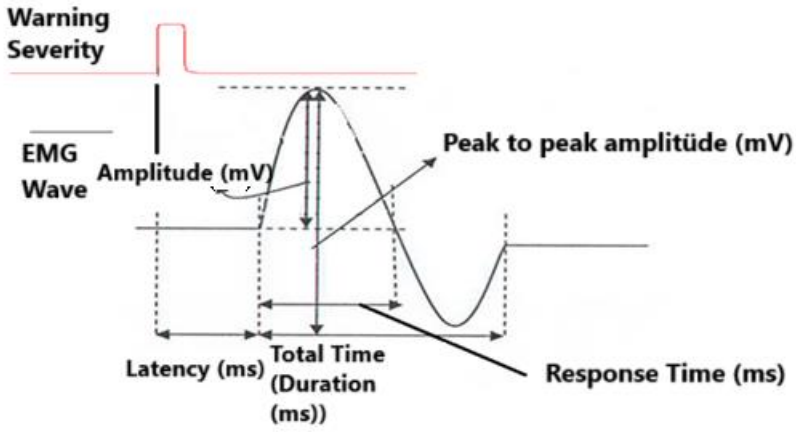

Figure 2. Parameters measured in EMG recordings

Statistical Analysis

The 'IBM SPSS 22' package program was used for statistical analysis. The data obtained by applying a one-way ANOVA test for multiple comparisons between groups, posthoc Tukey and Scheffe tests for all bilateral comparisons between groups, two independent samples t-test, and paired sample t-test for intra-group comparison were compared. These tests were performed according to the homogeneity of variance and the significance level was considered as $p<0,05$.

RESULTS

The Effect of Exercise on Sciatic Function Index (SFI): There was a significant difference between groups ($p=0.000$) in SFI values after nerve damage (day 3), and at the end (day 35) of the experiment (Figure 3). SFI values were significantly higher (less negative) on day 35 in group IntE ($p=0.000$) compared to the control (C) group in bilateral comparisons between groups.

Statistically significantly higher (less negative) SFI values were found in group OpE ($p=0.001$) compared to groups C and IntE before the experiment. SFI values of day 3 ($p=0.000$) and day 35 ($p=0.000$) was found significantly lower (more negative) in group OpE, according to groups C and IntE (Figure 3).

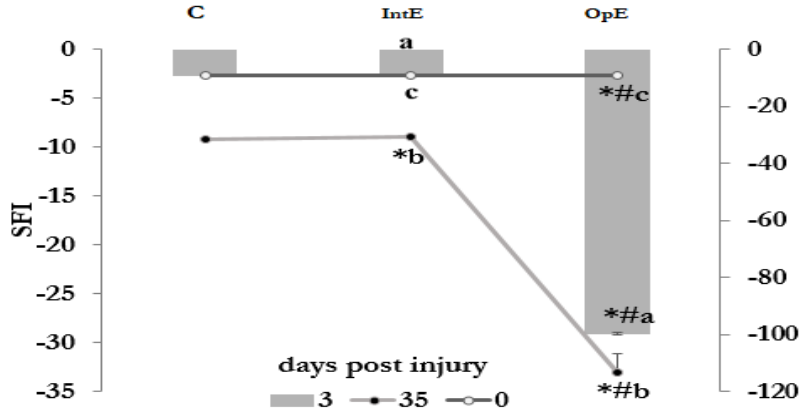

Figure 3. Sciatic Function Index (SFI) data of the experimental groups:
*Data are given as X ± Standard Error (SE). day 0: Before Experiment, 3. Day: After Injury, Day 35: After Experiment. C: Control, IntE: Intact Exercise, OpE: Operated Exercise. One-way ANOVA: (post-injury 0, 3 and 35th days; p=0.000) Symbols (C: *, IntE: #), according to other groups (Independent Samples t-test: in OpE group; day0; p=0,001, and days3 and 35: p=0.000), lower case letters (days 0-3: a, days 0-35: c, days 3-35: b, Paired Samples t-test: in OpE group; p=0.000) indicate the degree of significance compared to another day (p<0.05).*

The Effects of Exercise on Action Potential (CMAP and SNAP): The amplitude, peak-peak amplitude, latency, response time, and total duration values of the Action Potential (CMAP and SNAP) at the beginning of the experiment did not differ significantly (p>0.05), while they show a significant difference in the multiple comparisons between groups in day 3 (p=0.000) and day 35(p=0.000).

CMAP and SNAP amplitude levels showed a significant increase on day 35 (respectively, p=0.005; p=0.027) in group IntE according to group C. CMAP and SNAP amplitude and peak-peak amplitude levels revealed a significant decrease on the day 3 (p=0.000; p=0.000, respectively) and the day 35 (p=0.000; p=0.000, respectively) in group OpE according to the groups C and IntE.

