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RESEARCH ARTICLE

Morphological Alterations of Sciatic Nerve during Development from Rat Fetuses to Adults: A Histological, Stereological and Embryological Study

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Summary

Myelinated axon numbers of the sciatic nerves which were obtained from 16 and 20 days foetuses, newborn and 5 weeks ages rats were estimated in this study.. In all groups gender factor was not considered. Following induction of anaesthesia, the sciatic nerves were removed and myelinated axon numbers were estimated. Sciatic nerve samples were also histologically analyzed. Overall, there was a 249% increase in the total number of myelinated axons in the sciatic nerves obtained from 20 days rat foetuses as compare to 16 days foetuses. Axon number in sciatic nerves got from newborn rats was decreased 56% as compare to twenty days foetuses. Histological results in this study were parallel to our stereological results. There were more myelinated axons in 20 days foetuses than in 16 days foetuses. It was also detected lesser myelinated nerve in sections obtained from newborn sciatic nerve than to 20 days foetuses. Moreover; the most myelinated axon number was observed in sciatic nerve got from 5 weeks ages adult rats. In view of these results, this study indicated that myelinated axons were decreased in the last a few days of pregnancy as compare to newborn group, whereas myelinated axon numbers were increased during postnatal ages till 5 weeks adult ages.

Keywords: *Sciatic nerve, Embryology, Stereology, Rat, Light microscopy*

Siyatik Sinir Gelişimi Esnasında Görülen Morfolojik Değişimlerin Sıçan Model Üzerinde Araştırılması: Histolojik, Stereolojik ve Embriyolojik Bir Çalışma

Özet

Bu çalışmada, 16 ve 20 günlük sıçan fetüsleri, yeni doğan ve 5 haftalık erişkin sıçanlardan elde edilen siyatik sinirlerin myelinli akson sayıları hesaplandı. Çalışma için 16 ve 20 günlük fetüslerin yanı sıra yeni doğan ve 5 haftalık erişkin sıçanlar kullanıldı. Çalışma yapılırken cinsiyet farkı dikkate alınmadı. Anestezi altındaki deneklerden siyatik sinir örnekleri çıkarıldı ve myelinli akson sayıları da hesaplandı. Aynı zamanda siyatik sinir örnekleri histolojik olarak ta incelendi. Yirmi günlük sıçan fetüslerinden elde edilen siyatik sinirlerindeki akson sayısı 16 günlük fetüslerdekine kıyasla %249 artmıştı. Yeni doğanlardan elde edilen siyatik sinirlerindeki akson sayısı yirmi günlük sıçan fetüslerindekine kıyasla %56 azalmıştı. Bu çalışmadan elde edilen histolojik sonuçlar stereolojik sonuçlar ile paralellik göstermekteydi. Yirmi günlük fetüslerde 16 günlük fetüslere kıyasla daha fazla myelinli akson vardı. Yeni doğan siyatik sinirlerinden elde edilen kesitlerde ise 20 günlük fetüslerinkine kıyasla daha az myelinli akson görüldü. Ek olarak en fazla myelinli akson sayısı 5 haftalık erişkinlerden elde edilen siyatik sinirlerde görüldü. Bu çalışmadan elde edilen sonuçlar ışığında, gebeliğin son günlerinde yeni doğan grubuna kıyasla myelinli aksonların azaldığı; fakat postnatal dönemde erişkine ulaşana değin myelinli akson sayısının arttığı kanaatine varılmıştır.

Anahtar sözcükler: *Siyatik sinir, Embriyoloji, Stereoloji, Sıçan, Işık mikroskopi*

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INTRODUCTION

In the gross anatomical level, the nervous system is organized into the central nervous system (CNS), which comprises the brain and spinal cord, and the peripheral nervous system (PNS), which includes cranial, and spinal nerves, and their associated ganglia. In the microscopical level, the cells of the nervous system may be subdivided into two categories: i) neurons which are originated from neuroepithelium are responsible for the receptive, integrative, and motor functions of the nervous system, ii) neuroglial cells which are originated from three different embryological source, which are called mesoderm, neuroepithelium and neural crest are responsible for supporting and protecting neurons. Neural crest cells give rise to a variety of cell types, including Schwann cells of the peripheral nervous system [1]. Neural crest cells migrate away from the dorsal aspect of the neural tube along particular pathways to different locations in the embryo [1-3]. The survival of developing mammalian Schwann cells is regulated by a variety of factors [4] such as developmental age, autocrine factors including insulin-like growth factor-1, neurotrophin-3, and platelet-derived growth factor-BB [5].

During the development of the nervous system, the balance between mutagens, survival factors, and death signals is the most important factor of cell numbers in interesting area [6]. In the other word, Schwann cell numbers in developing nerves are associated with two main factor called proliferation and apoptosis.

In this study, it has been essentially interested in how the number of myelinated axons produced by Schwann cells in the rat sciatic nerve in both prenatal and postnatal stage of development because of exhibiting mitotic division in prenatal and postnatal stage were examined using histopathological methods under light and electron microscopy and also modern stereological methods.

Stereology is name of a new method that is used for estimating the properties of geometrical objects in space. To apply any stereological approach; it is necessary to carry out two step-procedures separated each other. One of these is sampling procedure, called systematic, uniformly random sampling in stereology literature which gives each field of view the same probability of being sampled [7]. Other steps are estimating procedure based on well defined mathematic and statistic background and the value of stereology for describing the three-dimensional (3D) structural composition and spatial arrangement of biological specimens from essentially two-dimensional (2D) thin sections [8].

