Article

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# Effects of eliminating tension by means of epineural stitches

## A comparative electrophysiological and histomorphometrical study using different suture techniques in an animal model

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## ABSTRACT

Epineural stitches are a means to avoid tension in a nerve suture. We evaluate this technique, relative to interposed grafts and simple neurorraphy, in a rat model. **Method:** Twenty rats were allocated to four groups. For Group 1, sectioning of the sciatic nerve was performed, a segment 4 mm long discarded, and epineural suture with distal anchoring stitches were placed resulting in slight tension neurorraphy. For Group 2, a simple neurorraphy was performed. For Group 3, a 4 mm long graft was employed and Group 4 served as control. Ninety days after, reoperation, latency of motor action potentials recording and axonal counts were performed. Inter-group comparison was done by means of ANOVA and the non-parametric Kruskal-Wallis test. **Results:** The mean motor latency for the simple suture (2.27±0.77 ms) was lower than for the other two surgical groups, but lower than among controls (1.69±0.56 ms). Similar values were founding in both group 1 (2.66±0.71 ms) and group 3 (2.64±0.6 ms). When fibers diameters were compared a significant difference was identified between groups 2 and 3 (p=0.048). **Conclusion:** Good results can be obtained when suturing a nerve employ with epineural anchoring stitches. However, more studies are needed before extrapolating results to human nerve sutures.

**Key words:** neurorraphy, tension, autologous graft, nerve suture, peripheral nerve.

### Avaliação dos efeitos da eliminação da tensão através de ancoramento epineural: estudo experimental comparando resultados eletrofisiológicos e histomorfométricos após diferentes técnicas de reparo no nervo

## RESUMO

A aproximação através de pontos epineurais é uma forma de se reduzir a tensão numa neurorrafia. Neste estudo esta técnica é avaliada através da sua comparação com a interposição de enxertos e neurorrafia simples num modelo experimental utilizando o rato. **Método:** Vinte ratos foram utilizados e divididos em 4 grupos. No Grupo 1, após a ressecção de 4 mm, os cotos do nervo foram aproximados através de pontos de ancoramento epineurais e suturados com tensão. No Grupo 2, uma neurorrafia simples foi realizada após secção do nervo. No Grupo 3, um enxerto de 4 mm foi utilizado para o reparo e o Grupo 4 foi utilizado como controle. Noventa dias após, os nervos foram novamente expostos e a medida da latência do potencial de ação motor e a contagem axonal foram realizados. A comparação entre os grupos foi realizada através da comparação entre as médias (ANOVA) e com o teste não-paramétrico de Kruskal-Wallis. **Resultados:**

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A média da latência motora na sutura simples (2,27±0,77 ms) foi menor em relação aos outros dois grupos onde o nervo foi seccionado e reparado e maior que o grupo controle (1,69±0,56 ms). Resultados semelhantes foram identificados nos grupos 1 (2,66±0,71 ms) e 3 (2,64±0,6 ms). Uma diferença significativa diâmetros das fibras foi identificada quando comparados os grupos 2 e 3 (p=0,048). **Conclusão:** Resultados equiparáveis aos obtidos com enxerto podem ser obtidos quando a neurorrafia é realizada com pontos epineurais de ancoramento com tensão, mas estudos adicionais são necessários antes desses resultados serem extrapolados para o reparo de nervo em seres humanos.
**Palavras-chave:** neurorrafia, tensão, enxerto autólogo, sutura nervosa, nervo periférico.

