Histological assessment of nerve lesions caused by epineurial electrode application in rat sciatic nerve

WERNER GIRSCH, M.D., RUPERT KOLLER, M.D., HELMUT GRUBER, M.D., JÜRGEN HOLLE, M.D., CHRISTIAN LIEGL, UDO LOSERT, M.D.V., WINFRIED MAYR, D.PH., AND HERWIG THOMA, D.PH.

Second Surgical Clinic and Third Department of Anatomy, University of Vienna, Vienna, Austria

The left sciatic nerve of 36 rats was exposed and four ring-shaped stainless steel wire electrodes were sutured to the epineurium of each nerve in the same manner as performed clinically for "carousel stimulation" in man. The rats were sacrificed 10 days (Group 1), 3 weeks (Group 2), or 3 months (Group 3) after implantation. The electrodes were excised, the nerves were embedded in Epon, and semithin sections were obtained for histological and planimetric assessment of lesions caused by the epineurally sutured electrodes. The right sciatic nerves served as controls. The total area of neural tissue within the epineurium was determined at three levels: at the site of the electrodes, 8 mm proximal, and 8 mm distal. The area of neural tissue damaged by the surgical procedure was expressed as a percentage of the total area. In Group 1, nine of 12 nerves showed lesions ranging from 0.39% to 25.39% of the total area of neural tissue, in Group 2 eight of 11 sciatic nerves showed lesions ranging from 0.24% to 13.03% of the total area, and in Group 3 five of 12 nerves showed lesions ranging from 0.21% to 4.96% of the total area. The pathologically altered area in Groups 2 and 3 exhibited distinct signs of nerve fiber regeneration. The reasons for the decrease in damage from Group 1 to Group 3 and the clinical implications of the results for long-term electrical stimulation are discussed.

KEY WORDS • morphometry • electrode, epineurial • nerve injury • diaphragm pacing • functional electrical stimulation

For many years, functional electrical stimulation of peripheral nerves has been applied successfully in the restoration of limb movement or respiratory function in para- and tetraplegic patients.\textsuperscript{4-7, 20-22} Since the first attempts at experimental and clinical use of this technique, many authors have investigated the question of whether functional electrical stimulation causes any change in the stimulated nerves. The effects of cuff electrodes, coiled wires, and intraneurally implanted electrodes on nerve integrity have been explored in many histological studies.\textsuperscript{1-3, 5, 9, 11, 13, 14, 16, 21-23}

The so-called "carousel stimulation" was developed in 1972 with the aim of reducing fatigue of the stimulated muscles.\textsuperscript{10, 20} It requires the application of at least four electrodes as close to the nerve as possible. Annular stainless steel electrodes are sutured to the epineurium of the peripheral nerve in a circular manner using microsurgical techniques. Despite successful clinical application for the purpose of the "Vienna phrenic pacemaker,"\textsuperscript{20} epineurally fixed ring electrodes have not become very common in functional electrical stimulation compared to other methods such as cuff electrodes. Previous experimental studies have revealed good functional and even good morphological results but have not provided satisfactory quantitative data concerning nerve alterations related to epineural electrode application.\textsuperscript{8, 15}

The present study was undertaken in order to quantify the extent and time course of lesions caused by electrode application. In a statistically relevant number of rats, annular stainless steel electrodes were sutured to the epineurium of the sciatic nerve and the extent of lesions was determined by computer-assisted image analysis.

Materials and Methods

Thirty-six female Sprague-Dawley rats, weighing an average of 255.7 gm each, were anesthetized with 12 to 14 mg ketamine hydrochloride/100 gm body weight for a standardized operation. Groups of four animals were housed together in cages and given a standard diet and water \textit{ad libitum}.
Nerve lesions caused by epineural electrodes

Electrode Implantation

Ring-shaped electrodes, 1 mm in inner diameter and connected to an electrode lead 1 cm in length, were used. Electrode and lead were made from stainless steel; the lead was covered with a Silastic tube. The left sciatic nerve was exposed in all animals. With microsurgical techniques, the nerve was isolated from the surrounding tissue and the electrode was implanted. Four electrodes were positioned around the sciatic nerve within a distance of 5 mm from each other. The electrodes were fixed to the epineurium of the nerve by a 8-0 nylon suture (Fig. 1). The most proximal electrode was situated about 5 mm distal to the ischial tuberosity and the most distal electrode at a minimum distance of 3.5 mm proximal to the division of the sciatic nerve into its main branches (Fig. 2). The electrode leads were placed at a right angle to the nerve between the dorsal and the adductor muscles of the thigh.

