—Regular Articles—

Does Collagenase Affect the Electrophysiological Parameters of Nerve Trunk?

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Collegenase is widely used in the process of teasing a nerve in order to perform single fiber action potential (SFAP) recordings. In this study, the effects of collagenase on nerve conduction parameters were investigated. To accomplish this, normal compound action potentials (nCAPs) were recorded from isolated frog sciatic nerve at various distances using the suction technique. Then, the same nerve was treated with collagenased Ringer's solution (3.5 mg/ml, Sigma Type XI) for 90 minutes and action potentials (cCAPs) were recorded again. Numerical analysis of these records was performed and the results were compared. Using the nCAP and cCAP recordings, the conduction velocity distributions (CVD) of the individual nerve trunks were determined by a method that we have previously described. Statistical results indicated significant differences ($p<0.05$) between the nCAP and cCAP CVD data. From these findings it is concluded that, when used for teasing the nerve fibers, collagenase may affect the nerve trunk conduction parameters. Specifically, a significant amount of decrease has been observed in conduction velocities of myelinated fibers having diameters smaller than 8 μm.

Key words——compound action potential; collagenase; fiber diameter distribution; suction technique

INTRODUCTION

Electrophysiological Parameters of Myelinated Nerve The internodal region of nerve fibers, conceptually thought to be composed of a passive resistance and capacitance connected in parallel, has an effective resistance of γ_{mye} which can be given as:

$$\gamma_{mye}=\frac{k.R_{my}}{2\pi}\ln\left(\frac{a}{b}\right) \quad (1)$$

where R_{my} is the specific resistance per unit length of a single layer of myelin sheath, k is the number of layers, and a and b are the internal and external diameters of the nerve fiber, respectively. Similarly, the space constant (λ) for the myelinated region can be defined as:

$$\lambda(k)=\sqrt{\frac{k.R_{my}}{8.\rho}a^2\ln\left(\frac{a}{b}\right)} \quad (2)$$

where ρ is the specific conductance of axoplasma. The magnitude of λ is an important indicator for the conduction velocity in myelinated fibers.[1,2] Another major parameter effecting the conduction velocity in nerve fibers is the time constant (τ), and can be given as:

$$\tau=R_{mye}C_{mye} \quad (3)$$

where R_{mye} and C_{mye} are the effective resistance and capacitance per unit length of an axon.[3] However, in the case of saltatoric conduction in myelinated nerve fibers, space constant is known to be much more determinant than the time constant.[1,4]

It is known that the myelin layer can be catabolized by chemical agents such as lysolecithin.[5] Apparently, catabolism of such type would modify the parameters seen in Eqs.1, 2 and 3, and consequently, the nerve conduction velocity.[6] It is also known from previous studies that demyelinization changes the excitability and kinetic properties of the membrane.[4,7]

Because of its unique ability to hydrolyze native collagen, collagenase has a specific use for the isolation of cells from animal tissue and removal of the surrounding collagen fibrils and basal laminae from nerve fibers. Chernousow et al.[8] proposed that digestion of Schwann cell extracellular matrix with collagenase effectively disrupts most of the matrix including fibronectin fibrils. However, no information has been reported on the Schwann membrane itself. In experiments aimed at recording single fiber action potentials (SFAP), there exist many studies in which collagenase has been used to tease the nerve fibers.[5,9—11] Yet no evidence has been found in the literature about any possible effect of this isolation procedure on the electrophysiological parameters mentioned above. This study sought to answer these

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questions.

