ELSEVIER

Burns Vol. 22, No. 8, pp. 602–606, 1996

Printed in Great Britain
0305-4179/96 $15.00 + 0.00

PII: S0305-4179(96)00055-1

Alteration in sensory nerve function following electrical shock

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A study of the effects of electrical shock on peripheral nerve fibres is presented. Strength and duration of the applied shocks were similar to those encountered in a typical industrial electrical accident. The purpose of this study is: (i) to identify the electrophysiological and morphological change in nerve fibres after the application of electrical current shocks; (ii) to examine the ability of the peripheral nerve fibres to spontaneously regain function and; (iii) to demonstrate the usefulness of the sensory refractory spectrum as an additional technique in assessing the damage.

Three groups of animals received twelve 4-ms electric field pulses of approximately 37 V/cm (n=5), 75 V/cm (n=9) and 150 V/cm (n=6), respectively. Group 4 was a control group and received a direct application of 2 per cent lidocaine over the sciatic nerve for 30 min. Thermal effects of the shocks were negligible. The sensory refractory spectrum shows that electrical shock damage was mainly to the large, fast myelinated fibres and that higher field strengths do more damage. Also in a histological examination it was found that the more heavily shocked myelinated fibres had sustained more damage. Copyright © 1996 Elsevier Science Ltd for ISBI.

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Introduction

Neurological symptoms and signs are quite common following electrical injury[1–3] and often serve as the basis for a patient's failure to return to work[4]. However, many victims have persistent symptoms despite nerve conduction patterns that look normal. More precise and sensitive methods of peripheral nerve injury detection would therefore be quite useful. This report shows how the spectrum of refractory periods of a nerve can pinpoint the damage done by an electrical shock and determine which fibres, the slow non-myelinated ones versus the fast myelinated ones, have been affected.

In theory, the degree of vulnerability of a peripheral nerve axon and a muscle cell to electroporation is determined by the field strength and the electrical space constant. The space constant depends on the diameter and degree of myelination of the axon. It has been hypothesized that an electrical shock may induce the greatest transmembrane potential among the largest diameter nerve fibres and those with the thickest membrane[5,6]. When exposed to electrical shock, the fastest axons (i.e. the ones with the largest space constants), or the large, myelinated nerve fibres, should be damaged more than the slower non-myelinated axons. However, each peripheral nerve contains myelinated and unmyelinated axons of various diameters and several different types. Thus, the pattern of injury in a peripheral nerve following electrical injury is likely to be non-homogeneous.

Comparing the postshock values of the amplitude of the action potential and the nerve conduction velocity with the preshock values does not give any information about the non-homogeneity of the damage. Smith[7,8] and Kimura[9,10] have developed a technique that can make the differentiation between the fibres in the peripheral nerve. They use the refractory period of transmission, i.e. the maximum interval between two stimuli where the fibre cannot conduct the second impulse. A sequence of stimuli is delivered to the nerve with increasing interstimulation intervals. As the interval between the stimuli increases, ever more nerves are activated until finally the full signal is re-established. The large, myelinated fibres have short refractory periods (<0.5 ms). The small non-myelinated fibres have long refractory periods (>1.0 ms). The amplitude of the action potential grows when the interval between stimuli is increased and each increment of the amplitude indicates the proportion of fibres that is able to transmit an impulse in the time that was added to the stimuli separation. The subsequent increments form a spectrum that 'takes the nerve apart'. In a damaged nerve the spectrum can help one to determine which fibres are functioning normally and which ones are not.

Material and methods

The experiments were performed in accordance with the standards described in *The Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication no. 86-23, 1985, Department of Health and Human Services). The experimental protocol was approved by the University of Chicago Institutional Animal Care and Use Committee.