Many studies were reported about sciatic nerve till to day. Some of these are anatomical, pathological, physiological etc. [9,10]. But a few of these studies were stereological [11] and also none of these was examined nerve development together with quantitative features of this nerve at embryological process and growing.

The aim of the present study was to investigate morphometrical and histological alterations in sciatic nerve myelination of rat fetuses, newborns, pups and adults.

MATERIAL and METHODS

This study was carried out in the animal laboratory of Ataturk University. We used 30 adult Sprague-Dawley rats regardless of gender. Animals were divided into five groups: 16 days old fetus group (Group A, n=6), 20 days old fetus group (Group B, n=6), newborn group (Group C, n=6), 1 week old litter group (Group D, n=6) and five weeks old adult group (Group E, n=6). In this study, for group A and B, fetuses were removed from pregnant females. The first day of pregnancy was determined according to whether vaginal plug is present or not. In C group, it was permitted to rats for ending of their pregnancy and thus newborn rats were obtained. Some of them were killed at the first day of their life (Group C, n= 6). Both 1 week old litter and 5 weeks old adult groups were fed with mother milk during one week. At the end of one week period, the animals of the group D (One week old litter) were sacrificed and rest of them (Group E) was fed with distilled water and commercial rat diet ad libitum for four week after mother milk. At the end of this period, adult rats in group E were killed and sciatic nerve samples were dissected. This experiment was carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

At the end of study, all animals were anesthetized via short inhalation of ether, the animals were washed blod out intracardially initially by 0.9% saline (30 ml) solution and fixed in a mixture of 2% para-formaldehyde + 2% glutaraldehyde (150 ml) in 0.1 M phosphate buffer, pH 7.4 at room temperature. The

sciatic nerves were removed and incubated in the same fixative overnight at 4ºC.

On the following day, samples were post-fixed in 1% osmium tetroxide for 1 h, dehydrated through a graded alcohol series, and embedded in Epon resin. Semi-thin sections were cut at a thickness of 1 µm. From each block, semi-thin sections were collected on slides for histopathological examination and stereological analysis. The semi-thin sections were stained with toluidine blue and viewed using a light microscope. Light micrographs of these specimens were taken at different magnifications to examine the morphological changes of the sciatic nerves.

For detection of DNA breaks, in situ cell death detection kits for the tunel method were purchased from Roche Applied Science (Penzberg, Germany). The sections were deparaffinized and treated with proteinase K solution (20 µg/ml in PBS) for 15 min at room temperature. Subsequently, the sections were washed in distilled water and immersed in 3% hydrogen peroxide for 15 min. After several washings in PBS (50 mM sodium phosphate and 200 mM NaCl at pH 7.4), the sections were immersed in equilibration buffer at room temperature for 20 min. The sections were then incubated in TdT enzyme (terminal deoxynucleotidyl transferase) at 37ºC for 1 h in a humidified chamber and the reaction was stopped by immersion in a stop/wash buffer. After several washings, the sections were incubated in antidigoxigenin-peroxidase for 30 min at room temperature. The reaction was revealed with 0.06% 3.3 -diaminobenzidine tetrahydrochloride (Sigma Chemical, St. Louis, MO) in PBS for 3-6 min and the sections were counterstained with Mayer's hematoxylin. These sections were examined and photographed in a light microscope Olympus BH-40.

Consecutive sections (section thickness: 1-µm and stained with toluidine blue) were obtained from each tissue block (about 10 sections for each sample). In these sections containing the sciatic nerves, the cross sectional area was estimated by Stereoinvestigator 6.0 (Microbrightfieeld; USA). This system reproduced microscopic images on the monitor of computer at a final magnification of 3220.

The cross sectional area of the sciatic nerves was evaluated by using a point grid, in which per point has 25 mm^2 representing area *(see to Fig 1 a1, b1, a2, a3 and b3 for detail)* [12]. In stereological study, five percent was accepted as the highest limit of significant coefficient error [12] and grid with 25 mm^2 representing area of per point was adequate in our study [13,14].

All points hitting the object of interest are counted according to a fixed rule. The area (a mm^2) of per test point (k mm) is equal k x k mm^2. Subsequently, the surface area of the nerve fibers in each section are estimated from following formula.

Cross Section Area (A) = k x k (mm x mm) x (ΣP) = mm^2

Where A is the area of the object of interest in one section plane, k x k interpoint area, and ΣP the number of point hitting the nerve section in that section. After the same formula is applied for entry other sections, mean area that is want to be estimated is obtained from

Total Area = A_1+ A_2+…+A_n

Finally, mean area of the sciatic nerve was obtained by dividing to section number in each block of estimated total area value.

In this study, it was used the fractionator technique to estimate the total number of myelinated axons *(see to Fig 1 a2, a3 and b3 for detail)* [12]. To obtain the