A universally-accepted concept referring to the suture of a nerve is that if one draws both ends together under tension at the site of union, the resultant tension jeopardizes the final outcome[1]. In the majority of peripheral nerve sections of traumatic origin, nerve tissue is lost; consequently, a gap is created that prevents the union of the two ends without tension. This gap is increased by the natural elasticity of the nerve stumps, which tend to retract and further separate over time. Innumerable mechanisms have been developed and tested over the last 100 years, in attempts to avoid this tension, including flexion of articulations[2], transpositions, the use of nerve grafts[3-5] and modern neurotubes[6-8]. Articular flexion is restricted to certain nerves, and only when injured at a site where they transverse a joint. A common complication of this technique is articular rigidity[5]. Transpositions are commonly used, but also limited to specific nerves at certain locations along their path as the ulnar nerve at the elbow. Neurotubes have proven successful, but only when the gap is short (up to 3 cm)[6,7]. Moreover, the high costs of neurotubes limit their utility. The gold standard for uniting two ends of a nerve when there is loss of substance between them is an interposing neurorraphy with an autologous graft. At present, this method has shown to be the most appropriate[4]. However, the use of grafts is not without complications, as is the loss of sensitivity in the territory innervated by the donor nerve, generally the sural nerve, thereby increasing operating time, pain due to neuromas, infection risk, etc. But perhaps the most important point that must be taken into account when evaluating sutures using a graft, is that its results are always inferior, in terms of reinnervation, to those involving direct suturing without tension or termino-terminal neurorraphy. In accordance with Saint Venant's principle, the epineurium can be used as a splint to distribute tension at a repair site[9]. If a small number of anchoring sutures are placed one nerve diameter away from the main repair site, this relieves tension and allows for placement of the remaining small stitches designated to maintain stump alignment. With this, fibrosis can be eliminated and axonal passage through the neurorraphy facilitated.

The objective of the present study is to compare, electrophysiologically and morphometrically the results of nerve sutures employing distal anchoring stitches in a rat model against the results obtained employing grafts and sutures without tension, as well as against non-injured controls without sutures.

## METHOD

This study was undertaken in the laboratory of the Program of Organ and Tissue Transplants, at the School of Medical Sciences for the National University of La Plata. The present study was approved by the local Ethic Committee. A total of 21 inbred male Wistar rats were used. For anesthesia, the following were used: induction with intraperitoneal ketamine/diazepam/atropine 75, 10 and 0.04 mg/kg, respectively; followed by maintenance with intravenous or intra-peritoneal ketamine. One male rat died with the first 24 hours post-surgery, and was not considered in results or statistics, generating a final sample of 20 rats. For each animal, each lower limb was considered an anatomical and physiological unit in itself, thereby resulting in 40 lower limbs, which were divided into 4 groups. The surgical position used was dorsal decubitus, securing the upper limbs with adhesive tape (while allowing for respiratory movement), and similarly securing the contralateral lower limb. Later, the free limb was transposed, crossing over the midline and secured to its contralateral counterpart. In this way, the totality of the gluteus region was exposed. After shaving and sterilizing this region, a cutaneous half-moon incision was made with a small concavity towards the distal segment of the limb. Subcutaneous cellular tissue was extracted and the gluteus muscle identified. The muscle then was split parallel to the direction of its muscle fibers at the union of the superior third with the inferior two thirds, taking special care not to injure the nerve. Traction elements were put into place, in order to enlarge the surgical field, two superior and two inferior at the edges of the muscle section. For the first 10 rats, on their right lower limb, 4 mm of the sciatic nerve was sectioned and discarded. Then, direct neurorraphy was performed under magnification with the microscope, previ-