Experimental Groups

The 36 animals were separated into three investigation groups consisting of 12 animals each. All 12 animals in each group received electrode implantation in the left sciatic nerve. For control data, two rats received microsurgical exposure of the right sciatic nerve (sham operation), and the right sciatic nerve was crushed in two other animals. Thus, the right sciatic nerve was exposed in 12 of the 36 animals. In six of these animals, the nerve was isolated from the surrounding tissue under the operating microscope, but electrode implantation was not carried out (sham operation); in six other rats the right sciatic nerve was crushed between the branches of a microsurgical forceps, and the remaining 24 animals received no surgical procedure on the right sciatic nerve.

Explantation and Histological Examination

The animals were sacrificed 10 days (Group 1), 3 weeks (Group 2), or 3 months (Group 3) after implantation. In each animal both sciatic nerves were exposed and excised between the sacrum and the knee joint. Under microscopic magnification, the four electrodes were removed from the epineurium of the left sciatic nerve. From each of the left sciatic nerves, specimens situated directly at the site of the electrodes and 8 mm proximal and 8 mm distal to them (Fig. 2) were taken for histological examination. The nerves of the right thigh served as controls and were harvested for histology at corresponding levels.

The nerves were fixed in 3% glutaraldehyde, post-fixed in 2% buffered osmium tetroxide, and embedded in Epon. For quantitative evaluation, 2-μm semithin sections were cut on an ultramicrotome and transmitted via television camera from the microscope to a personal computer for computer-assisted planimetry. The final magnification used for image analysis was × 780. Cross sections of the sciatic nerve at the three levels mentioned above were examined in regard to either signs of degeneration (such as myelin fragmentation, reduction of nerve fiber density, and increase in connective tissue) or signs of regeneration (for example, groups of small fibers with thinned myelin sheaths).

The following parameters were measured for each specimen of the sciatic nerve: total cross-sectional area of neural tissue within the perineurial sheath; area of nerve segments showing signs of degeneration or regeneration; cross-sectional area of 500 normal-appearing nerve fibers (expressed as the calculated diameter of a circle of the same area); and cross-sectional area of the remaining intact and regenerating fibers in pathologically altered segments (expressed as the calculated diameter of a circle of the same area). The following data were calculated from these measurements: altered area proportional to total area of neural tissue within the perineurium (percentage of altered area), and neural cross-sectional area occupied by myelinated fibers pro-
portion to that captured by connective tissue in normal and injured segments (nerve fiber density).

The density and diameters of nerve fibers were evaluated to confirm the classification of normal-appearing or pathologically altered regions of the sciatic nerve. In cases where it was difficult to differentiate between intact sensory fascicles and areas in an advanced state of degeneration, small nerve fiber diameters in combination with remnants of degenerated myelin sheaths were the relevant criteria for classifying segments as pathologically altered.

Results

Gross Findings

At the time of autopsy, a distinct increase in connective tissue around the electrodes was found in six of the 35 rats independent of investigation group (one animal in Group 2 died 1 week after electrode implantation). In six rats one electrode and in one rat (Group 3) three electrodes had not preserved their original position in contact with the epineurium and were found more distant from the nerve.

Histological Findings in Experimental Nerves

Sciatic Nerve. At 8 mm proximal to the electrodes, the sciatic nerve was always composed of one fascicle with a mean cross-sectional area of 482,566 ± 14,107 sq μm (standard error of the mean) within the perineurium. At the level of the electrodes two fascicles were usually found capturing a mean area of 505,533 ± 10,818 sq μm. At 8 mm distal to the four electrodes, the sciatic nerve was composed of at least three fascicles due to the varying level of division into its muscular and cutaneous branches (Fig. 3). The mean cross-sectional area of the nerve at this site was 491,721 ± 13,912 sq μm.