Female Sprague–Dawley rats weighing 324±29.7 g were anaesthetized using a combination of ketamine and xylazine (75 mg/kg and 10 mg/kg, respectively)

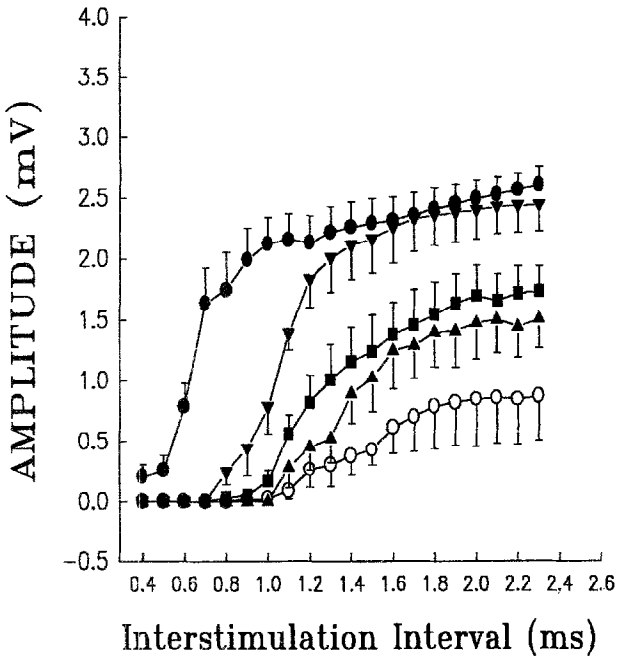

Figure 1. Results of the sensory refractory period test on the sciatic nerve after exposure to 2 per cent lidocaine. ●, Control; ▼, 1 min ($n=6$); ■, 5 min ($n=6$); ▲, 10 min ($n=6$); ○, 30 min ($n=5$) after the application of lidocaine.

dissolved in an isotonic saline solution. An intraperitoneal catheter was used to provide continuous anaesthetic infusion. Thermal support was provided by a circulating hot water pad that was kept at 35–36°C. Antidromic direct stimulation to the sciatic nerve was used, which, after surgical procedure, is placed in a 1.5-cm-long custom-built cylindrical polysulphonate chamber and fitted with circular platinum electrodes at each end[11]. Silver/silver

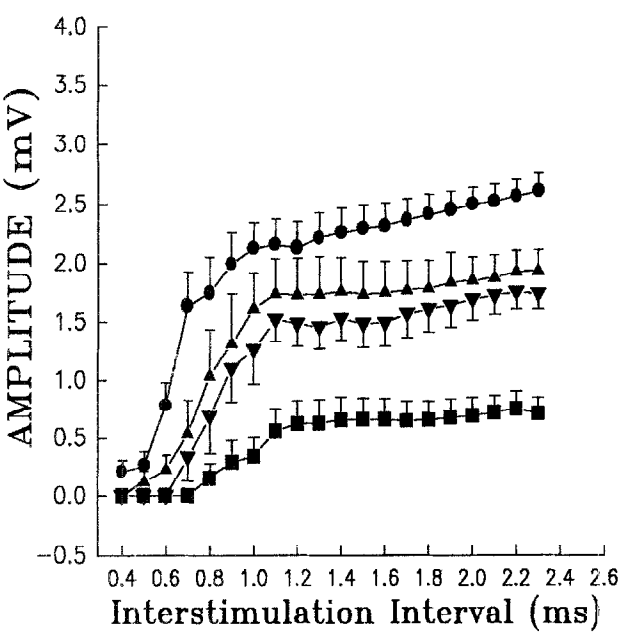

Figure 2. Results of the sensory refractory period test on the sciatic nerve after exposure to 2 per cent lidocaine. ●, Control; ■, 1 h ($n=5$); ▼, 2 h ($n=5$); ▲, 3 h after the application of lidocaine.

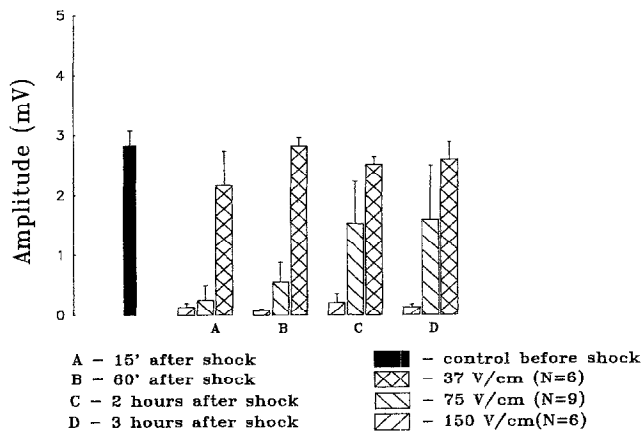

Figure 3. The recovery of the sciatic nerve's action potential amplitude after the application of electrical shocks of different strengths.