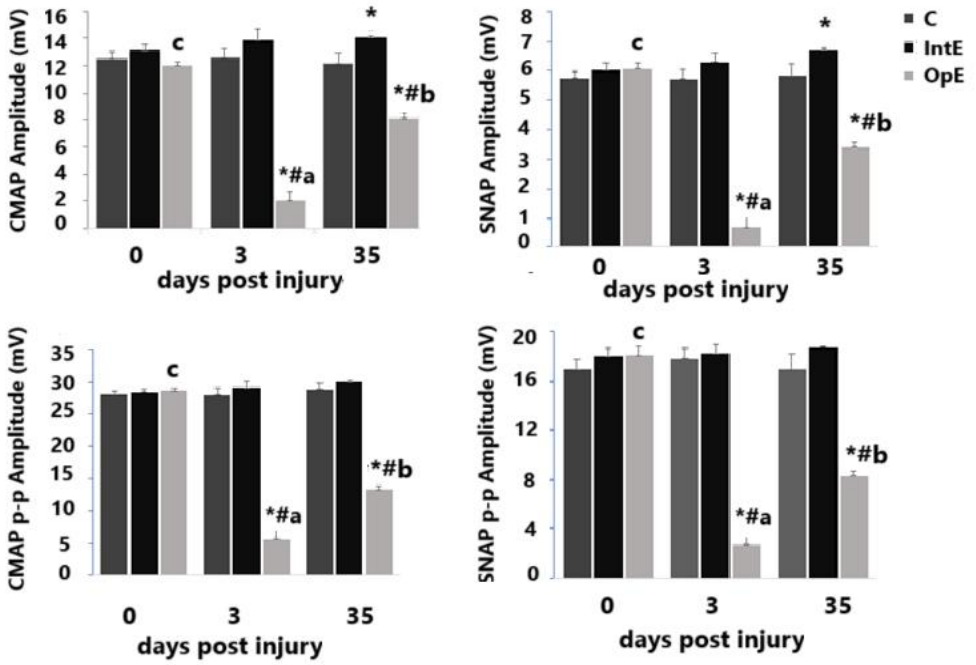

Figure 4. CMAP and SNAP amplitude and p-p amplitude change levels of experimental groups
*One-way ANOVA: (post-injury 3 and 35th days; p=0.000) Symbols (C: *, IntE: #), according to other groups (Independent Samples t-test: in OpE group; CMAP and SNAP amplitude and p-p amplitude; 3 and 35th days: p=0.000 and p=0.000), lower case letters (days 0-3: a, days 0-35:*

c, days 3-35: b, Paired Samples t-test: in OpE group; CMAP and SNAP amplitude and p-p amplitude; 3 and 35th days: p=0.000 and p=0.000) indicate the degree of significance compared to another day.

In the intra-group comparison, CMAP and SNAP amplitude and peak-peak amplitude values showed a significant increase on day 35 with exercise application (p=0.000; p=0.000, respectively) while it decreased significantly (p=0.000; p=0.000, respectively) on day 3 in group OpE (Figure 4).

The CMAP and SNAP latency duration, response time, and total duration levels showed a significant elongation in groups C and IntE on day 3 (p=0.000; p=0.000, respectively) and day 35 (p=0.000; p=0.000, respectively) according to group OpE (Figure 5).

The term of CMAP latency of group IntE showed a significant shortening on day 35 (p=0.000) in intra-group comparisons. CMAP and SNAP latency times, response time, and total time levels are significantly longer on day 3 (respectively, p=0.000; p=0.000), while they showed a significant shortening (respectively, p=0.000; p=0.000) on day 35 in group OpE (Figure 5).

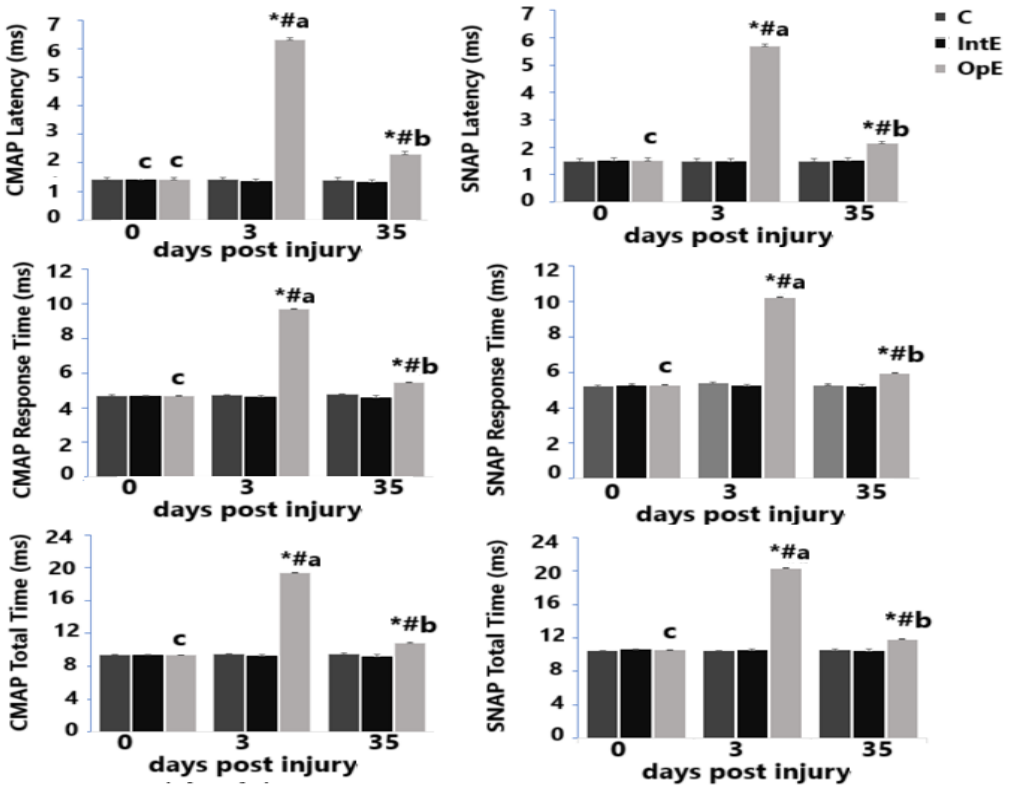

Figure 5. CMAP and SNAP Change Levels of Experimental Groups:

*One-way ANOVA: (post-injury 3 and 35th days; p=0.000) Symbols (C: *, IntE: #), according to other groups (Independent Samples t-test: in OpE group; CMAP and SNAP latency, response time and total time: 3 and 35th days: p=0.000, p=0.000, p:0.000), lower case letters (days 0-3: a, days 0-35: c, days 3-35: b, Paired Samples t-test: in OpE group; CMAP and SNAP latency, response time and total time: 3 and 35th days: p=0.000, p=0.000, p:0.000) indicate the degree of significance compared to other days.*

The percentage CMAP values showed significant differences on day 3 (p=0.000), and on day 35 in the multi-comparison between groups (p=0.000). Percentage CMAP levels were significantly lower in group IntE (p=0.014) according to group C at the beginning of the experiment. The percentage CMAP values showed a significant decrease in group OpE on day 3 (p=0.000) and day 35 (p=0.000) according to groups C and IntE. Percentage CMAP values showed a significant decrease on day 3 (p=0.000) while an increase is shown on day 35 (p=0.000) with exercise practices in intra-group comparison in group OpE (Figure 6).

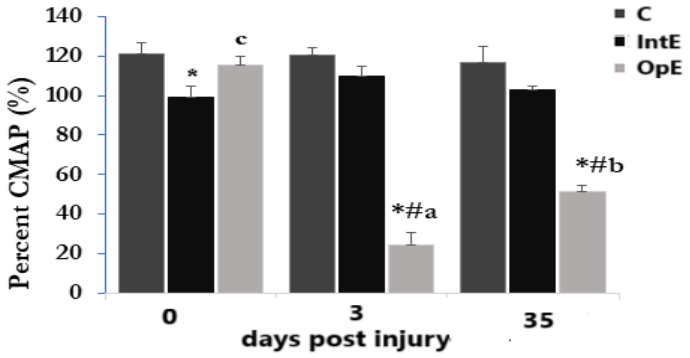

Figure 6. Percent CMAP values of experimental groups:

*One-way ANOVA: (post-injury 0. day: p=0.054, 3 and 35th days; p=0.000) Symbols (C: *, IntE: #), according to other groups (Independent Samples t-test: in OpE group; Percent CMAP-3 and 35th days: p=0.000), lower case letters (days 0-3: a, days 0-35: c, days 3-35: b, Paired Samples t-test: in OpE group; Percent CMAP: 3 and 35th days: p=0.000) indicate the degree of significance compared to other days.*

The Effect of Exercise on Motor and Sensory Nerve Conduction Rates (MNCV- SNCV): There was a significant difference in motor and sensory nerve conduction rates on day 3 (p=0.000) and day 35 (p=0.000) while there was no statistically significant difference at the beginning of the experiment (p>0.05), between the groups. MNCV and SNCV levels of group OpE decreased significantly on day 3 (p=0.000; p=0.000, respectively) and day 35 (p=0.000; p=0.000, respectively) according to groups C and IntE.

MNCV, and SNCV values of group OpE decrease significantly on day 3 (p=0.000; p=0.000, respectively) while they increase significantly (p=0.000; p=0.000, respectively) on day 35 in the intra-group comparison. Although it was not significant in group IntE, there was an increase in MNCV value (p>0.05) after exercise (Figure 7).

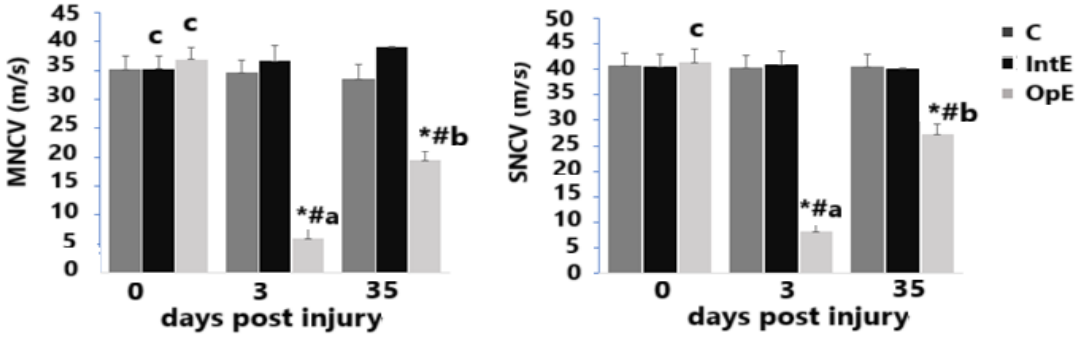

Figure 7. Motor Nerve ConductionVelocity (MNCV) and Sensory Nerve Conduction Velocity (SNCV) values of experimental groups

*One-way ANOVA: (post-injury 3 and 35th days; p=0.000) Symbols (C: *, IntE: #), according to other groups (Independent Samples t-test: in OpE group; MNCV and SNCV; 3 and 35th days: p=0.000 and p=0.000), lower case letters (days 0-3: a, days 0-35: c, days 3-35: b, Paired Samples t-test: in OpE group; MNCV and SNCV; 3 and 35th days: p=0.000 and p=0.000) indicate the degree of significance compared to other days.*

DISCUSSION and RESULT

Because sciatic nerve injuries affect our lives in many ways, more research is needed to ensure rapid nerve regeneration. Considering ineffective treatment methods, many protocols have been designed to improve motor function and repair nerve and muscle damage after peripheral nerve injury (Arabzadeh et al., 2022). However, the results and evidence on this subject differ due to the different modeling of nerve damage

and the type, intensity and duration of the designed exercise programs. For this purpose, this study aimed to investigate the effect of 45-minute swimming exercise during four weeks on the recovery level of sciatic nerve damage in Wistar Albino rats.

