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RESEARCH ARTICLE

Minimally Invasive Motor Nerve Conduction Study of the Rat Sciatic and Tail Nerves

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Summary

Human and veterinary scientific researchers widely use rats as experimental animals. The rat nerves conduction studies are used for investigations of neural tissue injury, neural regeneration, peripheral neuropathies etc. in experimental models. Nerve conduction studies on animals do not follow strict rules, as studies in humans. Various methods and lack of the single recognized technique of nerve conduction study on the animals often lead to difficulties in the interpretation and comparison of the results of similar studies. In the present study, we have described the minimally invasive motor nerve conduction study on the rat sciatic and tail nerves. Electrophysiological examination including nerve conduction velocity and compound muscle action potential amplitudes measurements were performed on six normal growing male adult Wistar Albino rats. The mean motor nerve conduction velocity of the sciatic nerves was 58.90±5.07 m/s and of the tail nerves was 40.23±2.39 m/s. The mean compound muscle action potential amplitudes of the sciatic and tail nerves were 17.91±6.75 mV and 1.89±0.49 mV, respectively. Similar results of previous *in vitro* and *in vivo* studies prove the objectivity and reliability of our nerve conduction technique by bipolar needle electrodes on the non-exposed nerves.

Keywords: *Nerve conduction study, Sciatic nerve, Tail nerves, Rat*

Rat Siyatik ve Kuyruk Motor Sinirlerinin Minimal İnvaziv İletim Çalışması

Özet

Beşeri ve veteriner bilimsel araştırmacılar, ratları deneysel hayvan olarak yaygınca kullanmaktadırlar. Rat sinir iletim çalışmaları sinir dokusu hasarı, sinir rejenerasyonu, periferik nöropati ve buna benzer deneysel araştırma modellerinde kullanılmaktadır. Hayvanlar üzerinde yapılan sinir iletim çalışmaları, insan çalışmalarından farklı olarak, katı kurallara uymaz. Hayvanlarda sinir iletim çalışmalarının çeşitli yöntemleri ve tek tanınan tekniğin olmayışı benzer çalışmaların sonuçlarının yorumlanmasında ve kıyaslanmasında zorluklara yol açar. Çalışmamızın amacı, rat siyatik ve kuyruk motor sinirlerinin minimal girişimsel iletim çalışma yöntemlerini tanımlamaktır. Altı erkek erişkin Wistar Albino ratın sinir iletim hızları ile bileşik kas aksiyon potansiyelleri ölçümlerini içeren elektrofizyolojik incelemesi yapıldı. Ortalama motor sinir iletim hızları siyatik sinirlerde 58.90±5.07 m/sn ve kuyruk sinirlerinde 40.23±2.39 m/sn idi. Siyatik ve kuyruk sinirlerin ortalama bileşik kas aksiyon potansiyellerinin genliği sırasıyla 17.91±6.75 mV ve 1.89±0.49 mV idi. Bipolar iğne elektrotlar ile ve sinir ekspozisyonu yapılmadan uyguladığımız bu sinir iletim tekniğimizin sonuçlarının daha önceki *in vitro* ve *in vivo* çalışmaların sonuçları ile benzerlik göstermesi bu tekniğinin objektiflik ve güvenilirliğini desteklemektedir.

Anahtar sözcükler: *Sinir iletim çalışması, Siyatik sinir, Kuyruk sinirleri, Rat*

INTRODUCTION

One of the methods for study peripheral nerves is electroneurography (ENG) or, in other words, nerve conduction velocity (NCV) study. This method is used for stimulating the various nerves and recording the potentials. It can be said that, the NCV is an indicator of the physiological or pathophysiological state of the nerves.