chloride recording electrodes were placed on the skin of the sole of the rat's hind foot, with 1.0-cm separation. The distance between stimulation and recording electrodes was 4.0±0.2 cm. A Dantec Counterpoint™ computerized neurodiagnostic machine was used to measure and record the baseline action potential amplitude and nerve conduction velocity.

After the baseline nerve conduction velocity and the action potential amplitude were established, all the electrodes were removed, the animal was placed in a special polysulphonate box and subjected to the electrical current shocks. A current-regulated high-voltage power supply was used to deliver a shock to an anaesthetized rat from the base of the tail to the ankle of the hind limb. Body contact was made using a 4-M KCl salt bridge connected to stainless steel electrodes. The epidermis was removed from the contacts using dermabrasion. Three groups received 0.5, 1.0 and 2.0 A shocks, corresponding to electrical field strengths of about 37, 75 and 150 V/cm, respectively, depending on the size, weight and anatomy of the animal. The electrical field strength produced by the 2.0 A current was approximately 150 V/cm and, in theory, this is a field strength similar to that exper-

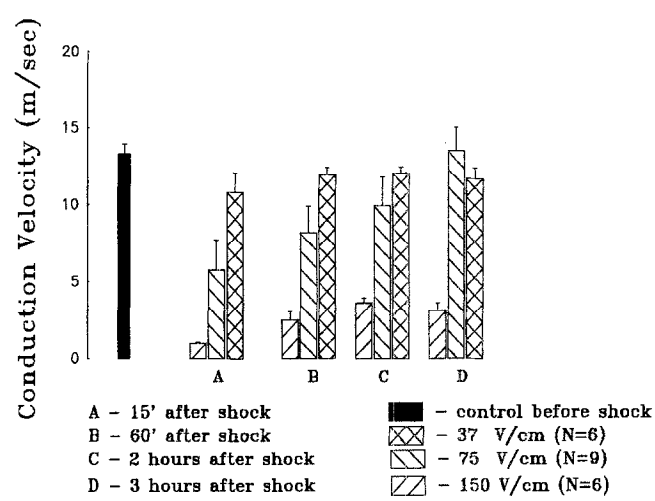

Figure 4. The recovery of the nerve's conduction velocity after the application of electrical shocks of different strengths.

ienced by many victims of high-voltage electrical trauma. Twelve shocks of 4-ms duration, with a 10-s interval between shocks, were applied. By giving many short pulses instead of one longer pulse, overheating and ensuing thermal injury are prevented. In all of the experiments regions of the rat's skin at the ankle of its left hind limb and at the base of its tail were abraded and wrapped in gauze soaked in a conducting and cooling gel: Parker Aquasonic 100 Ultrasound Gel (Parker Laboratories, Inc., Orange, NJ, USA). The monitoring of peripheral nerve function started 15 min after the delivery of the shocks and lasted for 3 h.

After the experiment the animals were killed, the sciatic nerve was dissected, placed in a formalin solution and stained with haematoxylin-eosin. Histological slides were made with the oil immersion technique and enlarged to a final magnification of ×800.

In order to calibrate our electrophysiological studies, experiments were performed in which a 2 per cent lidocaine solution was applied to the nerve. For the group receiving the lidocaine solution the procedure was as with the three shocked groups, but instead of receiving a shock they underwent a 30-min control period to secure stability of the action potential. During this period records were taken after 1, 5, 10 and 30 min. After lidocaine application was terminated, records were taken after 1, 2 and 3 hours.