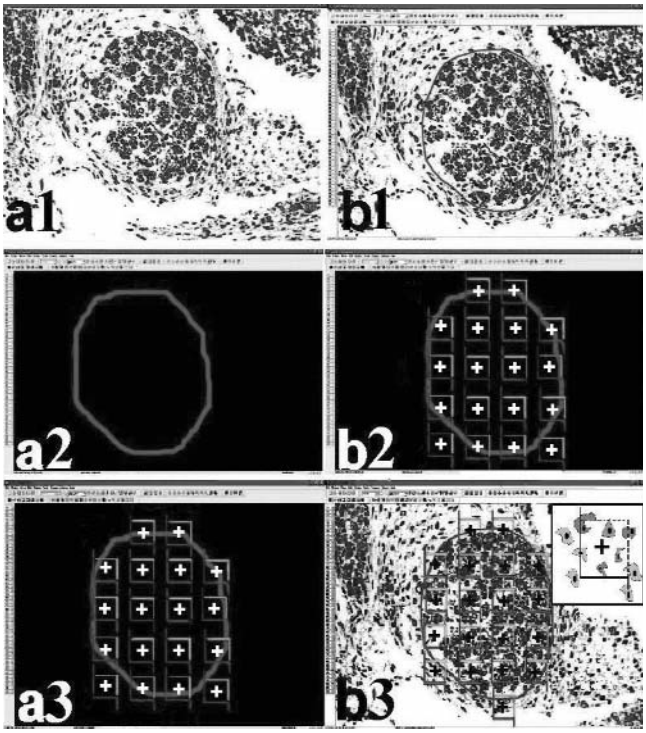

Fig 1. Sampling process and counting of myelinating axon in the light micrographs at sterioinvestigator software. Firstly, boundaries of sciatic nerve were drawn (a1, b1 and a2). Secondly, appropriate unbiased counting frames were prepared (b2, a3 and b3) and superimposed on the light micrographs (b3)

Şekil 1. Işık mikroskobik kesitler üzerinde Sterioinvestigator programı ile yapılan örnekleme işlemi ve myelinli akson sayım prosedürü görülmektedir. İlk olarak siyatik sinirlerin sınırları çizildi (a1, b1 and a2). İkinci olarak uygun tarafsız sayım çerçevesi hazırlandı (b2, a3 and b3). Son aşamada tarafsız sayım çerçevesi ışık mikrograflar üzerine yerleştirilerek sayımlar yapıldı (b3)

myelinated axon number of cross sections was used total 1600 time magnifications together with objective, ocular and camera (Stereoinvestigator 6.0). Myelinated axon number of the sciatic nerves was evaluated by using an unbiased counting frame *(see to Fig 1 a2, a3 and b3 for detail)* [15-17]. For this process, unbiased counting frame with known size (43200 mm^2) was automatically mounted on images by Stereo-investigator software and axon number was estimated according to basic sample procedure of stereological principle in the manner of systematic and random [18].

Finally; total number of myelinated axon profiles was found by multiplying section profiles in area with numerical density of myelinated axons.

To evaluate the difference between the groups, we used One Samples T Test for myelinated axon number and cross-section area of sciatic nerve. All P values were considered to be significant when smaller than 0.05.

RESULTS

In this study, sciatic nerve development was examined with histochemical and stereological methods in rat fetuses, newborns and adults. For this purpose, at the 16th day and 20th days of gestation, fetuses were removed from pregnant rats. Dissected sciatic nerves from fetuses, newborns and 5 weeks old postnatal rats were examined stereologically, microscopically and immunohistochemically at light and electron microscopical levels. Then, all results were analyzed statistically.

All stereological results were summarized in *Table 1*.

When these results were statistically analyzed, results of 16 and 20 days fetuses were significantly different from each other (P<0.01). There were significant differences both between 20 days fetuses and newborn (P<0.01) and between newborn and 5 weeks rats (P<0.01). In embryonic 16 days, it was observed initial organization model of a typical peripheral nerves consisted of epineurium, perineurium and endoneurium which were surrounded by several investments of connective tissue sheaths from the outermost to the innermost layer respectively.

Table 1. *Mean myelinated axon number of sciatic nerves in all groups ± SEM (standard error mean)*

Tablo 1. *Tüm gruplar için hesaplanan siyatiksinirlerdeki ortalama miyelinli akson sayıları aşağıdaki tabloda özetlenmiştir ± SEM (ortalama standart hata)*

Groups	Mean Myelinated Axon Number
Group A	1936.6±113.5
Group B	6765.0±587.4
Group C	6765.0±587.4
Group D	5330.7±468.9
Group E	10483.3±854.8

There were a lot of Schwann cell nuclei, and a few myelinated or unmyelinated axons at the light microscopic level *(Fig 2)*.

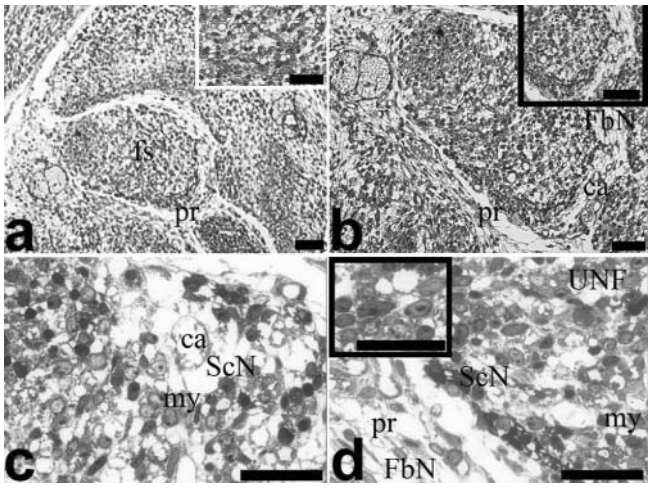

Fig 2. Light micrographs of the sciatic nerve in embryonic 16 days showing a few myelinated axons, and unmyelinated axons (UNF, unmyelinated fiber; SCN, Schwann cell nuclei; FbN, Fibroblast nuclei; fs, Fasicle; pr, perineurium; ca, capillary; my, myelin. Magnification bars: 50 µm

Şekil 2. Onaltı günlük sıçan fetüslerinden elde edilen ve az sayıda myelinli akson içeren ışık mikroskobik kesitler görülmektedir. (UNF, myelinsiz akson lifi; SCN, Schwann hücresi çekirdeği; FbN, Fibroblast çekirdeği; fs, sinir fasikülü; pr, perinöryum; ca, kapiller; my, myelin. Barlar: 50 µm

At electron microscopic level, the most important finding was present of Schwann cell precursors with thin cytoplasm at all field of sections. It was determined to possess cellular interactions of Schwann cell precursors with a lot of processes *(Fig 3)*.