The motor nerve conduction velocity (MNCV) is tested by stimulating the motor nerve with a supramaximal stimulus at two points of a nerve trunk. Recording electrodes are placed on the muscles innervated by the nerve and then record the potential which is called a compound muscle action potential (CMAP). CMAP is a

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maximally evoked electrical activity of all active neural fibers. The CMAP recorded from indicator muscle reflects the three values: the size of the motor unit innervated by the axons, the size of motor nerve fibers responding to the stimulus and the synchronization of their response. The greater number of active fibers increases the CMAP amplitude [1,2]. The amplitude of the CMAP recorded by the proximal and distal stimulations conventionally is measured from the peak of the negative deflection to the peak of the positive deflection [1]. The time required for the onset of CMAP with distal stimulation is called the terminal distal latency. The MNCV can be calculated by dividing the distance between proximal and distal stimulation site with the difference of onset latencies of two CMAP. The MNCV reflects the degree of myelination of the fastest axons [3].

As conventional, surface or needle electrodes are use for CMAP recording in human and animal practice. When placed to the skin, surface electrodes allow a non-invasive assessment of the nerve. Surface electrodes are adhered to the skin overlying the selected muscle for indirect measure of muscle generated potentials. Invasive methods consist of surgically placing electrodes on exposed nerve or introducing needles near to nerve. Needle electrodes convert direct signals from selected region of the muscle. Needle electrodes which used in the electrophysiological studies have monopolar, concentric and bipolar properties.

Human and veterinary scientific researchers widely use rats as experimental animals. Viability, fast reproduction and simplicity of these animals make them indispensable in experimental researches. In neurology, rats are used in models of human neurological diseases, neurological drug researches and for development new diagnostic neurological approaches. Particularly, rats are used for investigation of models of peripheral axonal and demyelinating neuropathies, neural tissue injury and regeneration [1-6]. Usually, in these studies scientists have used rats' sciatic nerve, because easy for stimulation, has the simple anatomy and relatively thicker than other peripheral nerves [1-3,6]. As known, the rat sciatic nerve originates from spinal segments L4-L5. In the middle of thigh sciatic nerve is separated into two major branches: the tibial and peroneal branches. The peroneal nerve passes laterally and innerves anterior muscles. The tibial nerve takes a depth course, passes between the lateral and medial heads of gastrocnemius muscle and innervates the flexor muscles [7].

Also, the rat tail nerves conduction studies are used for non-invasive investigations of peripheral neuropathies in experimental models [5,6]. Caudal nerves of rat tail originate from sacrocaudal spinal roots and pass along the length of the tail where they are divided into two minor dorsal and two major ventral nerves [8].

Frequently, for selective stimulation of the nerve and recording the CMAP's in rats are used surgical exploration of the sciatic nerve or other nerves and muscles. These methods can result in sacrificing the animal, because this trauma leads to severe disability or death. As a result, researchers can not track changes in nerves in the same animal over time. Also, multiple animals are needed for the same study.

On the other hand, there are some issues such as ethics and humanistic use of animals in studies. The most appropriate research method and minimum number of animals, which may lead to scientific results, must be preferred in animal studies. Minimizing the pain of the animal during the experiment and less traumatic and appropriate methods of anesthesia and analgesia must be used [9-11].

For these reasons we tried to describe two useful and objective methods of motor nerve conduction study of the sciatic and tail nerves in rats without exploration of nerves and animal sacrification or disability.

MATERIAL and METHODS

This study was performed on six normal growing male adult Wistar Albino rats, weighting 200-225 g. These rats were used as controls in previous study with the approval of the Ethic Committee on Research Animal Care at Veterinary Faculty of Kafkas University of Kars, Turkey (No 2012/30) [1].

All procedures were performed aseptically. The rats were anesthetized by using intraperitoneal 10 mg/kg xylazin HCl and 80 mg/kg ketamin HCl. The animals were positioned prone with maximally straightened hindlimbs. The rat's body temperature was maintained by keeping the animal on an electric pad that was switched off during recording. Core temperatures were measured by placing the probe on the sacrum. Rat's core temperature was maintained at between 36-38°C. Electrophysiological measurements were taken using Neuropack M1 MEB-9200 (Nihon-Cohden Corp, Tokyo, Japan) device. No animal were killed or disabled during the present study.