Although there are sufficient studies on sciatic nerve damage in the literature, studies with swimming exercises in terms of rehabilitation processes are limited. In this study, we aimed to present the contributions of swimming exercises performed on rehabilitation processes in rats with sciatic nerve damage.

This study shows that swimming exercises cause significant and positive improvements in terms of motor functional recovery, walking stability, and nerve regeneration after nerve damage. The types of sciatic nerve damage (Goulart et al., 2014; Andrade et al., 2020), type of exercise and duration of application affect SFI values in different ways (Pollari et al., 2018; Debastiani, Santana, Ribeiro, Brancalhão and Bertolini, 2019; Rosa Junior et al., 2016; Arabzadeh at al., 2022). Different exercise practices ; swimming exercises (Ozocak, 2022, Pollari et al., 2018; Debastiani et al., 2019; Rosa Junior et al.,2016; Arabzadeh at al., 2022), resistance exercises (Bobinski et al., 2011), treadmill exercises (Udina et al., 2011; Goulart et al., 2014; Bobinski et al., Cai, Na and Hwangbo, 2015; Minegishi et al., 2022; Tsai et al., 2012), endurance exercises (Ilha et al., 2008), balance and coordination exercises (Bonetti et al., 2011), eccentric exercises (Martins et al., 2018), active and passive cycling exercises (Oliveira et al., 2008), the intensity of exercise (low intensity-30min/ 5 days a week/2 and 4 weeks-10m/min)and high intensity improve nerve regeneration and functional recovery by increasing the SFI values (Bobinski et al., 2011; Cai et al., 2015). Low-intensity treadmill exercises (8 m/min/30 min/5 days) in the acute period after nerve damage and high-intensity treadmill exercises (20 m/min/30 min/5 days) in the late period provide motor functional improvement by increasing SFI values (Cai et al., 2015). Treadmill exercises performed after single and multiple sciatic nerve crush injuries (60 min/week 5 days/10 m/min) statistically improved SFI values on the 14th to 21st days for single nerve crush injury and on the 21st to 35th days for multiple nerve crush injury (Minegishi et al., 2022; Tsai et al., 2012). Treadmill exercises (10 min/5m/min for the first 2 weeks, 15 min/10 m/min for the 3rd and 4th weeks, 20 min/m/min for the 5th and 6th weeks) started at the 3rd week after sciatic nerve damage and repair, improved SFI values, similar results were obtained to the control group, nerve graft, and nerve repair after damage and repair of the sciatic nerve by nerve graft they stated that the graft+exercise groups did not improve the SFI values and did not modify nerve regeneration (Figueiredo et al., 2022). Van Meeteren et al. (van Meeteren, Brakkee, Hamers, Helders and Gispen, 1997) reported that long-term swimming exercises (24 days) performed after sciatic nerve injury provide restoration of muscle reinnervation. Accordingly, the current study showed that 20 days of swimming exercise improved nerve regeneration after sciatic nerve injury.

EMG provides important information in the detection of neurodegenerative disorders in the clinic along with the degeneration and regeneration processes after peripheral nerve injuries.

In this study, CMAP and SNAP latency, response, and total time in the left hind limbs of rats were prolonged with myelin sheath damage immediately after sciatic nerve injury, while these times were shortened on the 35th day with exercise application. The amplitude, peak-peak amplitude values, and percentage CMAP values from the MNCV, SNCV, CMAP, and SNAP parameters decreased immediately after sciatic nerve injury, while they increased with exercise practices at the end of the experiment.

Prolonged latency duration (English, Chen, Carp, Wolpaw and Chen, 2007; Selagzi, Buyukakilli, Cimen, Yilmaz and Erdogan, 2008), decreased CMAP amplitude levels after peripheral nerve injuries and diabetic peripheral neuropathy (English et al.,2007; Han, Lu, Xu L and Xu J, 2015) , swimming exercises (Pollari et al., 2018; Selagzi et al., 2008), low-intensity aerobic treadmill exercises (60 min/5 days per week/6 weeks) exercises (Park and Höke, 2014), intermittent or continuous treadmill exercises (5 days per week/4 weeks) in male and female rats (Wariyar, Brown, Tian, Pottorf and Ward, 2022), upward sloping at 11 weeks it has been shown that latency times are shortened and CMAP amplitude levels are increased with treadmill exercises (20% slope/16m/min) and flat slope (0% slope/16m/min) (Cannoy et al.,2016),