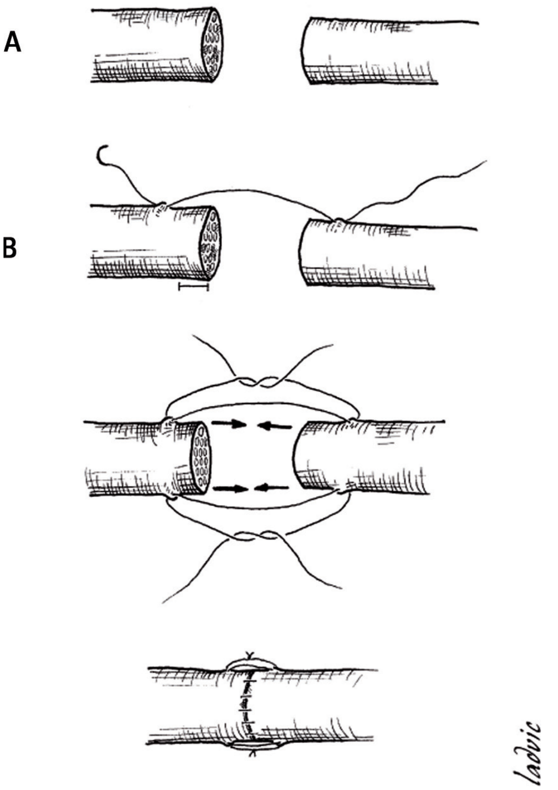

**Fig 1.** Scheme for anchoring epineural stitches. [A] Gap. [B] passage of inextensible suture string (for example, nylon 6.0) through epineurium at a site at least 5 mm distal to the suture. Once both stumps are drawn closer together by means of anchorage stitches, a microsuture with 10.0 stitches is performed by means of the common technique.

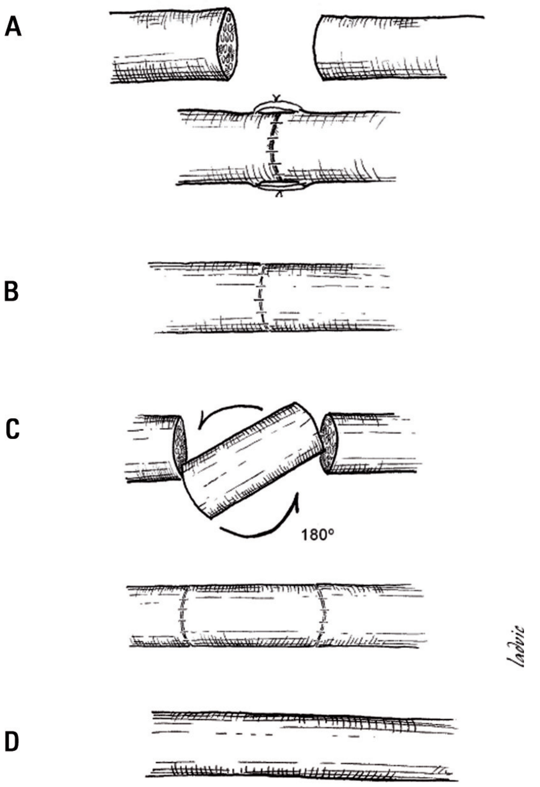

**Fig 2.** Suture technique employed for each group (n partial=10, n total=40) [A] Group 1: tension suture with epineural stitches after resecting a nerve segment of 4 mm. [B] Group 2: termino-terminal suture without tension, without resecting the nerve. [C] Group 3: suture with an autologous graft: after exposition of the sciatic nerve, a segment of 4 mm length is extracted, rotated 180º and sutured with common microsurgical techniques. [D] Group 4: control group, the nerve is not manipulated until neurophysiological measures are taken 90 days after the procedure performed on the contralateral lower limb.

ously placing one or two epineural stitches at a distance of 3 mm from the ends of both stumps (Fig 1). A knot was made with the objective of drawing the two ends nearer to each other and undertaking a termino-terminal neurorraphy without tension at the site of the suture (Group 1) (Fig 2A).

On the left side of the same 10 rats, the procedure was repeated; but instead of resecting 4 mm of the sciatic nerve, a direct termino-terminal suture under microscopy was performed with very little or no tension, coapting the extremes of the sectioned nerve (Group 2) (Fig 2B).

A second lot of 10 Wistar rats underwent intervention on the right side in much the same way as for Group 1, resecting a 4 mm long segment of the sciatic nerve, but then rotating it in the opposite direction, and later suturing it under a microscope, using a graft in an interposed form (Group 3) (Fig 2C). The left side of the second lot of rats was left intact, and later used as a control group (Group 4) (Fig 2D).

Animals were maintained in the lab under optimal conditions for food, light and sleep, to ultimately be re-operated upon 90 days after the first surgery. At the time of the second procedure, again under general anesthesia, the operated zone for Groups 1, 2 and 3 was explored; whereas the sciatic nerve was exposed for the first time in Group 4 limbs. Electrophysiological measurements and microscopic studies were performed, as described below. All animals ultimately were euthanized by means of an intra-peritoneal overdose of thiopental.