MATERIALS AND METHODS

Experiments were performed on 5 sciatic nerves isolated from male ''Rana Cateibana'' bullfrogs. All animals were treated in accordance with the EU directives and National Institutes of Health Guide for Care and Use of Laboratory Animals. Frogs were dissected just before the experiments were started. During the dissections, frog Ringer's solution was used to keep the nerve moist. The isolated nerve was transferred into an experimental suction bath. This method is considered useful because of its compatibility with the suction recording technique in cases of recording action potential from the whole nerve or individual fibers.[12] The suction bath had dimensions of 10 cm$\times$ 5 cm$\times$1 cm and serves 20 ml Ringer's solution. It also included a suction electrode, with a reference electrode in Ringer's solution for recording, and 5 pairs of Ag/AgCl electrodes for the stimulation process. Frog Ringer's solution that was used in the suction bath contained (mM) 115 NaCl, 2.8 KCl, 1.8 $CaCl_2$ and 5 Hepes, and its pH was adjusted to 7.4. The supra maximal square wave pulses used for stimulation were delivered by a Grass Model S88 stimulator through a Grass Model SIU5 stimulus isolation unit. Stimuli of 0.1 ms duration and 10 Hz frequency were applied at distances of 1.5; 2.0; 2.5; 3.0; 3.5; 4.0; 4.5; 5.0; 5.5 cm from the suction recording electrode in order to record normal compound action potentials (nCAPs). The resultant nerve compound action potential waveforms, obtained using the suction method[12,13], were amplified by a Grass P16 preamplifier and the recordings were taken over a 12-bit A/D converter (Advantech PCL-812PG) with 0.04 ms sampling period. The A/D converter is controlled by computer software called BIOSIG.[14] The same nerve was then put to rest for 90 minutes in another bath of collagenased Ringer's solution. The nerves were treated with collagenased solution of 3.5 mg/ml concentration for 90 minutes in accordance with the literature.[5,9,15] As a chemical, collagenase is available on the market in a variety of types having different activities. Since no information could be found in the literature about the type most suitable for use, Sigma Type XI was chosen after a trial period. In order to preserve the overall integrity of the nerves left in collagenase, no mechanical teasing of them was made over them. After a 90 minutes period, the nerve was again put into the suction bath and stimulated, and the cCAP recordings were taken following the same procedure as applied for nCAP recordings. Since the nerve remains fully viable for 24 hours or more time period[9,16], keeping it in Ringer's solution is not believed to have any extra effect on nerve parameters. All experiments were held at room temperature of 21—25°C.

Analysing Procedure A mathematical procedure was conducted on nCAP and cCAP recordings to investigate the status of neural function before and after the collagenase treatment. The maximum time derivatives of nCAPs and cCAPs (dV/dt), the areas under the CAPs, the latency periods between the stimulus artifacts, and the CAP onset points were computed. Maximum derivatives correspond to the maximum rate of change in a rising phase of CAPs with time, and the change in maximum derivatives can also be used as an index of conduction activity of nerve fibers in a bundle. Since the area under the CAP is proportional to the number of excited nerve fibers in that particular nerve, areas under CAPs were calculated. Latency periods from stimulus artifacts to onset points of CAPs were also computed to obtain the conduction velocity of the fastest nerve fibers.

Analysing the CAPs recorded from certain points over a suitable mathematical model, one can compute the fiber diameter distribution of that nerve.[17,18] To obtain the individual nerve fiber activity, a conduction velocity distribution histogram was developed using the mathematical model that we have enhanced[17] and taking the model proposed by Cummins et al.[19] as a base. The basic principle of our model is based on the statements of CAP, and can be expressed as

$$\mathrm{CAP}(t) = \sum_{i=1}^{N} w_i f_i(t-\tau_i) \qquad (4)$$

where $\mathrm{CAP}(t)$: the observed compound action potential as a function of time; N: the number of fiber classes; w_i: the amplitude weighting coefficients for class i; and $f_i(t)$: single fiber action potential in class i.[17,18,20]

The weighting coefficients (w_i) are general parameters to account for all influences on the contribution of each fiber class to the observed CAP.

This model procedure has been applied to all data on computer media, and the fiber diameter histograms of nerve (so the conduction velocity distribution, CVD) was obtained for nCAPs and cCAPs.

Statistical Procedures Linear regression ana-

lyses were performed on data of area, amplitude, duration, maximum derivative, and latency period of both nCAPs and cCAPs recorded for various distances. The derived regression line equations were compared with those by the regression line method. For the CVD data of nCAPs and cCAPs, sum of the area under the curves values were first calculated and then compared. All statistical analyses were performed using SPSS-10.0 for a Windows statistical software package with $p<0.05$ accepted as significant.

RESULTS

Relative change (%) in areas under the CAP versus recording distance in both collagenase treated and untreated CAP signals are plotted in Fig. 1 ($n=5$). Although there exist some fluctuations in the relative changes of both nCAP and cCAP integrals with distance, the data points could have been fit over straight lines. The equations of each regression line of the average of all 5 nerves for both groups have been computed, the slopes and the corresponding correlation coefficients (R^2) are given in the inset of Fig. 1 ($n=5$) and also in Table 1. There is no significant difference ($p<0.05$) between slopes of nCAPs and cCAPs (average slope $-0.13\ cm^{-1}$ for nCAP and $-0.16\ cm^{-1}$ for cCAP).