In embryonic 20 days of rat sciatic nerve, it was prominent more regular connective tissue and well developed tissue sheaths consisted of a lot of fibro-blast nuclei. It was detected increasing of myelinated axon number on the light micrographs *(Fig 4)* and not only a lot of apoptotic but also mitotic Schwann cell nuclei at light and electron micrographs *(Fig 4-6)*. In TUNEL method performed sections, many apoptotic Schwann cell nuclei were detected in day 20 fetuses *(Fig 6)*.

In newborn, it was observed well developed connective tissue sheaths with typical peripheral

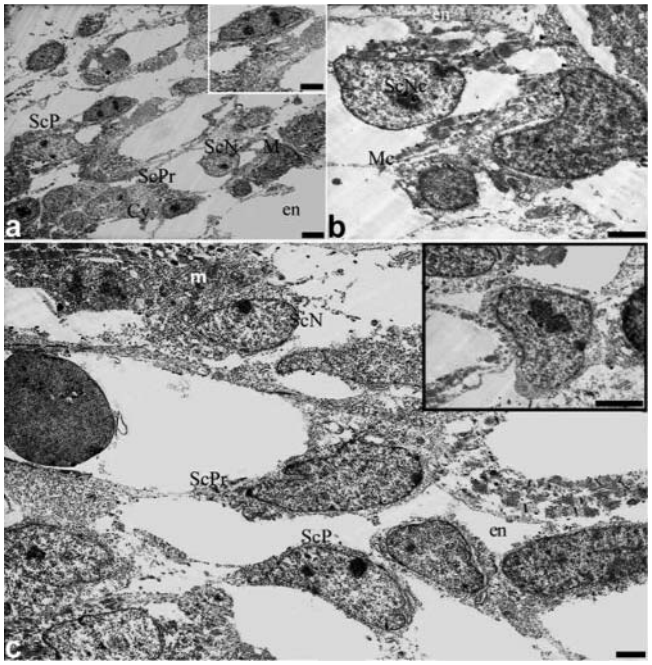

Fig 3. Electron micrographs of the sciatic nerve in embryonic 16 days showing precursors Schwann cell nucleus (ScN, Schwann cell nuclei; ScP, Schwann cell precursors; ScPr, Schwann cell processes; M, macrophage; Mc, microtubule; Cy, cytoplasm; en, endoneurium) Magnification bars: 3 µm

Şekil 3. Onaltı günlük sıçan fetüslerinden elde edilen ve Schwann hücresi prokürsörlerini içeren elektron mikroskobik kesitler görülmektedir. (SCN, Schwann hücresi çekirdeği; ScP, Schwann hücresi prekürsörleri; ScPr, Schwann hücresi uzantıları; M, makrofaj; Mc, mikrotübül; Cy, sitoplazma; en, endonöryum) Barlar: 3 µm

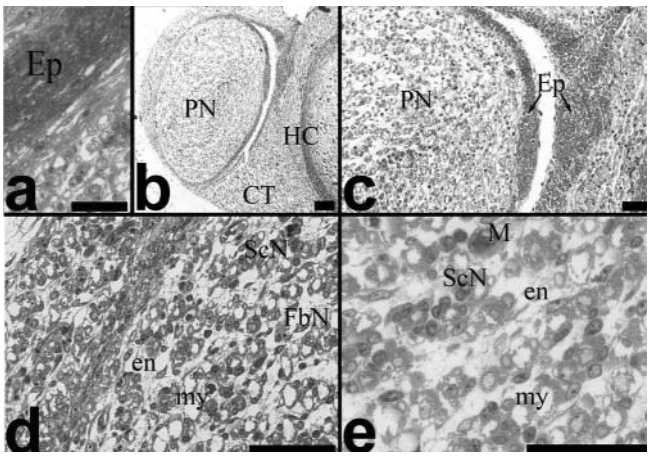

Fig 4. Light micrographs of the sciatic nerve in embryonic 20 days showing a lot of myelinated axons (SCN, Schwann cell nuclei; M, Macrophage; en, Endonorium; Ep, epineurium; my, myelin; FbN, Fibroblast nuclei) Magnification bars: 200 µm

Şekil 4. Yirmi günlük sıçan fetüslerinden elde edilen ve çok sayıda myelinli akson içeren ışık mikroskobik kesitler görülmektedir. (SCN, Schwann hücresi çekirdeği; FbN, fibroblast çekirdeği; M, makrofaj; en, endonöryum; Ep, epinöryum; my, myelin). Barlar: 200 µm

nerves appearances except endonerium and also nerve fascicles was clearly examined *(Fig 7)*.