treadmill exercises (Sabatier et al., 2008; Tsai et al., 2012; Boeltz et al., 2013; Brandt et al., 2015; Cobianchi, Casals-Diaz, Jaramillo and Navarro, 2013). After sciatic nerve incision and repair, the latency period at the start time of reinervation is extended, active treadmill exercises (EA-1 hour / 5 days per week/1 month) and passive cycling exercises (EP-two sets of 5 days per week/45rpm/30 minutes/1 month) shorten this period. The permanent prolongation of the initial latency and decrease in peak amplitude levels after a sciatic nerve crushing injury was probably caused by demyelination, which caused abnormal CMAP activity, the latency period and CMAP amplitude levels were shorter, and higher in the swimming exercise group than in the untreated group (Yang et al., 2015) like this study. It has been stated that long-term swimming exercises (30 min/7 days/ water temperature 30°C) performed alone after complete sciatic nerve incision don't change the parameters of SFI, CMAP, amplitude, latency, but only mesenchymal stem cell applications improve these parameters (Wang, Yang, Chen and Hsieh, 2010). Intermittent or continuous treadmill exercises (5 days a week, 4 weeks) after sciatic nerve incision and repair shorten the response time (latency), increase CMAP amplitude levels, increase functional development and axon elongation by increasing functional vascularization, axon elongation, and functional improvement if the nerve is repaired with fibrin glue together with at least 10 days of exercise therapy (Wariyar et al.,2022). Swimming exercises (3 times a week-10 minutes/3 times a week- 20 minutes/3 times a week for 30 minutes/ 4 weeks) started from the 7th day after sciatic nerve incision did not significantly change the CMAP amplitude, latency, and nerve conduction rate (NCV) levels in rats (Liao et al., 2017). Myelin sheath destruction after peripheral nerve damage begins on the 2nd day after sciatic nerve damage, while macrophages that phagocyte myelin reach a peak level on the 7th day after nerve damage. Myelin formation starts from the 5th day after nerve damage, while the myelin sheath repair and the healing process take about two weeks (Hirata and Kawabuchi, 2002; Kavlak et al., 2014).

The latency period increases in the damaged group while the latency periods decrease with exercise practice. In studies, even with different types of exercise, the duration of latency varies according to the created nerve damage model (English et al., 2007). Although 4-hour daily challenging maximal exercises (van Meeteren et al., 1997) after sciatic nerve injury improved MNCV values towards near normal values (80-120%) in the early period (on the 100th day). It was determined that swimming exercises (45 min/5 days/4 weeks) applied after sciatic nerve injury improved MNCV values (52%) and SNCV values more (65%) in this study.

It was observed that MNCV values, which decreased after sciatic nerve crush injury, returned to normal levels after swimming exercise application (Kavlak et al., 2014). In rats with peroneal nerve injury, it has been stated that treadmill exercises performed twice a day for 1.5 hours for 10 weeks (5 days/week), improve fast nerve fibers and increase nerve conduction speed (Marqueste, Alliez, Alluin, Jammes and Decherchi, 2004).

In recent years, there has been an increasing interest in evaluating the effects of exercise on the peripheral nervous system. The absence of guidelines on the type, duration, and intensity of exercises makes it difficult to evaluate results. Also, the involved processes of nerve repair are not the main focus of most studies. Therefore, advances from animal studies can be used to further develop the field of exercise rehabilitation.

The paucity of literature on the relationship between sciatic neuropathy biomarkers and swimming exercise in humans makes it necessary to extend the research on animal studies to better understand the mechanisms of exercise-related nerve regeneration. The purpose of this study was to determine swimming exercise play a role in sciatic-peripheral nerve repair and regeneration, motor functional recovery, and electrophysiological parameters and affect recovery. Further, the aim was to indicate the impact, timing, type, and dosage of these specific strategies in humans and animals with peripheral neuropathy or nerve damage, particularly regarding when to start this therapy.

REFERENCES

Andrade, I. R. S., Nakachima, L. R., Fernandes, M., Fernandes, C. H., Santos, J. B. G. D., & Valente, S. G. (2020). Assessment of the effects of swimming as a postoperative rehabilitation on nerve regeneration of wistar rats submitted to grafting of autologous nerves after injury to the sciatic nerve. *Revista brasileira de ortopedia, 55*(3), 323–328. https://doi.org/10.1055/s-0039-1692711

Arabzadeh, E., Rahimi, A., Zargani, M., Feyz Simorghi, Z., Emami, S., Sheikhi, S., Zaeri Amirani, Z., Yousefi, P., Sarshin, A., Aghaei, F., & Feizolahi, F. (2022). Resistance exercise promotes functional test via sciatic nerve regeneration, and muscle atrophy improvement through GAP-43 regulation in animal model of traumatic nerve injuries. *Neuroscience letters, 787*, 136812. https://doi.org/10.1016/j.neulet.2022.136812

Asensio-Pinilla, E., Udina, E., Jaramillo, J., & Navarro, X. (2009). Electrical stimulation combined with exercise increase axonal regeneration after peripheral nerve injury. *Experimental neurology, 219*(1), 258–265. https://doi.org/10.1016/j.expneurol.2009.05.034

Ashoura, FA., Elbazb, AA., Sabeck, NA., Hazzaad, SM., Metwallyd, EM. (2015). Effect of electrical stimulation and stem cells on experimentally induced peripheral nerve injury in rats. *Menoufia Med J,* 28, 742-747.