Sciatic Nerve Conduction Study

In this study, the sciatic nerve was stimulated percutaneously from bipolar needle electrodes which were placed at the level of hip joint and popliteal fossa. Recording bipolar needle electrodes were placed in medial gastrocnemius muscle belly *(Fig. 1)*.

Accurate placement of the recording needle on the gastrocnemius muscle is very important, because the volume conduction from neighboring muscles could result in false positive signals, mostly from the biceps femoris muscle [3,12]. The common reference (ground electrode) was placed on the basis of tail. Electrical stimulation was square pulse with an frequency of 1 Hz and duration of 0.2 m/s. The stimulus current intensity was increased gradually up to

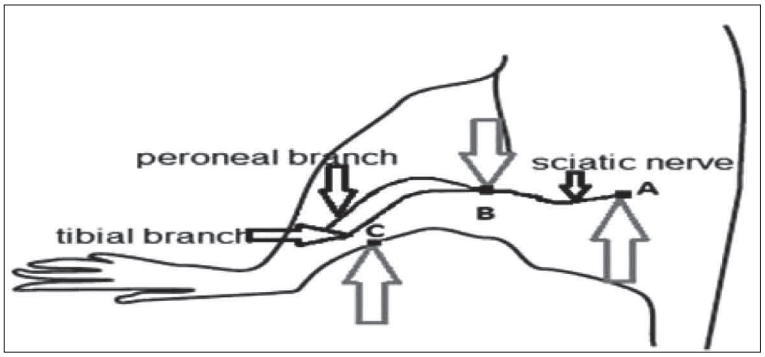

Fig 1. Stimulation and recording points of rat sciatic nerve motor conduction study; **A-** proximal stimulation point, **B-** distal stimulation point, **C-** recording point

Şekil 1. Rat siyatik sinir motor iletim çalışmasında uyarım ve kayıt noktaları; **A-** proksimal uyarım noktası, **B-** distal uyarım noktası, **C-** kayıt noktası

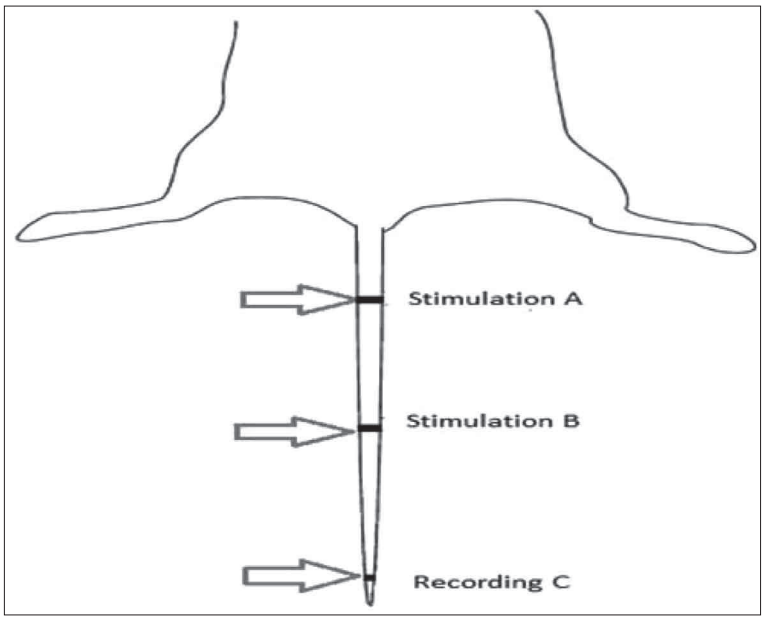

Fig 2. Stimulation and recording points in motor nerve conduction study of rat tail; **A-** proximal stimulation point, **B-** distal stimulation point, **C-** recording point

Şekiil 2. Rat kuyruk motor sinirlerinin iletim çalışmasında uyarım ve kayıt noktaları; **A-** proksimal uyarım noktası, **B-** distal uyarım noktası, **C-** kayıt noktası

supramaximal intensity (current intensity 30% above the value to evoke the maximal CMAP). The stimulation was repeated 5 times for all measurements and the average values were recorded. Then the rat's hindlimbs were straightened and the distance between the stimulating electrodes were ascertained with a tape measure to the nearest millimeter.