Space between cells was occupied by non organized premyelinating structures *(Fig 7, 8)*. It was

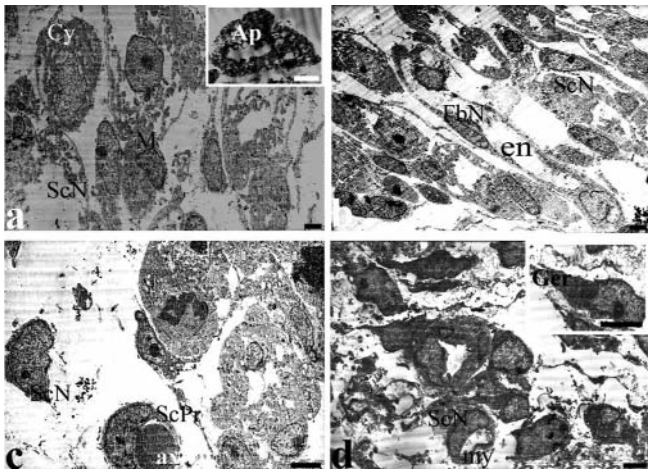

Fig 5. Electron micrographs of the sciatic nerve in embryonic 20 days showing increasing myelinated axon fibers, apoptotic and mitotic Schwann cell nuclei (ScN: Schwann cell nuclei, ScPr: Schwann cell processes, M: macrophage, Ap: apoptotic cell, ax axon; en: endonorium, FbN: fibroblast nuclei, my: myelin) Magnification bars: 3 µm

Şekil 5. 20 günlük sıçan fetüslerinden elde edilen ve artan myelinli akson fibrillerinin yanısıra apoptotik ve mitotik Schwann hücrelerini içeren elektron mikroskobik kesitler görülmektedir. (SCN, Schwann hücresi çekirdeği; ScPr, Schwann hücresi uzantıları; M, makrofaj; Ap: apoptotik hücre, ax akson; en, endonöryum) Barlar: 3 µm

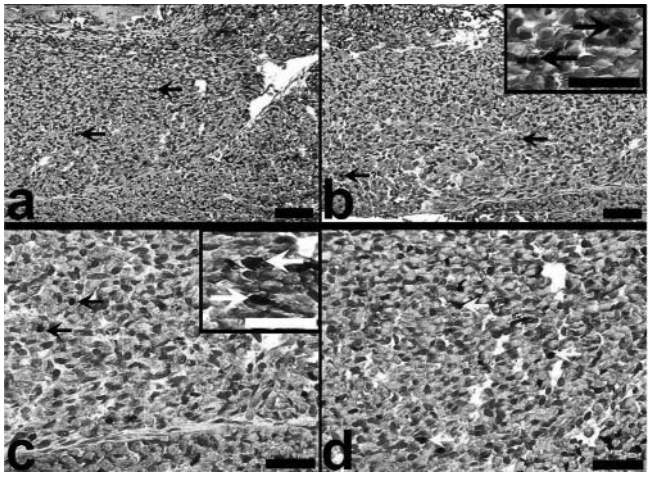

Fig 6. Light micrograph of the sciatic nerve in embryonic 20 days showing a lot of TUNEL-positive, apoptotic cell nuclei (Arrows). Magnification bars: 40 µm

Şekil 6. Yirmi günlük sıçan fetüslerinden elde edilen ve çok sayıda TUNEL-pozitif apoptotik hücre çekirdeği (Oklar) içeren ışık mikroskobik kesitler görülmektedir. Barlar: 40 µm

shown fusing with each other of a lot of sheaths called myelin at premyelinating stage at light microscopy *(Fig 7)*. On the most of sections profiles, it was examined both more dominate mitotic and a few apoptotic cell nuclei at light *(Fig 7)* and electron microscopical level *(Fig 8)*.

There were also many unmyelinated nerve fibers in electron micrographs of newborn rats *(Fig 8)*. However, there were a few apoptotic Schwann cell

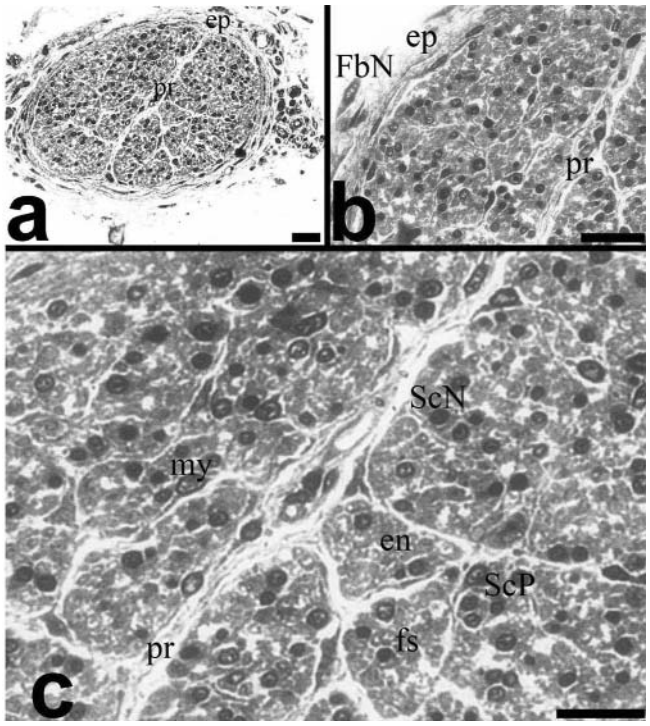

Fig 7. Light micrographs of the sciatic nerve in newborn rat showing a lot of myelinated axons (SCN, Schwann cell nuclei; ScP, Schwann cell precursors; en, endoneurium; pr, perineurium; Ep, epineurium; my, myelin; fs, nerve fascicle; FbN, fibroblast nuclei). Magnification bars: 50 µm

Şekil 7. Yenidoğan sıçan yavrularından elde edilen ve çok sayıda myelinli akson içeren ışık mikroskobik kesitler görülmektedir. (SCN, Schwann hücresi çekirdeği; ScP, Schwann hücresi prokürsörleri; en, endonöryum; pr, perinöryum; Ep, epinöryum; my, myelin; fs, sinir fasikülü; FbN, Fibroblast nükleusu). Barlar: 50 µm