Bain, J. R., Mackinnon, S. E., & Hunter, D. A. (1989). Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. *Plastic and reconstructive surgery, 83*(1), 129–138. https://doi.org/10.1097/00006534-198901000-00024

Bobinski, F., Martins, D. F., Bratti, T., Mazzardo-Martins, L., Winkelmann-Duarte, E. C., Guglielmo, L. G., & Santos, A. R. (2011). Neuroprotective and neuroregenerative effects of low-intensity aerobic exercise on sciatic nerve crush injury in mice. *Neuroscience, 194*, 337–348. https://doi.org/10.1016/j.neuroscience.2011.07.075

Boeltz, T., Ireland, M., Mathis, K., Nicolini, J., Poplavski, K., Rose, S. J., Wilson, E., & English, A. W. (2013). Effects of treadmill training on functional recovery following peripheral nerve injury in rats. *Journal of neurophysiology, 109*(11), 2645–2657. https://doi.org/10.1152/jn.00946.2012

Bonetti, L. V., Korb, A., Da Silva, S. A., Ilha, J., Marcuzzo, S., Achaval, M., & Faccioni-Heuser, M. C. (2011). Balance and coordination training after sciatic nerve injury. *Muscle & nerve, 44*(1), 55–62. https://doi.org/10.1002/mus.21996

Brandt, J., Evans, J. T., Mildenhall, T., Mulligan, A., Konieczny, A., Rose, S. J., & English, A. W. (2015). Delaying the onset of treadmill exercise following peripheral nerve injury has different effects on axon regeneration and motoneuron synaptic plasticity. *Journal of neurophysiology, 113*(7), 2390–2399. https://doi.org/10.1152/jn.00892.2014

Cai, J., Na, S., & Hwangbo, G. (2015). The effects of exercise intensity and initial timing on functional recovery after sciatic nerve crush injury in rats. *Journal of the Korean Society of Physical Medicine. The Korean Society of Physical Medicine.* https://doi.org/10.13066/kspm.2015.10.3.1

Cannoy, J., Crowley, S., Jarratt, A., Werts, K. L., Osborne, K., Park, S., & English, A. W. (2016). Upslope treadmill exercise enhances motor axon regeneration but not functional recovery following peripheral nerve injury. *Journal of Neurophysiology, 116*(3), 1408–1417. https://doi.org/10.1152/jn.00129.2016

Celebi, MT. (2013). *The effects of low dose radiation and VEGF (Vascular Endothelial Growth Factor) on the repairment of sciatic nerve injury in rats.* Unpublished Master Thesis. Pamukkale University, Medical Faculty Department Of Plastic Reconstructive and Aesthetic Surgery, Denizli.

Chatzi, C., Zhang, Y., Hendricks, W. D., Chen, Y., Schnell, E., Goodman, R. H., & Westbrook, G. L. (2019). Exercise-induced enhancement of synaptic function triggered by the inverse BAR protein, *Mtss1L. eLife,* 8, e45920. https://doi.org/10.7554/eLife.45920

Cobianchi, S., Casals-Diaz, L., Jaramillo, J., & Navarro, X. (2013). Differential effects of activity dependent treatments on axonal regeneration and neuropathic pain after peripheral nerve injury. *Experimental neurology, 240*, 157–167. https://doi.org/10.1016/j.expneurol.2012.11.023

Dai, C., Tang, S., Li, J., Wang, J., & Xiao, X. (2014). Effects of colistin on the sensory nerve conduction velocity and F-wave in mice. *Basic & clinical pharmacology & toxicology, 115*(6), 577–580. https://doi.org/10.1111/bcpt.12272

Debastiani, J. C., Santana, A. J., Ribeiro, L. F. C., Brancalhão, R. M. C., & Bertolini, G. R. F. (2019). Sericin silk protein in peripheral nervous repair associated with the physical exercise of swimming in Wistar rats. Neurological research, 41(4), 326–334. https://doi.org/10.1080/01616412.2018.1564187

English, A. W., Chen, Y., Carp, J. S., Wolpaw, J. R., & Chen, X. Y. (2007). Recovery of electromyographic activity after transection and surgical repair of the rat sciatic nerve. *Journal of neurophysiology, 97*(2), 1127–1134. https://doi.org/10.1152/jn.01035.2006

Farzamfar, S., Salehi, M., Tavangar, S. M., Verdi, J., Mansouri, K., Ai, A., Malekshahi, Z. V., & Ai, J. (2019). A novel polycaprolactone/carbon nanofiber composite as a conductive neural guidance channel: an in vitro and in vivo study. *Progress in biomaterials, 8*(4), 239–248. https://doi.org/10.1007/s40204-019-00121-3

Figueiredo, G. S. L., Fernandes, M., Atti, V. N., Valente, S. G., Roth, F., Nakachima, L. R., Santos, J. B. G. D., & Fernandes, C. H. (2022). Use of aerobic treadmill exercises on nerve regeneration after sciatic nerve injury in spontaneously hypertensive rats. *Acta Cirurgica Brasileira, 37*(8), e370804. https://doi.org/10.1590/acb370804

Goulart, C. O., Jürgensen, S., Souto, A., Oliveira, J. T., de Lima, S., Tonda-Turo, C., Marques, S. A., de Almeida, F. M., & Martinez, A. M. (2014). A combination of Schwann-cell grafts and aerobic exercise enhances sciatic nerve regeneration. *PloS one, 9*(10), e110090. https://doi.org/10.1371/journal.pone.0110090

Han, D., Lu, J., Xu, L., & Xu, J. (2015). Comparison of two electrophysiological methods for the assessment of progress in a rat model of nerve repair. *International Journal of Clinical and Experimental Medicine, 8*(2), 2392–2398.