In each animal, both sciatic nerves on all 12 hindlimbs were investigated.

Tail Nerve Conduction Study

Fig. 2 shows schematic illustration of the bipolar needle electrode arrangement for the measurement of MNCV in the tail nerves. All electrodes were inserted intramuscularly. CMAPs were evoked by stimulation at point B on the middle part of the tail and point A in the proximal part of the tail. CMAP was recorded at point C, located about 2-3 cm from the end of tail. Frequency, duration and intensity of the electrical stimulation were performed as on the sciatic nerve. The distance between the stimulation and recording electrodes was measured by the tape measure in the straight tail position. MNCV was calculated by the conventional method: the distance was divided by the difference between proximal and distal latencies.

We performed all measurements as accurately as possible because the small error in distance may lead to incorrect results in MNCV. Also, we tried to minimize the artifacts and to record a response, when the electro-negativity from the isoelectric line was started.

Fig. 3 and *Fig. 4* show sample traces of sciatic and tail nerves motor conduction studies.

Statistical Analysis

The results were analyzed using Minitab-12 statistical software. For tests normality of distributions were used the Anderson-Darling test. The following variables were considered: MNCV and CMAP amplitude. All results were expressed as the mean ± standard deviation (SD).

RESULTS

The mean MNCV of the sciatic nerves were 58.90±5.07 m/s and of the tail nerves were 40.23±2.39 m/s *(Fig. 5)*. The mean MNCV were statistically slow in the tail nerves than in the sciatic nerves.