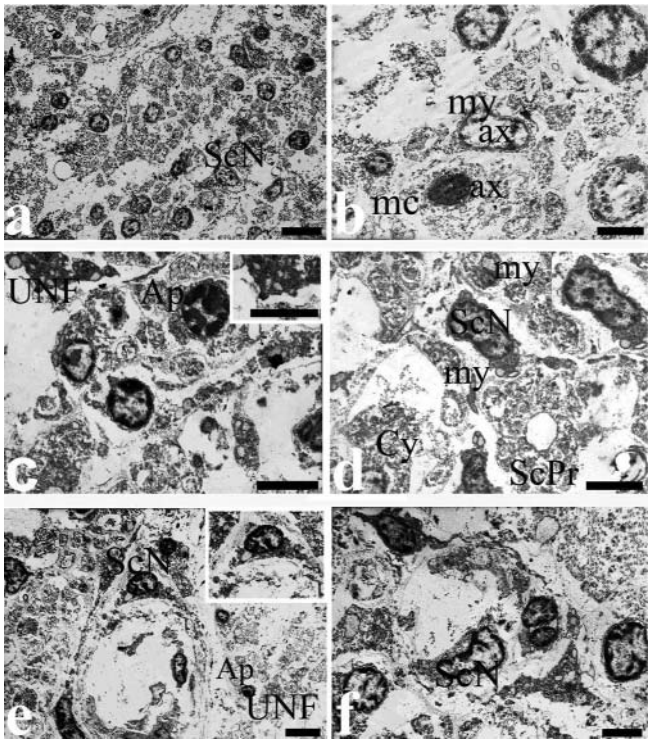

Fig 8. Electron micrographs of the sciatic nerve in newborn rat showing decreasing myelinated axon fibers, and apoptotic Schwann cell nuclei (ScN, Schwann cell nuclei (and in inset of e); ScPr, Schwann cell processes; mc, microtubule; Ap, Apoptotic cell; ax, axon; my, myelin; UNF; unmyelinated fibers (and in inset of c), Cy, cytoplasm of Schwann cell). Magnification bars: 8 µm

Şekil 8. Yenidoğan sıçan yavrularından elde edilen ve azalan myelinli akson fibrillerinin yanısıra apoptotik Schwann hücrelerini de içeren elektron mikroskobik kesitler görülmektedir. (SCN, Schwann hücresi çekirdeği (ve e'deki küçük resimde); ScPr, Schwann hücresi uzantıları; mc, mikrotübül; Ap: apoptotik hücre, ax akson; my, myelin; UNF, myelinsiz akson (ve c'deki küçük resimde); Cy, Schwann hücresinin sitoplazması) Barlar: 8 µm

nuclei in TUNEL method applied sciatic nerve samples of newborn rats *(Fig 9)*.

When light microscopical sections of 5 week's adult rats were evaluating, bigger sections (approximately 3-4 times) with two lobes were found. Epineurium, nerve fascicles, perineurium which is among these fascicles, regularly myelinated axons and vessels in endoneurium were seen *(Fig 10)*.

In electron microscopical sections of this group, axons were well-developed and myelinated. Boundaries of myelinated sheaths of axons were regular and there were axon bodies in these sheaths. Both myelinated and rare unmyelinated axons were surrounded by mature Schwann cells *(Fig 11)*.

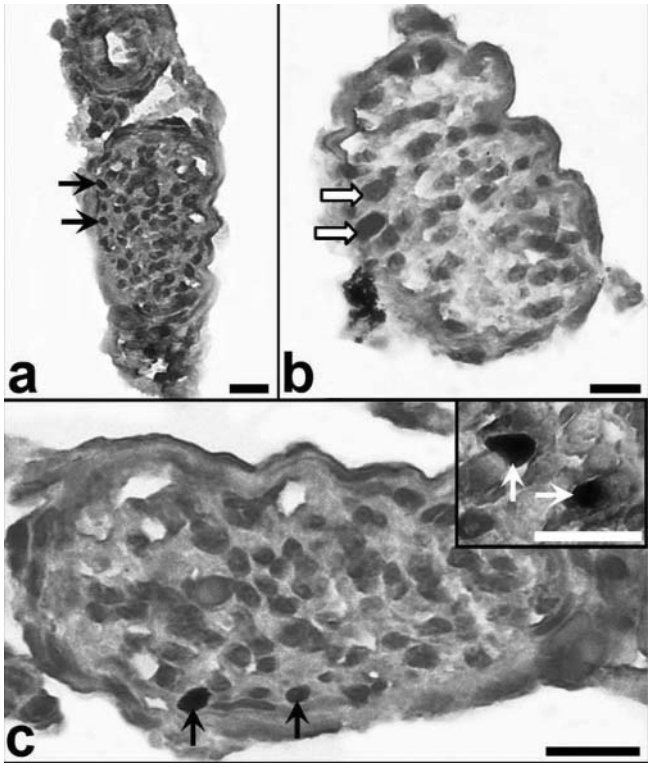

Fig 9. Light micrographs of the sciatic nerve in postnatal first day showing a fewTUNEL-positive, apoptotic cell nuclei (Arrows). Magnification bars: 45 µm

Şekil 9. Yenidoğan sıçan yavrularından elde edilen ve çok sayıda TUNEL-pozitif apoptotik hücre çekirdeği (Oklar) içeren ışık mikroskobik kesitler görülmektedir. Barlar: 45 µm