The integral of a signal, in our particular case meaning the area under CAP, is an important parameter for the contribution of SFAPs to the reconstruction of CAP. For an untreated normal nerve trunk, the area under the CAP signal remains unchanged with the recording distance, in cases of the volume conduction effect of the surrounding tissue being neglected and/or corrected.[21] However, unchanged area with distance alone does not necessarily mean that there is no structural or functional change in a nerve trunk. Therefore, in order to increase the reliability of a suggestion, additional analysis should be performed on the subject.

The change in the peak amplitude of CAPs vs. distance for both groups is given in Fig. 2. Assuming the functional relation is linear, the corresponding regression lines are computed and plotted in the figure. The slopes and the correlation coefficients are also given in Table 1. The slopes of the two curves are almost the same; however, the overall decrease in the peak amplitude which is about 5% in collagenase treated nerve trunks compared with untreated nerve trunks is significant ($p<0.05$).

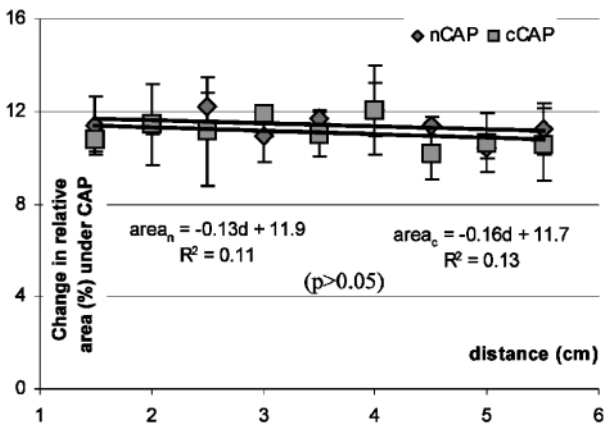

Fig. 1. The Change in nCAP and cCAP Relative Areas with Distance, before and after Treatment with Collagenase ($n=5$)

Equations of regression lines are also given on the graph. The error bars in this and following figures represent standard deviations.

Table 1. The Slope of Equations for Regression Lines Relating to CAP Integrals, Maximum Derivatives, Latencies, Amplitude and Duration with Distance and the Corresponding Correlation Coefficients (R^2) for the Nerves before and after Treatment with Collagenase (n=5)

	Slope of regression lines			
	Before treatent (nCAP)		After treatment (cCAP)	
	(value ± sd)	R^2	(value ± sd)	R^2
Relative area (%) slope (ms. mV/cm)	−0.13±0.05	0.11	−0.16±0.06	0.13
Maximum derivative (V/s. cm)	−6.47±3.42	0.99	−5.35*±2.60	0.97
Latans (ms/cm)	0.36±0.05	0.99	0.34±0.06	0.99
CAP amplitude (mV/cm)	−0.71±0.10	0.99	−0.70*±0.11	0.95
CAP durartion (ms/cm)	0.25±0.11	0.94	0.23*±0.07	0.94

* indicates $p<0.05$

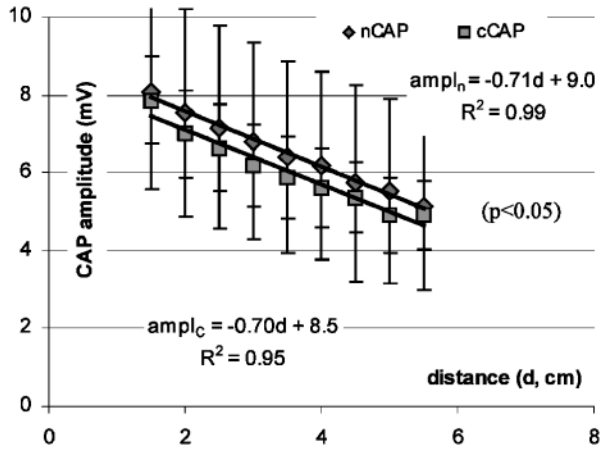

Fig. 2. The Change in Amplitude of nCAP and cCAP with Distance (n=5)