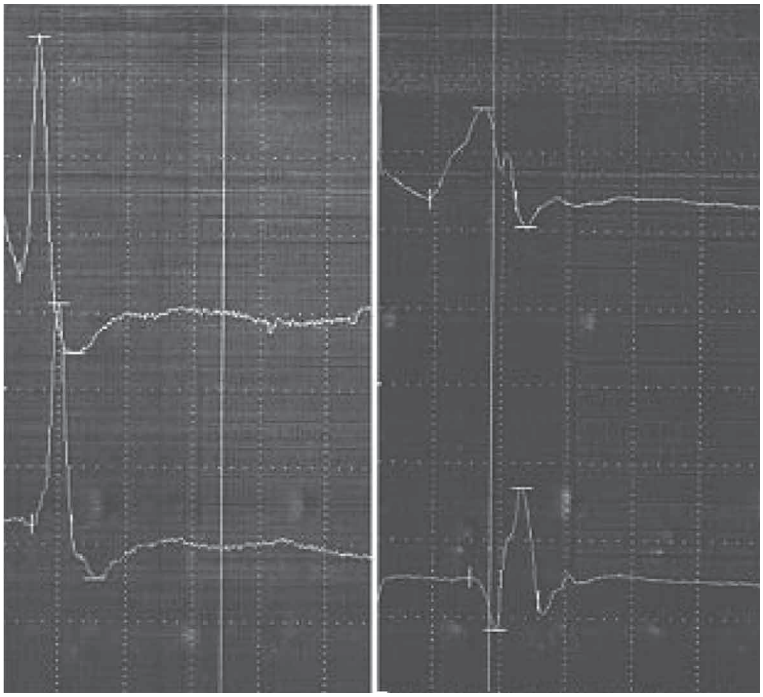

Fig 3. Sample traces of sciatic motor nerve conduction study

Şekil 3. Siyatik sinir motor iletim çalışmasının örnek traseleri

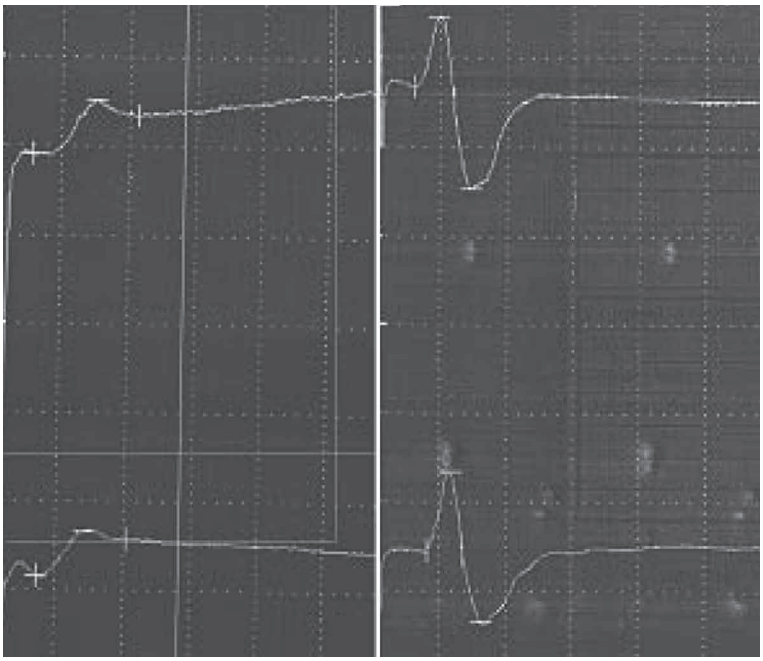

Fig 4. Sample traces of rat tail motor nerve conduction study

Şekil 4. Rat kuyruk motor sinirlerinin iletim çalışmasının örnek traseleri

The mean CMAP amplitudes of the sciatic and tail nerve were 17.91±6.75 mV and 1.89±0.49 mV, respectively *(Fig. 6)*. The CMAP amplitudes of the tail nerves were statistically lower compared with the amplitudes of the sciatic nerves.

DISCUSSION

Nerve conduction studies in rats are used in animal experimental models of chronic or acute axonal and demyelinating neuropathies, in nerve injury and regeneration studies and for better understanding effects and toxicity of several drugs [1-6,8,12]. In the human and animal medical literature, particularly in rats, numerous nerve conduction techniques have been described. Nerve conduction studies on animals do not follow strict rules, as studies in humans [13]. Various techniques of nerve conduction were described in each animal study. Most of these techniques were performed on the exposed nerves *in vivo* or on the nerve segments *in vitro* [1-6,14,15]. Sometimes, researchers preferred recording from the different muscles (gastrocnemius, tibialis anterior or interosseous muscles) and by different type of electrodes (surface or needle electrodes). Various methods and the lack of the single recognized technique of nerve conduction study often

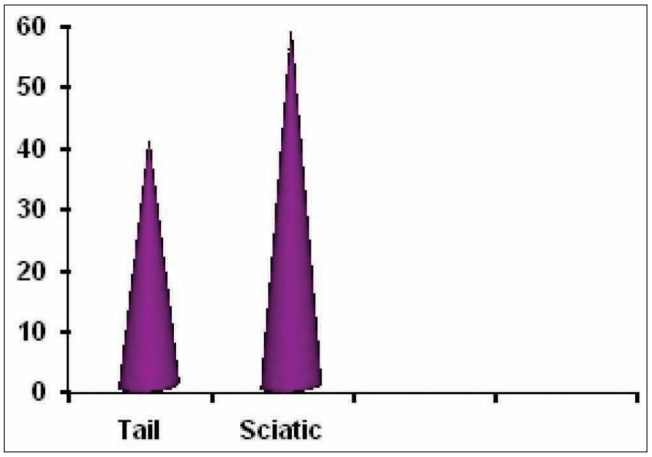

Fig 5. Sciatic and tail MNCV (m/s)
Şekil 5. Siyatik ve kuyruk motor sinirlerinin iletim hızları (m/sn)