## Electrophysiological measurements

Electrophysiological studies were performed to measure the latency of the motor action potentials (MAP)[10]. For this evaluation, we designed two ground electrodes; one electrode to administer the stimulus; and a fourth for recording. One of the ground electrodes was constructed of stainless steel wire, 316L gauge and 0.40 mm in diameter, with one end shaped in an helicoidal fashion, so positioned as to surround the nerve, thereby increasing the area of contact. The other ground electrode consisted of a monopolar needle with a diameter of

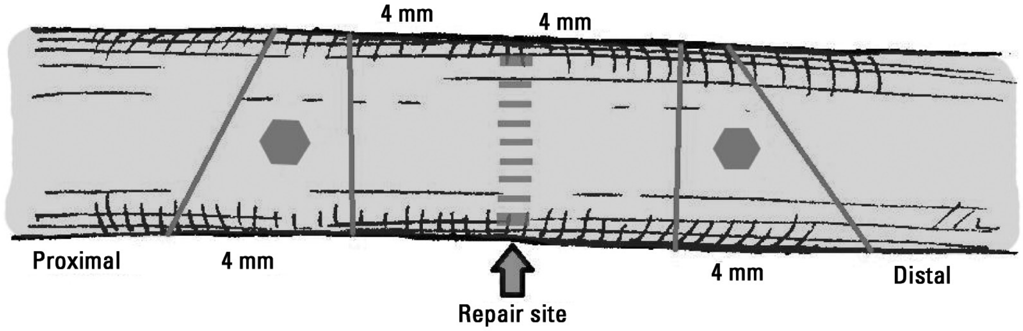

**Fig 3.** Segments of the nerve obtained after Karnovsky fixation solution application. The proximal and distal portions marked with an hexagon were analyzed.

0.40 mm, length of 25 mm, and gauge (G) 26[11]. The electrodes for stimulation were made of wire formed from a voltaic arc of stainless steel 316L, 0.5 mm in diameter, with a distance of one mm between the anode and cathode. For recording, a coaxial needle, 0.30 mm in diameter, 25 mm long and 30G, as well as a recording area of 0.019 square mm was employed. Records were made by means of a portable electroneuromyography device with two channels (Medtronic, Denmark).

After exposing the sciatic nerve, the ground electrodes were installed, the helicoidal ground electrode placed half the distance between the electrodes for stimulation and recording. The recording electrode was installed in the gastrocnemius muscle by means of a percutaneous puncture using a coaxial needle. The distance between the stimulating and recording electrodes was always 30 mm. Short duration supra-maximal stimulation (1 millisecond) was applied, so as to generate an action potential. To determine the supra-maximal current of stimulation, the stimulation values were increased gradually, at intervals of 0.1 milli-amperes (mA), until the scope of the last two potentials remained unaltered. From this potential record, called MAP, the latency were estimated and called LATM. All electrophysiological procedures were undertaken by the electrophysiologist on our research team. Obtained results were analyzed by a different researcher (RSM).

## Histomorphometric measurements

Immediately after the electrophysiological evaluation, both sciatic nerves were fixed *in vivo* with instillation of Karnovsky fixative solution (paraformaldehyde, 2.5% glutaraldehyde and 0.1M buffer at pH=7.32%). After 3 minutes, a 2 cm segment of nerve was sectioned, including the suture site; in the suture with graft group, a 3 cm segment was cut. Both 5 mm distal and proximal to the suture, sectioning with a scalpel, 2 segments 4mm in length were obtained (Fig 3). In the graft group, this entire graft area was considered the 'repair zone', instead of just the suture lines, as in Groups 1 and 2. These segments were stored in labeled bottles containing the same fixative solution. Each segment then was post-fixed in 2% osmium tetroxide solution, dehydrated in growing solutions of ethyl alcohol, clareated with ethylene oxide, infiltrated, and finally added to an epoxy resin, which allowed us to obtain 1mm transversal cuts, toluidine blue-included, suitable for microscopic analysis.