Results

Sensory refractory transmission spectra were taken from lidocaine-exposed nerves in order to see how well these spectra describe the well-known non-homogeneous blocking by lidocaine. Lidocaine provides an immediate, but short-lasting, block on the bigger faster myelinated fibres and a delayed, but longer-lasting, block on the smaller slower non-myelinated fibres. Data from the lidocaine control group indeed show that after 1 min a significant portion of the faster, myelinated fibres is blocked and that after 30 min no signal at all can be transmitted when the interstimulation interval is smaller than 1 ms (*Figure 1*). This means that the large fast myelinated fibres are completely blocked. In *Figure 2* it is seen that the prelidocaine control data and the 3-h postlidocaine data develop statistically significant differences when the interstimulation interval becomes bigger than 2.0 ms, indicating that after 3 h the slower non-myelinated fibres are blocked by the lidocaine, whereas the faster myelinated ones have recovered. Therefore the known effect of lidocaine on different types of fibres within a nerve can be retrieved with Smith's sensory refraction method.

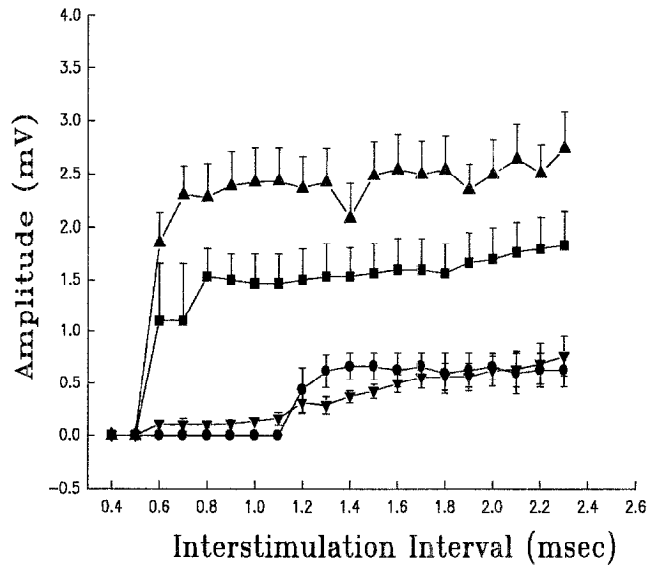

Figure 5. Results of the sensory refractory period test 3 h after the electrical shock. ▲, Control ($n=9$); ■, 37 V/cm ($n=6$); ▼, 75 V/cm ($n=6$); ●, 150 V/cm ($n=4$).

Tables I and *II* and *Figures 3* and *4* show that 3 h postshock the 37-V/cm group did not exhibit an action potential amplitude and a nerve conduction velocity that were different from the preshock values in a statistically significant manner. In the 75- and 150-V/cm groups the amplitude of the action potential was significantly decreased ($P<0.001$) (*Figure 3*). Nerve conduction velocity following the 3-h recovery

Table I. Recovery of the sciatic nerve's action potential amplitude (in mV) after the application of electrical shocks of different strengths

Applied current	*Control before shock*	*15 min after shock*	*60 min after shock*	*2 h after shock*	*3 h after shock*
37 V/cm	2.83±0.57	2.15±0.57	2.82±0.13	2.51±0.28	2.59±0.28
75 V/cm	2.83±0.57	0.25±0.24	0.55±0.32	1.55±0.70	1.58±0.91
150 V/cm	2.83±0.57	0.13±0.06	0.07±0.03	0.21±0.15	0.13±0.04

Table II. Recovery of the nerve's conduction velocity (in m/s) after application of different currents

Applied current	*Control before shock*	*15 min after shock*	*60 min after shock*	*2 h after shock*	*3 h after shock*
37 V/cm	13.3±0.62	10.08±0.62	11.9±0.46	12.0±0.39	11.6±0.69
75 V/cm	13.3±0.62	5.7±1.94	8.18±1.69	9.9±1.88	13.5±1.5
150 V/cm	13.3±0.62	0.11±0.06	2.52±0.53	3.63±0.31	3.1±0.5