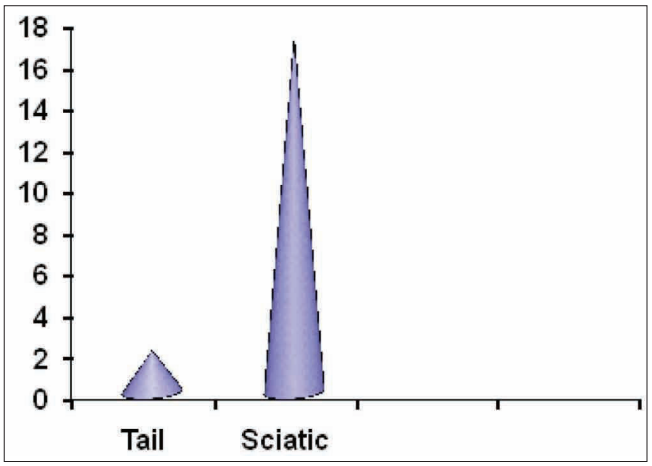

Fig 6. Sciatic and tail CMAP amplitudes (mV)
Şekil 6. Siyatik ve kuyruk bileşik kas aksiyon potansiyeli amplitüdleri (mV)

lead to difficulties in the interpretation and comparison of the results of similar studies.

In the present study we have described the motor nerve conduction study methods of the sciatic and tail nerves and attempted to verify the results of our study, comparing with the results of previous researches. Although the purpose of this study is not to compare the velocities and CMAP amplitudes between the sciatic and the tail nerves, we found that the sciatic MNCV investigated by needle electrodes from gastrocnemius muscle were faster than the tail MNCV. Also, the sciatic CMAP amplitudes were higher than the tail CMAP amplitudes. Those were the expected results and explained by the "size principle" of Henneman-larger muscles (gastrocnemius muscle) have bigger motor unites with thicker nerve fibers and thus, have faster conduction velocities than small muscles (tail muscles) [16].

Some electrophysiological studies on rats were performed using surface recording and stimulating electrodes [4,8,17] As known, surface electrodes non-invasive, painless, easy and quick to use [18,19]. Surface electrodes record the activity of a large area and as a result, give the information about electrophysiological activity from the large muscle groups. On the other hand, surface electrodes lack specificity because they sample a large volume of muscle and thus, are not be able to distinguish signals from the target muscle or neighboring muscles. Also, surface electrodes detect signals from the superficial muscles and not from the deep muscles.

Nevertheless, using of needle electrodes is an invasive procedure and can lead to pain, neural and muscular injury, we preferred to use the bipolar needle electrodes, because they can record and distinguish signal from selected muscle, especially from the thin muscles in the small laboratory animals, such as the gastrocnemius muscle in the present study [9]. According to our knowledge, this is the first study where were used bipolar needle electrodes for stimulation non-exposed nerves and recording from the gastrocnemius muscle.

The sciatic and tail nerves conduction studies by needle electrodes were performed in some studies on rats [3-6]. Relatively long distance between two stimulation pointes, thickness of these nerves, easy to stimulation, simple anatomy of these nerves are advantages to use in nerve conduction studies [20]. Study technique, sciatic and tail MNCV and CMAP amplitude values in previous studies were similar to our results. Oğuzhanoğlu et al.[4] found that sciatic nerve motor conduction velocities were 55.12±9.63 m/s and CMAP amplitudes of gastrocnemius muscle were 31.87±9.38 mV. Also, Kasselman et al.[6] reported the motor nerve conduction velocities in the tail and sciatic nerves were similar to our study results. Another author reported that the sciatic- tibial MNCV was 46.3±12.4 m/s and CMAP amplitudes were 10.2±3.9 mV; and the tail MNCV was 21.9±3.2 and CMAP amplitudes were 8.3±3.3 mV in rats at the 9th week of age [17]. Additionally, some studies, which were performed on isolated sciatic nerve segments and on the exposed nerve, support our results. Dalkılıc et al.[15] performed sciatic nerve conduction study *in vitro* on isolated nerve segment. Conduction velocities in their study were 55.7±2.6 m/s similar to our values. The study on exposed sciatic nerve was performed by Wang et al. and the results were close to ours [14]. Another study, performed on exposed sciatic nerve, reported that the sciatic nerve MNCV as 46.9±2.2, 53.0±2.1 and 48.9±3.8 m/s depending on dietary supplementation [21]. Similar results *in vitro* and *in vivo* studies prove the objectivity and reliability of our nerve conduction technique by bipolar needle electrodes on the non-exposed nerves.