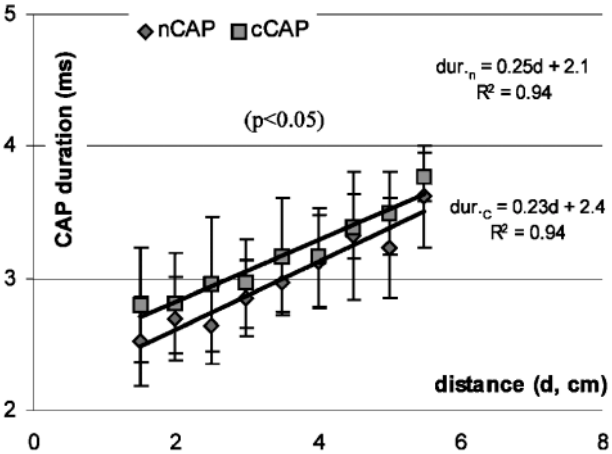

Fig. 3. Change in Duration of nCAP and cCAP (n=5)

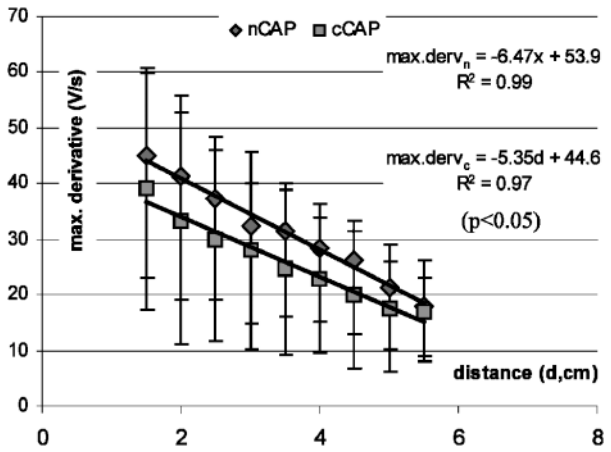

Fig. 4. The Change in nCAP and cCAP Maximum Derivatives with Distance (d) and Equations of Regression Lines before and after the Treatment with Collagenase (n=5)

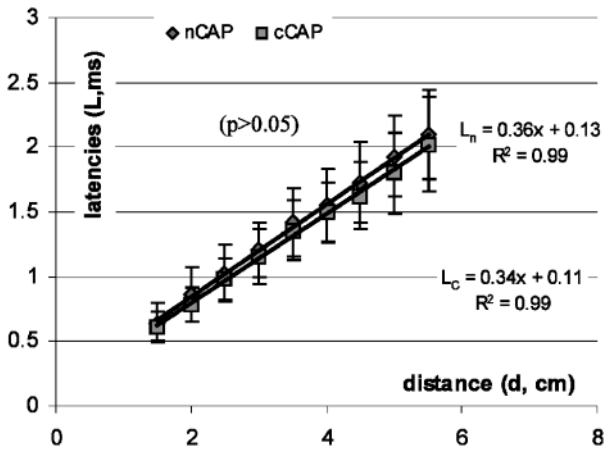

Fig. 5. The Change in nCAP and cCAP Latencies (L) with Distance (d) before and after the Treatment with Collagenase (n=5)

Similarly, the change in CAP duration for both groups is shown in Fig. 3 (n=5). Corresponding linear regression lines are computed and plotted in the figure. The slopes and the correlation coefficients are also given in Table 1. It is apparent that the average increase of about 14% in CAP duration in a collagenase treated nerve trunk is significant ($p<0.05$).

The maximum values of the derivatives of nCAP vs distance and cCAP vs distance curves are given in Fig. 4 (n=5). Assuming that the functional relationship is linear, the equations of the regression lines of nCAPs and cCAPs are computed and their parameters are given in Table 1. Statistical results show that there is a significant difference ($p<0.05$) between the change in maximum derivatives of nCAPs and cCAPs in all of the 5 experiments. The time at which maximum derivative occurs fits the AP travel time of approximately 8—9 μm fiber diameter. This is complementary information that supports our previous studies.[17]

The change in latency periods and the time elapsed between pulse artifact and CAP onset, were also measured in order to evaluate the effects of collagenase on fast conducting fibers. The change in the latency of nCAPs and cCAPs with distance are both linear and given in Fig. 5 (n=5). The equations for the regression lines are also given in the figure. The average slopes for all 5 experiments and correlation coefficients are also given in Table 1. Statistical results show that there is no significant difference ($p<0.05$) between the values of nCAPs and cCAPs in

any of the experiments.