Number of Altered Nerves. The number of sciatic nerves showing pathologically altered segments was nine in Group 1, eight in Group 2, and five in Group 3 (Table 1). Lesions were seen at all levels of examination. Only one specimen showed an altered region proximal to the electrodes. At the level of the electrodes, 14 of the 35 nerves examined exhibited alterations. Distal to the site of the electrodes the number of sciatic nerves exhibiting pathologically altered segments was nine in Group 1, six in Group 2, and three in Group 3. In four cases (two in Group 1 and two in Group 2), alterations at the electrode level were not accompanied by altered nerve segments distal to the electrodes.

Extent of Lesions. At the site of the electrodes, between 3.46% and 12% of the total area was damaged by electrode implantation in Group 1 (Table 2). Pathologically altered areas ranged between 0.7% and 13.03% in Group 2, and between 0.38% and 4.96% in

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TABLE 1

<table>
<thead>
<tr>
<th>Number of sciatic nerves showing altered sectors at three time intervals following electrode implantation</th>
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<tr>
<td>Factor</td>
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</tr>
<tr>
<td>no. of rats</td>
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<tr>
<td>no. of sciatic nerves with lesions</td>
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<tr>
<td>location of lesions</td>
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<tr>
<td>proximal to electrode level</td>
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<tr>
<td>at electrode level</td>
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<td>distal to electrode level</td>
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TABLE 2

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<tr>
<th>Percentages of total fascicular cross-sectional areas altered by electrode implantation*</th>
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<tr>
<td>Location of Altered Areas</td>
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<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>at electrode site</td>
</tr>
<tr>
<td>distal to electrodes</td>
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*Percentage expressed as range; numbers in parentheses denote the median.
Nerve lesions caused by epineural electrodes

<table>
<thead>
<tr>
<th>Location of Altered Areas</th>
<th>Group 1 (10 days)</th>
<th>Group 2 (3 weeks)</th>
<th>Group 3 (3 months)</th>
</tr>
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<tr>
<td>at electrode site</td>
<td>4.74%</td>
<td>2.18%</td>
<td>0.57%</td>
</tr>
<tr>
<td>distal to electrodes</td>
<td>6.43%</td>
<td>2.62%</td>
<td>0.27%</td>
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*Altered areas include areas with and without detectable lesions.

Group 3. Distal to the site of electrodes the injured sectors occupied between 0.39% and 25.4% of the total area of neural tissue within the perineurium in Group 1. Corresponding ranges were 0.24% to 5.74% for Group 2 and 0.21% to 2.36% for Group 3. When all animals in each group are combined, the average pathologically altered areas at the electrode level involved 4.74% (Group 1), 2.18% (Group 2), and 0.57% (Group 3) of the total fascicular area (Table 3); corresponding values determined from cross sections distal to the electrodes were 6.43% in Group 1, 2.62% in Group 2, and 0.27% in Group 3.

Appearance of Lesions. Lesions referred to electrode application were usually confined to segments and never widely disseminated over the whole cross-sectional area of the sciatic nerve. Normal and pathologically altered regions could be distinguished easily in Groups 1 and 2, whereas in Group 3 differentiation was not as apparent in some cases. In regions classified as normal (Fig. 3), the mean nerve fiber diameter was 6.91 ± 0.09 μm and the mean nerve fiber density (neural cross-sectional area occupied by myelinated fibers) was 64.57% ± 1.02%.

Regions classified as pathologically altered appeared differently in the various rat groups. Ten days after electrode implantation (Group 1), the injured sectors exhibited fragmented myelin sheaths, myelin globules, signs of myelin phagocytosis, and a reduction of nerve fiber density due to an increase in connective tissue and edematous swelling. Within these sectors a small amount of relatively large normal-appearing nerve fibers could be observed (Fig. 4A). Normal and pathologically altered sectors were distinguished on the basis of the mean nerve fiber density, which was reduced to 10.97% ± 1.44% in damaged areas. Three weeks after electrode application (Group 2), damaged nerve segments usually contained several small nerve fibers with thin myelin sheaths and remnants of degenerated myelin sheaths (Fig. 4B). In this group, the mean nerve fiber density was 16.71% ± 1.22% and the mean nerve fiber diameter was 4.84 ± 0.46 μm in pathologically altered areas. Both criteria allowed a clear distinction (Fig. 5). Three months after the implantation procedure (Group 3), remnants of degenerated myelin sheaths in combination with small nerve fibers indicated pathologically altered areas in an advanced state of regeneration in four rats (Fig. 4C). However, in two specimens of this group the altered segments still exhibited pronounced fibrosis and only a few regenerating nerve fibers were seen.
Histological Findings in Control Specimens