Hirata, K., & Kawabuchi, M. (2002). Myelin phagocytosis by macrophages and nonmacrophages during Wallerian degeneration. *Microscopy Research and Technique, 57*(6), 541–547. https://doi.org/10.1002/jemt.10108

Hu, J., Zhu, Q. T., Liu, X. L., Xu, Y. B., & Zhu, J. K. (2007). Repair of extended peripheral nerve lesions in rhesus monkeys using acellular allogenic nerve grafts implanted with autologous mesenchymal stem cells. *Experimental Neurology, 204*(2), 658–666. https://doi.org/10.1016/j.expneurol.2006.11.018

Iijima, Y., Ajiki, T., Murayama, A., & Takeshita, K. (2016). Effect of artificial nerve conduit vascularization on peripheral nerve in a necrotic bed. plastic and reconstructive surgery. *Global Open, 4*(3), e665. https://doi.org/10.1097/GOX.0000000000000652

Ilha, J., Araujo, R. T., Malysz, T., Hermel, E. E., Rigon, P., Xavier, L. L., & Achaval, M. (2008). Endurance and resistance exercise training programs elicit specific effects on sciatic nerve regeneration after experimental traumatic lesion in rats. *Neurorehabilitation and Neural Repair, 22*(4), 355–366. https://doi.org/10.1177/1545968307313502

Kavlak, E., Belge, F., Unsal, C., Uner, A. G., Cavlak, U., & Cömlekçi, S. (2014). Effects of pulsed electromagnetic field and swimming exercise on rats with experimental sciatic nerve injury. *Journal of Physical Therapy Science, 26*(9), 1355–1361. https://doi.org/10.1589/jpts.26.1355

Kuzay, D., Kestane, S., Kemerli MZ., Ozocak, O., Aktoprak, M., Coksevim, B (2022). The effect of exercise and mesenchimal stem cell application on plasma oxidative stress levels in experimental neuropathy. *Turkish Journal of Health and Sport. 3* (1), 20-25.

Lee, S. K., & Wolfe, S. W. (2000). Peripheral nerve injury and repair. *The Journal of the American Academy of Orthopaedic Surgeons, 8*(4), 243–252. https://doi.org/10.5435/00124635-200007000-00005

Liao, C. F., Yang, T. Y., Chen, Y. H., Yao, C. H., Way, T. D., & Chen, Y. S. (2017). Effects of swimming exercise on nerve regeneration in a rat sciatic nerve transection model. *BioMedicine, 7*(1), 3. https://doi.org/10.1051/bmdcn/2017070103

López-Álvarez, V. M., Modol, L., Navarro, X., & Cobianchi, S. (2015). Early increasing-intensity treadmill exercise reduces neuropathic pain by preventing nociceptor collateral sprouting and disruption of chloride cotransporters homeostasis after peripheral nerve injury. Pain, 156(9), 1812–1825.

Marqueste, T., Alliez, J. R., Alluin, O., Jammes, Y., & Decherchi, P. (2004). Neuromuscular rehabilitation by treadmill running or electrical stimulation after peripheral nerve injury and repair. *Journal of applied physiology (Bethesda, Md. : 1985), 96*(5), 1988–1995. https://doi.org/10.1152/japplphysiol.00775.2003

Martins, D. F., Martins, T. C., Batisti, A. P., Dos Santos Leonel, L., Bobinski, F., Belmonte, L. A. O., Mazzardo-Martins, L., Cargnin-Ferreira, E., & Santos, A. R. S. (2018). Long-Term regular eccentric exercise decreases neuropathic pain-like behavior and improves motor functional recovery in an axonotmesis mouse model: the role of insulin-like growth factor-1. *Molecular Neurobiology, 55*(7), 6155–6168. https://doi.org/10.1007/s12035-017-0829-3

Mendonça, A. C., Barbieri, C. H., & Mazzer, N. (2003). Directly applied low intensity direct electric current enhances peripheral nerve regeneration in rats. *Journal of Neuroscience Methods, 129*(2), 183–190. https://doi.org/10.1016/s0165-0270(03)00207-3

Minegishi, Y., Nishimoto, J., Uto, M., Ozone, K., Oka, Y., Kokubun, T., Murata, K., Takemoto, H., & Kanemura, N. (2022). Effects of exercise on muscle reinnervation and plasticity of spinal circuits in rat sciatic nerve crush injury models with different numbers of crushes. *Muscle & Nerve, 65*(5), 612–620. https://doi.org/10.1002/mus.27512

Mohammadi, R., Vahabzadeh, B., & Amini, K. (2014). Sciatic nerve regeneration induced by transplantation of in vitro bone marrow stromal cells into an inside-out artery graft in rat. Journal of cranio-maxillo-facial surgery : official publication of the European Association for Cranio-Maxillo-*Facial Surgery, 42*(7), 1389–1396. https://doi.org/10.1016/j.jcms.2014.03.031

Oliveira, L. S., Sobral, L. L., Takeda, S. Y., Betini, J., Guirro, R. R., Somazz, M. C., & Teodori, R. M. (2008). Electrical stimulation and swimming in the acute phase of axonotmesis: their influence on nerve regeneration and functional recovery. *Revista de Neurologia, 47*(1), 11–15.

Ozocak, O. (2022). *The effect of exercise and mesenchymal stem cell (MSC) Applications on muscular performance in sciatic nerve damage.* Unpublished Doctoral Thesis. Erciyes University Health Sciences Institute. Kayseri.