To evaluate the number of myelinized axons, 5 photographs of each cut were obtained with an 8.3 megapixel digital camera, with a 100× zoom. The final number express the addition of counting axons from these five samples. The samples were analyzed using the program Sigma Scan Pro 5.0 (SPSS Inc., Chicago, IL) for axon counts. After measuring the proximal and distal segments, it was possible to generate an index called the *regeneration index* (RI) by dividing the total number of regenerated axons in the distal segment by the total number of regenerated axons in the proximal segment. This index was used to evaluate the number of axons that had crossed the repair site. The fiber diameter distal to repair in the operated groups was also evaluated using the same program. After all these studies, the animals were sacrificed via the intra-peritoneal administration of an over-dose of pentobarbital.

## Statistical analysis

The results were expressed as mean±standard deviation. Analysis of variance (ANOVA) with Tukey's *post hoc* test was used to compare values of motor latency. The non-parametric Kruskal-Wallis test was used to compare RI results. A p value of 0.05 was considered significant.

# RESULTS

All 20 animals who survived beyond 24 hours exhibited an excellent post-operative course, regaining mobility of their limbs within less than 24 hours. With respect to surgical wounds, while the stitches of cutaneous sutures were self-extracted, only one female presented with dehiscence, a few millimeters in length; since the length of dehiscence was so short, it was decided not to re-operate on her. No infection at the surgical site or self-mutilation behaviors was reported. Over the duration of the experiment, the animals increased their average body weight by between 7 and 10%.

Electromyographic measurements were obtained in all animals, tabulated and analyzed, so as to calculate the conduction velocity of motor action potentials. In decreasing order, we noticed the best results concerning motor latency after simple sutures (2.27±0.77 ms), followed by sutures under tension (2.66±0.61 ms), and followed by nerve grafts (2.64±0.6 ms). The conduction velocity for the control group was 1.69±0.56 ms (Table 1). The comparison between groups showed statistical difference when comparing group 1 (suture with epineural stitches) and 4 (control) with p=0.013, and group 3 (graft) and control (p=0.024). No statistical difference was founded when comparing group 1 and 2 (simple suture) with p=0.732, when comparing group 1 and 3 (p=0.982), when comparing group 2 and 3 (p=0.505) and when comparing group 2 and 4 (p=0.252).

Table 2 presents the results of axon counts. The means for the RI were 1.04±0.3, 0.94±0.16, 0.93±0.11 and 0.97±0.17 for Groups 1, 2, 3 and 4, respectively, with no significant difference identified between the four groups (Kruskal-Wallis test, p=0.91). The fiber diameter distal to the repair site was 5.3±1.32, 6.9±2,46 and 4,97±1.11 μm in groups 1, 2 and 3, respectively. A significant difference was identified between groups 2 and 3 (p=0.048).

## DISCUSSION

The mechanism by which tension interferes with axoplasmatic passage through a suture site is fibrosis, which occurs at both stumps and prevents the passage of growing axons towards the distal end. Anchoring epineural traction stitches could be a mechanism by which one could avoid this fibrosis and retraction. Of course, this method only can be employed in nerves with a limited loss of tissue resulting in short intervals, since it is impossible to draw two stumps that are separated by a large gap into close proximity without generating a severe lesion due to traction at both ends, or lacerations involving the epineurium, which would definitely prevent connection by this means. Furthermore, it has been shown that both stumps of a sectioned nerve continue to retract over the first couple of weeks after section, until they find a point of neutral elasticity[12]. This could be another mechanism behind the failure of a suture under tension. After suturing under tension, both ends continue to separate until they are so far away that regeneration is prevented.