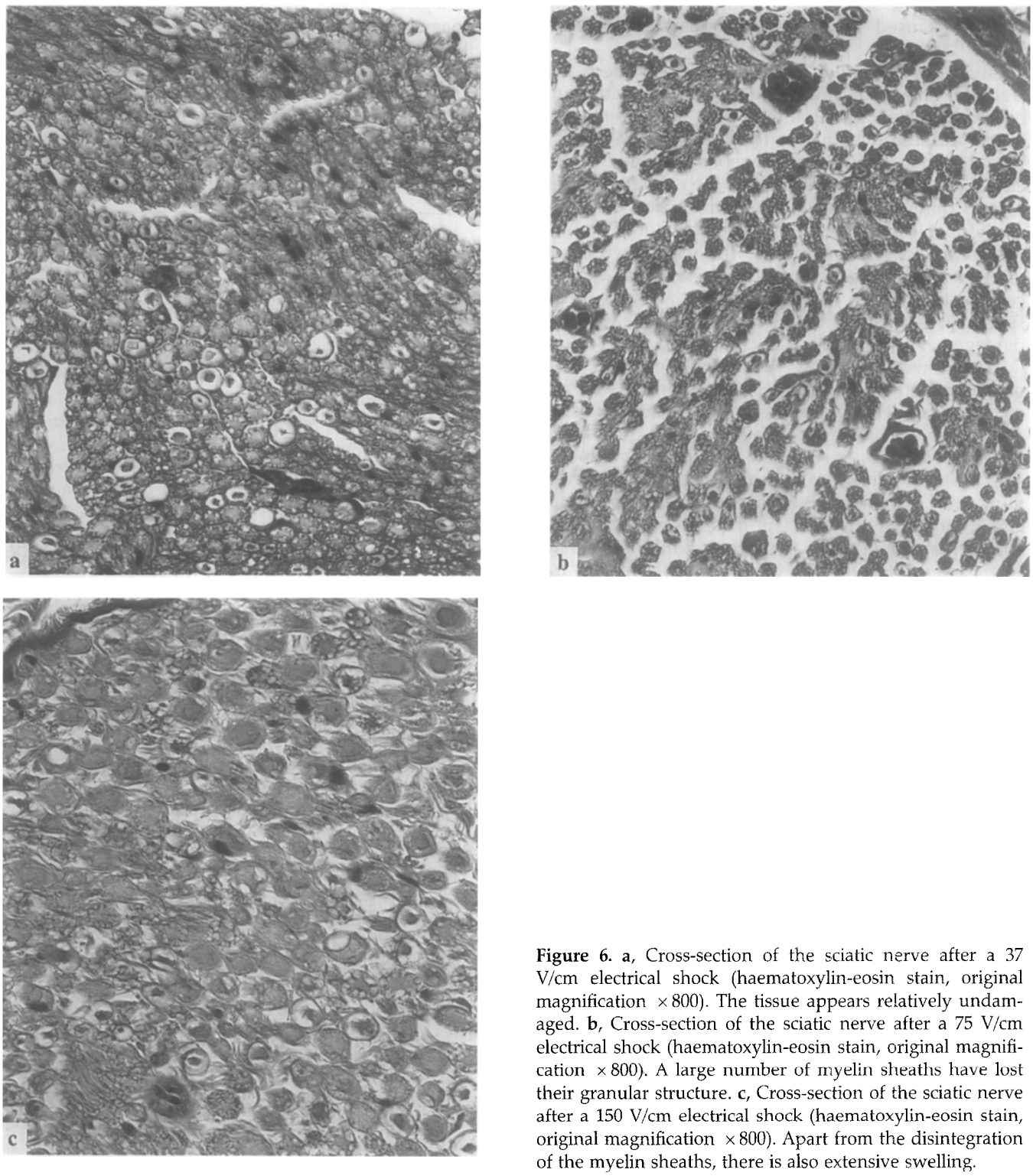

Figure 6. **a**, Cross-section of the sciatic nerve after a 37 V/cm electrical shock (haematoxylin-eosin stain, original magnification ×800). The tissue appears relatively undamaged. **b**, Cross-section of the sciatic nerve after a 75 V/cm electrical shock (haematoxylin-eosin stain, original magnification ×800). A large number of myelin sheaths have lost their granular structure. **c**, Cross-section of the sciatic nerve after a 150 V/cm electrical shock (haematoxylin-eosin stain, original magnification ×800). Apart from the disintegration of the myelin sheaths, there is also extensive swelling.

period appeared to have returned to the preshock values in the 37- and 75-V/cm groups, as shown in *Figure 4*, but remained decreased in the 150-V/cm group ($P < 0.001$).