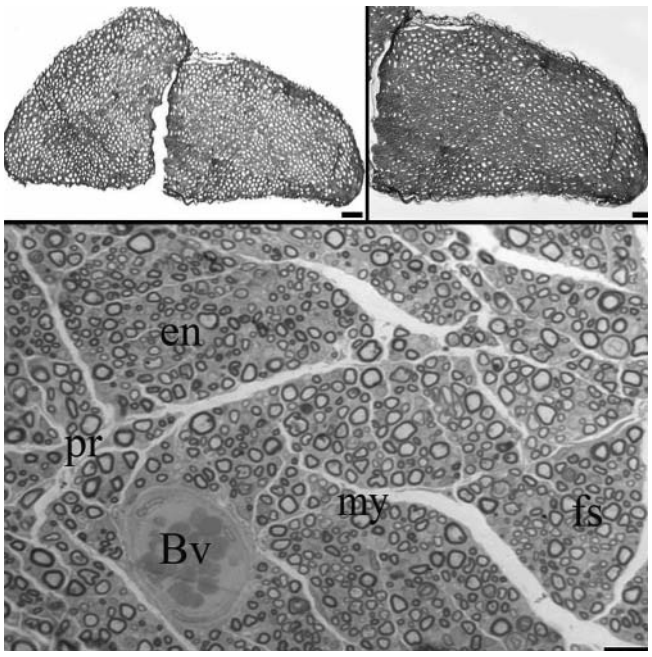

Fig 10. Light micrographs of the sciatic nerve in 5 weeks old rat showing a lot of myelinated axons. (en, Endoneurium; my, myelin; fs, nerve fascicle; pr, Perineurium; Bv, blood vessel). Magnification bars: 80 µm

Şekil 10. İçerdiği aksonların neredeyse tamamı myelinli olan ve 5 haftalık sıçanlardan elde edilen ışık mikroskobik kesitler görülmektedir. (en, Endonöryum; my, myelin; fs, sinir fasikülü; pr, Perinöryum; Bv, kan damarı). Barlar: 80 µm

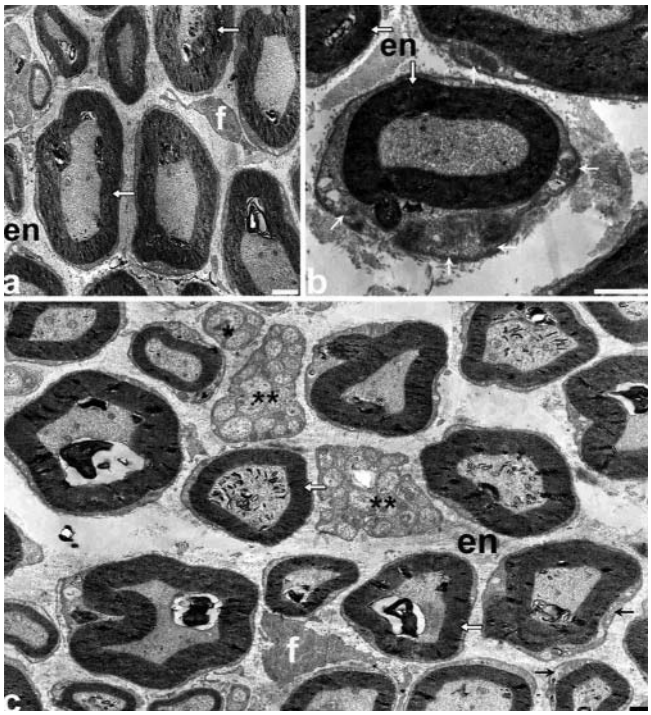

Fig 11. Electron micrographs of the sciatic nerve in 5 weeks old rat showing increasing myelinated axon fibers (en, Endoneurium; f, fibroblast; asterisk, unmyelinated nerve fibers; white arrows, Schwann cell; black arrows, Schwann cell cytoplasm; transparent arrows, myelin sheets). Magnification bars: 2 µm

Şekil 11. 5 haftalık sıçanlardan elde edilen ve artan myelinli akson fibrillerini içeren elektron mikroskobik kesitler görülmektedir. (en, Endonöryum; f, fibroblast; yıldız, myelinsiz sinir lifleri; beyaz oklar, Schwann hücresi; siyah oklar, Schwann hücresi sitoplazması; şeffaf oklar, myelin kılıf). Barlar: 2 µm

DISCUSSION

The differentiation of neurons or glial cells is accompanied by cell loss via programmed cell death which is depends on trophic factors that are provided by their targets in the developing both central nervous system (CNS) and peripheral nervous system (PNS) [19].

Neuronal and glial cell death result from competition for limited amounts of growth factors and may serve various purposes, such as eliminating inappropriate connections or matching the number of neurons to the size of their target [20]. It is also known that die during development Schwann cells which is glial cell of the PNS. Development stages of Schwann cells may be divided by three categories during embryogenesis, precursor from the neural crest, differentiation of these precursors into immature Schwann cells and, post-natally, their divergence into the two Schwann cell types, myelinating and the nonmyelinating Schwann cells [21].

In this study, we have particularly interested in four topics associated with each other following below;

i) to estimate stereologically the number of myelinated axons ii) to examine histopathologically myelin production with structural and ultrastructural techniques of myelin and, iii). To investigate development of Schwann cells in rat sciatic nerves during embryological 16-20 days and 1-7 days of postnatal life and finally in 5th weeks of life (adults) iv) to try to explain possible mechanisms of myelin production and elimination according to data of our study and other current literatures. It was used stereological, conventional light and electron microscopical and immunocytochemical methods.