However, Rupp et al.[22] considered that recording CMAPs from the gastrocnemius muscle in rats should be treated with caution, especially, if monopolar needle electrodes are used for recording. On the other hand, recordings from the gastrocnemius muscle have been applied in various studies. These studies do not contain

any inconsistencies about recording from gastrocnemius muscle [23-25].

In addition to the above, the minimally invasive measurements may be valuable tool to displaying peripheral nerve changes and it was confirmed by histomorphometric evaluation in the previous study [1].

In conclusion, the results of present study and other similar studies show that minimally invasive sciatic and tail nerves conduction studies with bipolar needle electrodes may be the suitable methods in rat models of peripheral nervous system diseases. These methods of nerve conduction may be preferable in cases where researchers need further observation of surviving animals after examination. On the other hand, from an ethical point of view, these methods are minimally invasive and not the cause of death or disability of animals, such as in the present study.

REFERENCES

1. Hüseyinoğlu N, Özaydın İ, Yayla S, Yıldırım CH, Aksoy Ö, Kaya M, Şengöz A, Taşdemiroğlu E: Electrophysiological assessment of the effects of silicone tubes and hyaluronic acid on nerve regeneration in rats with sciatic neurorrhaphy. *Kafkas Univ Vet Fak Derg*, 18 (6): 917-922, 2012.

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

3. Korte N, Schenk HC, Grothe C, Tipold A, Haastern- Talini K: Evaluation of periodic electrodiagnostic measurements to monitor motor recovery after different peripheral nerve lesions in the rat. *Muscle Nerve,* 44 (1): 63-73, 2011.

4. Oğuzhanoğlu A, Erdoğan C, Tabak E, Cenikli U: Comparison of conduction velocities of nerve fibers to smaller and larger muscles in rats. *Int J Neurosci,* 120 (1): 76-79, 2010.

5. Misumi J, Nagano M: Experimental study on the enhancement of the neurotoxicity of methyl n-butyl ketone by non-neurotoxic aliphatic monoketones. *Br J Ind Med,* 42 (3): 155-161, 1985.

6. Kasselman LJ, Veves A, Gibbons CH, Rutkove SB: Cold exposure exacerbates the development of diabetic polyneuropathy in the rat. *Exp Diabetes Res*, 2009:827943, E-pub 2010 Jan 14, DOI: 10.1155/2009/827943.

7. Schmalbruch H: Fiber composition of the rat sciatic nevre. *Anat Rec,* 215 (1): 71-81, 1986.

8. Canta A, Meregalli C, Chiorazzi A, Carozzi VA, Crippa L, Marmiroli P, Cavaletti G: The ventral caudal nerve: a physiologic-morphometric study in three different rat strains. *J Peripher Nerv Syst* 15 (2): 140-146, 2010.

9. Ulman YI, Ulus IH, Ozpinar A, Genc V: Preliminary notes for ethical conduct of animal experimentation with special reference to studies in Turkey. *Kafkas Univ Vet Fak Derg,* 17 (6): 1051-1056, 2011.