The refractory transmission spectrum was recorded after a 3-h recovery period and is shown in *Figure 5*. It appears that the sciatic nerves of the 75- and 150-V/cm exposure groups are no longer able to carry any signal with a refraction time of less than 1.0 ms. This suggest a general malfunction of the fast myelinated fibres. The spectrum of the 37-V/cm group seems unchanged when compared to the preshock control spectrum.

Histological pictures (*Figure 6*) from the nerve fibres show disintegration of the myelin sheath and swelling of the nerve tissue after exposure to electrical shock. The 37-V/cm shocks appears to have almost no effect on the tissue and the damage from a 150-V/cm shock is more extensive than from a 75-V/cm shock.

Discussion

Increasing electrical shock currents were applied to anaesthetized rats to examine the hypothesis that non-thermal electrical injury damages nerve fibres

selectively. The spectrum of refractory periods of the nerve was used to assess the damage to different fibres.

Gaylor et al.[5] and Lee et al.[6] postulated that electrical shock may induce the greatest transmembrane potential among the largest diameter nerve fibres and those with the thickest membrane. This would imply non-homogeneous damage after electrical injury. The extent to which different fibres sustain different damage can be measured using the method of Kimura et al.[9,10] and Smith et al.[7,8] With this technique the spectrum of the refractory periods of the fibres that constitute the nerve can be constructed. When comparing pre- and postshock spectra it is possible to see which fibres have been damaged.

The slower and the smaller peaks that were observed in the damaged nerves can be explained as an effect of electroporation, i.e. pores being formed in the lipid bilayer membrane during the shock. When such pores are formed significant amounts of Na^+ ions can follow the electrochemical potential and flow into the axon. During the first phase of an action potential the current through an open Na^+ channel is proportional to the logarithm of the ratio of the concentration of external and the concentration of internal Na^+, i.e. $\log([Na^+]_{outside}/[Na^+]_{inside})$. When the current through an open Na^+ channel is decreased the change in membrane potential due to an open channel will be slower. This means that the positive feedback at the beginning of an action potential is weaker and that it will thus take more time to get to the smaller amplitude. This effect was shown by Hodgkin and Katz[12], who found slower and smaller action potentials for smaller $[Na^+]_{outside}/[Na^+]_{inside}$ in a squid axon.

From *Figure 3* it is apparent that after a 75-V/cm shock a number of axons re-establish their ability to transmit a signal in the course of a few hours. The hypothesis that the damaged axons are the myelinated fibres can explain this duration. The membrane of the myelinated axons is thinnest at the nodes between the myelinated parts and it is likely that extensive poration and the ensuing inflow of Na^+ occurs there. At first the concentration of Na^+ at these nodes is so high that no signal transmission can occur. It can be easily calculated how long it takes the Na^+ to spread homogeneously along the axon. The diffusion coefficient for Na^+ ions in cytosol is about $D = 8.0\ 10^{-10}$ m^2/s (about half of the value in water[13]) and the nodes are about 2 mm apart. Applying the formula $d^2 = 2Dt$ for the mean square distance diffused in time t, we find that it takes about an hour to diffuse over a distance of 2 mm and thus for the Na^+ ions to homogenize over the axon. In *Figure 2* it is seen that it takes about 2 h to get back to half of the preshock amplitude.

These results support the hypothesis that faster myelinated axons are more sensitive to electrical shock. Electrophysiological data are consistent with histological studies which show that the number of damaged myelinated axons grows proportionally with the applied electrical current. The spectrum of refractory periods could be useful in evaluating and understanding patients with peripheral nerve damage. Furthermore, this study explains how extensive nerve damage occurs in patients with minimal thermal injury and clearly demonstrates a non-thermal mechanism of electrical shock injury.

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Paper accepted 30 April 1996.

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