Precursor that is considered the earliest Schwann cells appear at approximately embryonic day 14 (E14) [19], in which stage may be determined changes in morphology, cell-cell interactions, survival regulation, response to mutagens and molecular exp,. In this study, Schwann cell precursors were identified at light and electron microscopical levels in embryonic 16 days rat sciatic nerve. In these nerve sections, it was clearly observed a typical peripheral nerve appearance with bundles of nerve fibers which surrounded by connective tissue sheaths, epineurium, and perineurium are the outermost and the middle layer of the connective tissue respectively at light microscopical level and also was observed the innermost layer of the connective tissue at particularly electron microscopical level, endoneurium. In this stage, the most obvious features were present a lot of mitogenic Schwann cell precursor nuclei and a few and thin myelinated nerve fibers. It was observed slightly flattened and small nucleus and cytoplasm of a small amount around nuclei of cells which is contained a few sparse mitochondria, abundant ribosome clusters, many light and dense cytoplasmic vesicles and intermediate filaments. The most remarkable finding about this group was cell-cell interactions between cytoplasmic processes determined the most of cells. It was only estimated mean 1.936, 6 myelinated axons in this group.

In embryonic 20 days rat sciatic nerve according to our study findings, except of 16 days findings, we determined a few different and important morphological findings. One of these was increasing myelinated axon fibers and other was observed a lot of apoptotic Schwann cell nuclei at both light and electron microscopical examinations. First finding about number of myelinated axon fibers was tested and confirmed with stereological quantitative analysis. It was estimated mean 6765 myelinated axon fibers. Unal et al. reported that there were 5290 myelinated axons in weanling rats [11]. At this point, our results were compatible with their results. Increasing in myelinated axon fibers was approximately 249%. Second finding about apoptotic Schwann cells was tested and confirmed with TUNEL method. In this stage, both mitotic and apoptotic process were shown to carry out together in rat sciatic nerves. In literature about late embryonic stage in the rats, it was shown that is present of mitotic and apoptotic Schwann cells and also two Schwann cell types, myelinating and the nonmyelinating Schwann cells, the most of which are nonmyelinating Schwann cells in a lot of study [24,25]. In this group, although we determined more myelinated axons than those of embryonic 16 days rat sciatic nerve, the most of myelinated axons have thin myelin sheath called premeditating axons. It has been claimed that premeditating axons would have been eliminated at next step of development [19,26].

In newborn, we have found the apoptotic Schwann cells and myelinated axon fibers in sciatic nerve sections; but not Schwann cell precursors. Number of myelinated axon fibers was found mean 3796.7. It was determined decreasing (approximately 50%) of myelinated axon fibers compared with embryonic 20 days rat sciatic nerve. These data indicate that myelinated axon fibers are eliminated by programmed cell death in not only embryological period but also postnatal life too [19].

At this point, there were currently two hypotheses for composition of sciatic nerve of newborn rats. The first hypothesis which was reported by literature that was a subpopulation of premeditating Schwann cells which disappeared after the initial stages of development in neonatal nerves that was susceptible to apoptosis [19,26] and it was clearly shown in our study. The most important result of balance between Schwann cell proliferations and apoptosis were establishing of correct Schwann cell numbers in the mature nerve [27]. To provide 1:1 relationship between axonal numbers and myelinating Schwann cells finally, it is clearly observed removing supernumerary Schwann cells [27]. In this elimination or proliferation processes mentioned above about regulation of cell numbers, it must be tightly controlled to provide function efficiently of PNS via decreasing or increasing of same trophic factors [28-30].

According to our opinion, another hypothesis which is appeared in this study can be explained following:

i. spaces among to the Schwann cells was occupied by non organized premyelinating structures

in the light microscopical section of newborn rats and it was shown fusing with each other of a lot of sheaths called myelin at premyelinating stage under light microscope.

ii. Also there were many unmyelinated nerve fibers in electron microscopic slides of new born sciatic nerves.

Thus, the cause of less myelinated axon number estimated in this study than predicted is either unmyelinated nerves which are could not seen in toluidine blue dyed sections or fused myelinated fibers that are considered as only one axon while counting.

However, all referred reasons above were partially effective on stereological results of this study. So we suggested that number of myelinated axons in newborn rats was less than those of 20 days rat fetuses.

It has been shown that several factors, contributing to development and differentiation Schwann cell. These factors may be listed following; i). neuregulins - β having a pleiotropic effect on axonal signals suppress the generation of neurons from neural crest cells [31] and also support the survival of both Schwann cell precursors and Schwann cells [32]. Except of these, it has been claimed that there are some important autocrine factors induced developing of Schwann cell too such as insulin growth factor 2 (IGF-2), neurotrophin-3 (NT-3), platelet-derived growth factor-BB (PDGF-BB), leukemia inhibitory factor and lysophosphatidic acid (LPA) [33-36].

In the first weeksof postnatal life in the rat sciatic nerves, the more myelinated axon number was detected than that of newborn rats. The cell cycle and elimination finished, and Schwann cells tend to produce progressively more myelin. Thus, with age, an increase both in the number and diameter of myelinated axons is observed on histological sections as mentioning of Jeronimo et al.[37].

In our study, the most myelinated axon number was detected in 5 week's rats. Axons were generally myelinated. Myelin sheets were more thick and regular. Rarely unmyelinated nerves were found. Schwann cells localized in near the axons, peripherally. In this group, neither immature, precursor glial cells, nor apoptotic Schwann cell nuclei were observed at both light and electron microscopical examinations. This finding indicates that, although Schwann cell proliferation and elimination mechanisms continue until the early neonatal period (postnatal first days).

Finally, in this study, embryological development of sciatic nerve which is a part of the peripheral nervous system and the widest peripheral nerve of body was investigated with microscopical, stereological and immunohistochemical methods. In light of the rat model, a general opinion was obtained about peripheral nerve development of mammalian. Thus we think that our findings may provide important contributions about this point to literature.

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