Interpreting our other findings, it was apparent that any type of repair of a nerve alters neurophysiological conduction relative to a nerve that has undergone no manipulation. Moreover, this is further proof that if a nerve suture must be performed, it is better to leave it without tension at the same level, such being the ideal way to repair an injured nerve (Group 1). In cases where there is a loss of tissue from a nerve due to trauma, according to what has been extensively published in the literature to date, the tension generated by drawing the two severed ends together for suturing appears to be detrimental to procedural success, since the absence of re-innervation is verified[5]. An alternative way to correct such an imperfection is to anchor epineural stitches at a distance, so as to draw the two stumps closer together and avoid concentrated tension at the nerve suture site itself, displacing all tension to parts further away from the nerve. This suture technique is no novelty, having already been described in several papers[13,14].

The results of the experiment described in this paper showed that regarding motor latency, there was no statistical difference when employing this technique (epineural suture under tension) when compared with the use of grafts. The advantages of the epineural suture

**Table 1.** Mean latency of the motor action potential in each group.

| Study group | Motor latency (mean±standard deviation) |
|---|---|
| 1: tension suture | 2.66±0.71 ms |
| 2: suture without tension | 2.27±0.77 ms |
| 3: graft | 2.64±0.60 ms |
| 4: control | 1.69±0.56 ms |

ms: milisecond.

**Table 2.** Results of histomorphometric evaluation.

| Group | PS | DS | RI | DD |
|---|---|---|---|---|
| 1: suture with tension | 590.7 | 591.9 | 1.04±0.3 | 5.3±1.32 μm |
| 2: suture without tension | 620.7 | 584.6 | 0.94±0.16 | 6.9±2.46 μm |
| 3: graft | 562.6 | 522.6 | 0.93±0.11 | 4.97±1.11 μm |
| 4: control | 603.2 | 573.4 | 0.97±0.17 | – |

DD: fiber diameter in the distal nerve segment; DS: mean value of axonal counting in the distal segment; PS: mean value of axonal counting in the proximal segment; RI: regeneration index.

under tension when compared with grafts, i.e. no morbidity related to graft harvesting, easier surgical technique, less surgical time, make us strongly recommend the former when a nerve gap has to be reconstructed. Nevertheless, the negative effects of tension are displayed by the fact that tension in our rats generated worse results than a simple suture where there was no loss of nerve substance and, therefore, it was possible an adequately coaptation of the two stumps.

The final values for axon counts and, therefore, the regeneration index (RI), were similar across all 4 groups. It was initially presumed that the axonal count should be less in the distal versus proximal segment, reflecting axonal loss that occurs at the repair site. Nevertheless, multiple sprouts of axons from the proximal stump may have passed through the repair site without achieving effective re-innervation, and this increment of axons should compensate for those lost axons that could not cross the repair site[15-17]. The largest fiber diameter in group 2 compared to other two operated groups are in according to electrophysiological results since no differences were identified between groups 2 and control concerning latency of the motor action potential.

From a practical point of view, if the surgeon places a 6.0 or 7.0 stitch and, when drawing nerve stumps together, the epineurium lacerates from excessive traction, it should be accepted that this technique is of no use and one should resort to an interpose grafts. However, if one achieves correct coaptation with epineural traction stitches, the outcome achieved will be better than that obtained installing an autologous graft.

Our study has some limitations. First, we used an experimental rat model; consequently, the results we obtained cannot be extrapolated directly into usual surgical practice in humans. Also, the 4mm imperfection that was created artificially in the sciatic nerve of a rat may not correlate well with gaps in humans. Nonetheless, this study still demonstrates that the deleterious effect of a suture under tension can be eliminated or minimized by means of anchoring with epineural stitches, suggesting that it may be more effective to apply this technique than to employ interposed homologous grafts. It remains to be seen whether these findings can be extrapolated to larger animals with bulkier nerves. Other studies has been done in the last decades, analysing the same topic with different protocols, animal models, and methods of validation of their results[18-21], arriving at the same conclusion than ours: a limited tension at the repair site seems to be preferable than a suture with interposed grafts.

In conclusion, in this experimental model, it was made apparent that the negative effects of tension on the suture of a sectioned nerve can be minimized using anchoring epineural traction stitches. In this way, the results obtained, in terms of re-establishing electrical conduction, are similar than if one uses an interposed graft. These results could be extrapolated to the suture of nerves in common surgical practice by means of further studies on animals of greater weight.

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