10. Russell W, Burch R: The principles of humane experimental technique, Great Britain 1959; cited by Flecknell P: Replacement, reduction and refinement. *Altex*, 19 (2): 73-78, 2002.

11. Ülker K, Hüseyinoglu Ü: Animal ethics and animal use in laparoscopic surgery. *KJMS,* 2 (3): 89-93, 2012.

12. Siconolfi LB, Seeds NW: Mice lacking tPA, uPA, or plasminogen genes showed delayed functional recovery after sciatic nerve crush. *J Neurosci*, 21 (12): 4348-4355, 2001.

13. Oh SJ: Nerve conduction studies. In, Oh SJ (Ed): Clinical Electromyography. 3rd ed., Lippicott, Williams and Wilkins, Philadelphia, 2003.

14. Wang KK, Nemeth IR, Seckel BR, Chakalis- Halley DP, Swann DA, Kuo JW, Bryan DJ, Cetrulo CL: Hyaluronic acid enhances peripheral nerve regeneration *in vivo. Microsurg,*18 (4): 270-275, 1998.

15. Dalkilic N, Tuncer S, Bariskaner H, Kiziltan E: Effect of tramadol on the rat sciatic nevre conduction: A numerical analysis and conduction velocity distribution study. *Yakugaku Zasshi*, 129 (4): 485-493, 2009.

16. Henneman E, Olson CB: Relations between structure and function in the design of skeletal muscles. *J Neurophysiol,* 28, 581-598, 1965.

17. Kurokawa K, de Almedia DF, Zhang Y, Hebert CD, Page JG, Schweikart KM, Oh SJ: Sensory nerve conduction of the plantar nerve compared with other nerve conductions tests in rats. *Clin Neurophysiol*, 115 (7): 1677-1682, 2004.

18. Giroux B, Lamontagne M: Comparisons between surface electrodes and intramuscular wire electrodes in isometric and dynamic conditions. *Electromyogr Clin Neurophysiol* 30 (7): 397-405, 1990.

19. Waite DL, Brookham RL, Dickerson CR: On the suitability of using surface electrode placements to estimate muscle activity of the rotator cuff as recorded by intramuscular electrodes. *J Electromyogr Kinesiol* 20 (5): 903-911, 2010.

20. Leandri M, Saturno M, Cilli M, Bisaglia M, Lunardi G: Compound action potential of sensory tail nerves in the rat. *Exp Neurol,* 203 (1): 148-157, 2007.

21. Head RJ, McLennan PL, Raederstorff D, Muggli R, Burnard SL, McMurchie EJ: Prevention of nerve conduction deficit in diabetic rats by polyunsaturated fatty acids. *Am J Clin Nutr*, 71 (1 Suppl): 386S-392S, 2000.

22. Rupp A, Dornseifer U, Fischer A, Schmahl W, Rodenacker K, Jütting U, Gais P, Biemer E, Papadopulos N, Matiasek K: Electrophysiologic assessment of sciatic nerve regeneration in the rat: Surrounding limb muscles feature strongly in recordings from the gastrocnemius muscle. *J Neurosci Methods*, 166 (2): 266-277, 2007.

23. Negredo P, Castro J, Lago N, Navarro X, Avedano C: Differential growth of axons from sensory and motor neurons through a regenerative electrode: A stereological, retrograde traces, and functional study in the rat. *Neuroscience,*128 (3): 605-615, 2004.

24. Udina E, Rodriguez FJ, Verdu E, Espejo M, Gold BG, Navarro X: FK506 enhances regeneration of axons across long peripheral nerve gaps repaired with collagen guides seeded with allogeneic Schwann cells. *Glia*, 47 (2): 120-129, 2004.

25. Meek MF, van der Werff JFA, Klok, Robinson PH, Nicolai JPA, Gramsbergen A: Functional nerve recovery after bridging a 15 mm gap in rat sciatic nerve with a biodegradable nerve guide. *Scand J Plast Reconstr Hand Surg,* 37 (5): 258-265, 2003.