Finally, applying our previously described[16] mathematical model on nCAPs and cCAPs, the corresponding CVD histograms were computed. Relative number of fibers (%) was determined for 20 different fiber diameter intervals (between 3—22 μm) and, considering the linear relationship between fiber diameter and conduction velocity,[3] the corresponding conduction velocities for each interval were computed using the formula:

$$\text{conduction velocity (m/s)} = \text{fiber diameter } (\mu\text{m}) * (0.06T + 0.6) \quad , \quad (5)$$

where T is the temperature in Celsius.[21]

The histograms obtained for one of the nerves and the average of five nerves are given in Figs. 6(a) and (b). The histograms of the same nerve computed for the nCAPs and cCAPs are shown on the same graphs. For comparison of the histograms, the sum of area under curve values has been calculated and compared. The average area under conduction velocity histogram curve for nCAP and cCAP has been found to be 21.25±2,4 and 27.3±4,0, respectively ($n=5$). The results show that there is significant difference ($p<0.05$) between the two groups. However, from the graphs it is also apparent that the fibers with conduction velocity greater than 18 m/s (in other words fibers with diameter greater than 9 μm) seem to be unaffected by the collagenase treatment (arrow in Fig. 6).

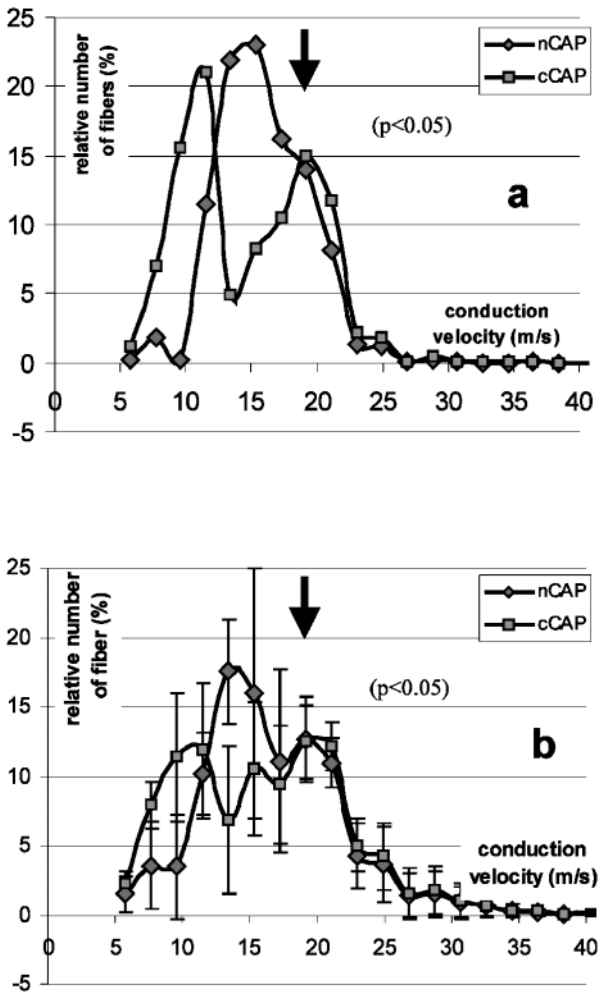

Fig. 6. The Conduction Velocity Distribution Histograms Computed Using the Mathematical Model for a Nerve and the Average ($n=5$) of Five Nerves (a and b)

Histograms for nCAP and cCAP are shown on the same curve. Standard deviations of five nerves are also given on the average graph (b) for both nCAP and cCAP. Arrows in both figures separate the partially affected and unaffected regions in respect to fiber diameter.

DISCUSSION

The most important task of nerve fibers is to transmit the information from one side to the other in a reliable way. When a myelinated axon suffers damage to some partial demyelinization zone(s) or in cases where no myelin exists at all, the axon may still continue to transmit but the transmitted signal arrives at its destination somewhat degenerated.[23]

It is known that the area under the CAP can be used as an index of the number of activated nerve fibers. If there is no relative change in the area under the CAP recorded at successive distances, it means that the CAPs are composed of the same number of fibers. In other words, the number of fibers contributing to the CAP signal remains unchanged. With this finding it may be said that there is no conduction block in any fibers at all on the way to their destination.