Park, J. S., & Höke, A. (2014). Treadmill exercise induced functional recovery after peripheral nerve repair is associated with increased levels of neurotrophic factors. *PloS one, 9*(3), e90245. https://doi.org/10.1371/journal.pone.0090245

Pollari, E., Prior, R., Robberecht, W., Van Damme, P., & Van Den Bosch, L. (2018). In vivo electrophysiological measurement of compound muscle action potential from the forelimbs in mouse models of motor neuron degeneration. *Journal of visualized experiments : JoVE, (136)*, 57741. https://doi.org/10.3791/57741

Rodinskii, A. G., Serdyuchenko, I. Y., & Demchenko, T. V. (2013). Electrical and force responses of the shin muscles of rats after unilateral compression of the sciatic nerve and systemic introduction of gamma-hydroxybutyrate. *Neurophysiology, 45*(2), 157-167. https://doi.org/10.1007/s11062-013-9352-y

Rosa Junior, G. M.; Magalhães, R. M. G.; Rosa, V. C.; Bueno, C. R. de S.; Simionato, L. H.; Bortoluci, C. H. F. (2016). Effect of the laser therapy in association with swimming for a morphological nerve repair and functional recovery in rats submitted to sciatic axonotmesis, *SciELO Journals*. Dataset. https://doi.org/10.6084/m9.figshare.20015447.v1

Sabatier, M. J., Redmon, N., Schwartz, G., & English, A. W. (2008). Treadmill training promotes axon regeneration in injured peripheral nerves. *Experimental Neurology, 211*(2), 489–493. https://doi.org/10.1016/j.expneurol.2008.02.013

Sakar M. (2010). *The effect of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) PHBHHX and mesenchymal stem cell (MSC) on axonal regeneration in experimental sciatic nerve damage*. Master Hacettepe University Faculty Of Medicine, in Neurosurgery. Ankara.

Selagzi, H., Buyukakilli, B., Cimen, B., Yilmaz, N., & Erdogan, S. (2008). Protective and therapeutic effects of swimming exercise training on diabetic peripheral neuropathy of streptozotocin-induced diabetic rats. *Journal of Endocrinological Investigation, 31*(11), 971–978. https://doi.org/10.1007/BF03345634

Silva, L. E., Valim, V., Pessanha, A. P., Oliveira, L. M., Myamoto, S., Jones, A., & Natour, J. (2008). Hydrotherapy versus conventional land-based exercise for the management of patients with osteoarthritis of the knee: a randomized clinical trial. *Physical Therapy, 88*(1), 12–21. https://doi.org/10.2522/ptj.20060040

Teodori, R. M., Betini, J., de Oliveira, L. S., Sobral, L. L., Takeda, S. Y., & de Lima Montebelo, M. I. (2011). Swimming exercise in the acute or late phase after sciatic nerve crush accelerates nerve regeneration. *Neural Plasticity,* 783901. https://doi.org/10.1155/2011/783901

Tsai, S. W., Chen, C. J., Chen, H. L., Chen, C. M., & Chang, Y. Y. (2012). Effects of treadmill running on rat gastrocnemius function following botulinum toxin A injection. *Journal of Orthopaedic Research : Official Publication of the Orthopaedic Research Society, 30*(2), 319–324. https://doi.org/10.1002/jor.21509

Udina, E., Cobianchi, S., Allodi, I., & Navarro, X. (2011). Effects of activity-dependent strategies on regeneration and plasticity after peripheral nerve injuries. *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft, 193*(4), 347–353. https://doi.org/10.1016/j.aanat.2011.02.012

Van Meeteren, N. L., Brakkee, J. H., Hamers, F. P., Helders, P. J., & Gispen, W. H. (1997). Exercise training improves functional recovery and motor nerve conduction velocity after sciatic nerve crush lesion in the rat. *Archives of Physical Medicine and Rehabilitation, 78*(1), 70–77. https://doi.org/10.1016/s0003-9993(97)90013-7

Wang, J., Yang, C. C., Chen, S. C., & Hsieh, Y. L. (2010). No synergistic effect of mesenchymal stem cells and exercise on functional recovery following sciatic nerve transection. *Functional Neurology, 25*(1), 33–43.

Wariyar, S. S., Brown, A. D., Tian, T., Pottorf, T. S., & Ward, P. J. (2022). Angiogenesis is critical for the exercise-mediated enhancement of axon regeneration following peripheral nerve injury. *Experimental Neurology, 353*, 114029. https://doi.org/10.1016/j.expneurol.2022.114029

Widick, M. H., Tanabe, T., Fortune, S., & Zealear, D. L. (1994). Awake evoked electromyography recording from the chronically implanted rat. *The Laryngoscope, 104*(4), 420–425. https://doi.org/10.1288/00005537-199404000-00005

Yang, C. C., Wang, J., Chen, S. C., Jan, Y. M., & Hsieh, Y. L. (2015). Enhanced functional recovery from sciatic nerve crush injury through a combined treatment of cold-water swimming and mesenchymal stem cell transplantation. *Neurological Research, 37*(9), 816–826.

Financial support

Erciyes University Scientific Research Projects Unit (BAP) (TDK-2019-9480)

Conflict of Interest

The authors have no conflict of interest to declare

Authors' Statement of Contribution to the Article

Idea/Concept: O. Ozocak, B. Coksevim **Article design:** O. Ozocak, S. Kestane, B. Coksevim **Consulting:** B. Coksevim **Data Collection and Processing:** O. Ozocak, S. Kestane **Analysis/Comment:** O. Ozocak, S. Kestane B. Coksevim **Literature review:** O. Ozocak, S. Kestane **Article writing:** O. Ozocak, S. Kestane **Critical Analysis:** O. Ozocak, S. Kestane H.H. Duran, B. Coksevim

Ethics Committee Approval: All experiments were performed according to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory after the approval of the Animal Ethics Committee of Erciyes University (ERU) (approval date and no: 15.05.2015-19/100).

Acknowledgements: The authors wish to thank Prof. Dr .Bekir Çoksevim for guiding during this study, the participants who supported the study and the Erciyes University support.