Normal Sciatic Nerves. In sciatic nerves on the right side, which had not received any surgical manipulation, the mean diameter was 6.83 ± 0.17 \( \mu \text{m} \) and the mean percentage of fascicular area occupied by nerve fibers was 62.7% ± 1.71%.

Sham-Operated Nerves. No signs of degeneration or regeneration could be detected in the six right sciatic nerves that had been exposed but did not receive electrodes. The mean diameter of myelinated fibers and the mean percentage of nerve fiber density in these specimens were 7.81 ± 0.19 \( \mu \text{m} \) and 70.11% ± 1.93%, respectively.

Crush-Injured Nerves. Contrary to nerve segments altered by electrode implantation, no intact nerve fibers were seen in sciatic nerves of Group 1 crushed 10 days before. Three weeks after being crushed, the regenerating sciatic nerves exhibited nerve fibers with a mean diameter of 3.37 ± 0.13 \( \mu \text{m} \) and a mean nerve fiber density percentage of 20.31% ± 3.31%. The corresponding data 3 months after crushing was 5.44 ± 0.19 \( \mu \text{m} \) and 49.82% ± 1.32%, respectively. In Groups 2 and 3 the appearance of alterations resulting from electrode application generally corresponded well with that of the specimens of the crushed right sciatic nerves in Group 1 (Fig. 5).

Discussion

The results of the present study reveal that ring-shaped stainless steel electrodes sutured to the epineurium of a peripheral nerve do not alter the morphological aspect of the nerve to a great extent, provided that microsurgical techniques are carefully used. The nerve lesions observed in this investigation were clearly visible, but in general were circumscribed, not extensive, and clearly decreased over time.

Previous Studies

In all previous studies dealing with the influence of cuff, coiled wire, or intraneurally implanted electrodes on nerve integrity, nerve fiber damage and myelin degeneration, or at least an increase in connective tissue due to electrode application was observed. Since minimal morphological alterations of nervous tissue seem to be an inevitable feature of functional electrical stimulation in peripheral nerves, even if lesions do not always occur. In some of the previous studies, an electrical current was applied to the nerves prior to histological examination. The question remained whether the influence of the electrical current or mechanical factors were responsible for the alterations observed in peripheral nerves after functional electrical stimulation. According to relevant publications, alterations of nervous tissue are obviously dependent on the intensity and the frequency of electrical current.

The present study was undertaken in order to quantify morphological lesions caused by epineurial electrode application. Since no electrical current has been applied, lesions can only be due to mechanical factors, either the operative procedure or chronic mechanical irritation by the implanted electrodes.

Arrangement of Lesions

It may be surprising on first sight that lesions associated with electrode application were usually arranged in sectors or segments and were never widely disseminated over the whole cross-sectional area of the nerve. According to relevant publications about nerve injuries, there is no intermingling of fibers in the distal portion of a nerve trunk. Our findings provide an excellent explanation as to why mechanical stress to a segment of the nerve results in segmentally localized lesions at a more distal level.

In the some cases, lesions at the electrode level were not followed by detectable alterations distal to the site of the electrodes. Thus, there is every reason to believe that in these cases the mechanical stress of electrode implantation did not cause Wallerian degeneration but resulted in segmental demyelination.

Time Course of Lesions

Both the number and extent of damaged areas decreased distinctly from Group 1 to Group 3, possibly due to full nerve regeneration in areas that had been only slightly damaged by the implantation procedure. Since the Group 3 animals had undergone electrode implantation before the two other investigation groups, an increase in the surgeon's experience is not responsi-
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be a method of fixing electrodes in a stable position to the nerve as close as possible without causing extensive and permanent alterations in nervous tissue.

References

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Address reprint requests to: Werner Girsch, M.D., 2nd Surgical University Clinic: Spitalgasse 23, A-1090 Vienna, Austria.