As can be seen from Table 1, slopes and correlation coefficients of both nCAP and cCAP area curves are nearly zero. This indicates that the relative area does not change with distance in either of the cases. The fluctuation seen in Fig. 1 at both nCAPs and cCAPs may originate from the suction recording condition. When we compare the curves of nCAPs and cCAPs statistically, there appears to be no significant difference ($p>0.05$) between the two cases. This means that collagenase does not alter the number of fibers which are activated.

On the other hand, significant differences and similarities between the two groups are faced when focused on time course of area curves. As pointed out above, one of the similarities is the equality of the relative area under CAP signals. The integral value of

the collagenase treated group reaches its maximum with considerable delay. This is also apparent in CAP duration vs distance relation given in Fig. 3. If this finding is considered with the aid of the information that the CAP area did not change, gathered from Fig. 1, it can be expected that there should also be some amount of decrease in peak amplitudes of CAPs in order to keep the CAP area relatively constant with distance. The data given in Figs. 2 and 3 satisfies this rule. Therefore, we may suggest at least that there is a considerable slowing in the conduction velocity of some groups of fibers in the collagenase treated nerve trunk.

The second similarity is the times at which integral values start to have non-zero value. This time corresponds to the latency period at which the fastest conducting fibers first contribute to CAP.

The latency vs. distance curve for the same nerves of nCAPs and cCAPs are given in Fig. 5. Both curves have a tendency to linearly increase with distance ($R^2=0.99$). Statistical results show that there are no significant differences between the latency periods of the conditions before and after the treatment ($p>0.05$); yet the slopes of latency curves for nCAPs and cCAPs appear to be nearly equal (0.36 ms/cm for nCAPs and 0.34 ms/cm for cCAPs). This may result from the fact that the latency periods are measured as the time lag between the pulse artifacts and CAP onsets, so they give information about the fastest conducting nerve fibers. It is well known that the degeneration of the myelin sheath causes an increase in the latency of axonal propagation.[24—26] Therefore, we suggest that there may be no severe myeline degeneration in the fastest fibers. However, comparison of the overall decrease in the peak amplitude and the maximum derivative (Fig. 4); relation with the distance may suggest that a conduction slowing effect happened in one of the groups of fibers, namely 7—8 μm.

Going further, in order to assess the affected fibers, we have determined the fiber diameter distribution histograms calculated using suction nCAP and cCAP recordings for the same nerves by a method we previously described.[16] The relative fiber number vs. conduction velocity group histograms were calculated for the same nerve before and after collagenase treatment. Plotting of one typical case (Fig. 6(a) and the average of all five nerve trunk (Fig. 6(b), $n=5$)) plots are given in Fig. 6. Statistical results show that there are significant differences ($p<0.05$) between nCAP and cCAP histograms in all of the 5 experiments. As can be seen from figure, there is an overall shift to left in conduction velocity.

Figure 6 supports and summarizes the overall effects on the electrophysiological parameters of the nerve trunk treated with collagenase. While the right side of the arrow in Fig. 6(b) representing fiber diameter greater than 8 μm remains unchanged, the left side broadens, yielding to a conduction which gets slower in smaller diameter fibers.

It is likely that the significant difference is due to the reason of collagenase primarily affecting the physical parameters of the myelin sheath of axons yielding an alteration in space constant. In the case of collagenase demolishing the myelin sheath, the parameter b, which is defined as the external diameter of a fiber in Eqs.1 and 2, will be lowered causing the effective resistance (γ_{mye}) and space constant (λ) of the internodal region to be reduced. As a result of this serious demolishing effect, either a slowing in the conduction velocity may increase or a full conduction block may occur.

It can be concluded from the results that our findings coincide with those of Chernousow et al.[8] and can be summarized as follows. When a nerve trunk is exposed in a bath of collagenase (3.5 mg/ml, Sigma Type XI) in order to tease it into fibers, the conduction parameters may be affected by this process. This conclusion implies that at least 90 minutes of collagenase treatment may destroy the part of the myelin sheath of fibers smaller than 8 μm in diameter, resulting in a considerable slowing in the conduction. However, with the actual results obtained in this study, no prediction can be made about the cases of treatment processes which are more than 90 min.

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