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Centrocentral anastomosis in the prevention and treatment of painful terminal neuroma

An experimental study in the rat

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In this experimental study, microsurgical centrocentral anastomosis was applied to an experimental model of painful terminal neuroma resulting from left sciatic nerve section in the rat. The anastomosis consisted of end-to-end suturing of the sciatic nerve fascicles to the tibial branch, with the interposition of a nerve graft taken from the same anastomosed fascicle. As a control parameter for the experiment, the autotomy which follows sciatic nerve section in the rat was evaluated. Autotomy is considered an objective indication of abnormal sensations that are provoked by the formation of a terminal neuroma. Histological study of the proximal stump of the sciatic nerve was also performed. The observation period was 10 weeks. The study demonstrates that centrocentral anastomosis reduces the size of the neuroma formation and the incidence of autotomy.

KEY WORDS • **neuroma** • **sciatic nerve** • **centrocentral anastomosis** • **rat**

TERMINAL neuromas result from the section of main nerve trunks and therefore can follow surgical or traumatic amputation of limbs. Some terminal neuromas are painful; this pain has been related to many causes, but there exist clinical and experimental data that support the belief that peripheral factors play a major role.

Samii[14] considers the major cause of postamputation pain to be the terminal neuroma. After resection of the terminal neuroma, he performs a centrocentral anastomosis in patients with chronic postamputation pain in an attempt to prevent recurrence of the neuroma and the pain. Lagarrigue, *et al.*,[11] have used the same technique in the treatment of other types of painful neuroma. Clinical results have been encouraging, with disappearance or alleviation of the pain.

We have examined this technique in rats with terminal neuromas resulting from transection of the sciatic nerve. In this experimental model, a painful terminal neuroma forms, provoking changes in the animal's behavior, and self-mutilation or autotomy of the denervated limbs.[1,21,23] This behavior pattern has been related to abnormal sensory impulses resulting from the neuroma formation[21] and has been modified through the administration of drugs[6,23,24] and transcutaneous electrical stimulation.[25] The aim of this study was to investigate the effect of centrocentral anastomosis on the formation of terminal neuromas and on the time course of autotomy following experimental transection of the sciatic nerve in the rat.

Materials and Methods

A total of 90 adult Sprague-Dawley male rats, each weighing 250 to 300 gm, were used for the study. The animals were anesthetized with ketamine and separated into five experimental groups. In Group I (control group of 20 rats), the left sciatic nerve was sectioned in the mid thigh and 5 mm of the distal stump was removed to prevent spontaneous reinnervation. Group II included 20 rats that underwent centrocentral anastomosis immediately following transection of the left sciatic nerve. The centrocentral anastomosis consisted of suturing microsurgically the cutaneous, sural, and peroneal branches of the sciatic nerve to the tibial nerve. A 5-mm nerve graft, taken from each anastomosed branch, was interposed (Fig. 1). In Group III, 20 rats underwent anastomosis 5 days after the left sciatic nerve was sectioned. The developing terminal neuroma was

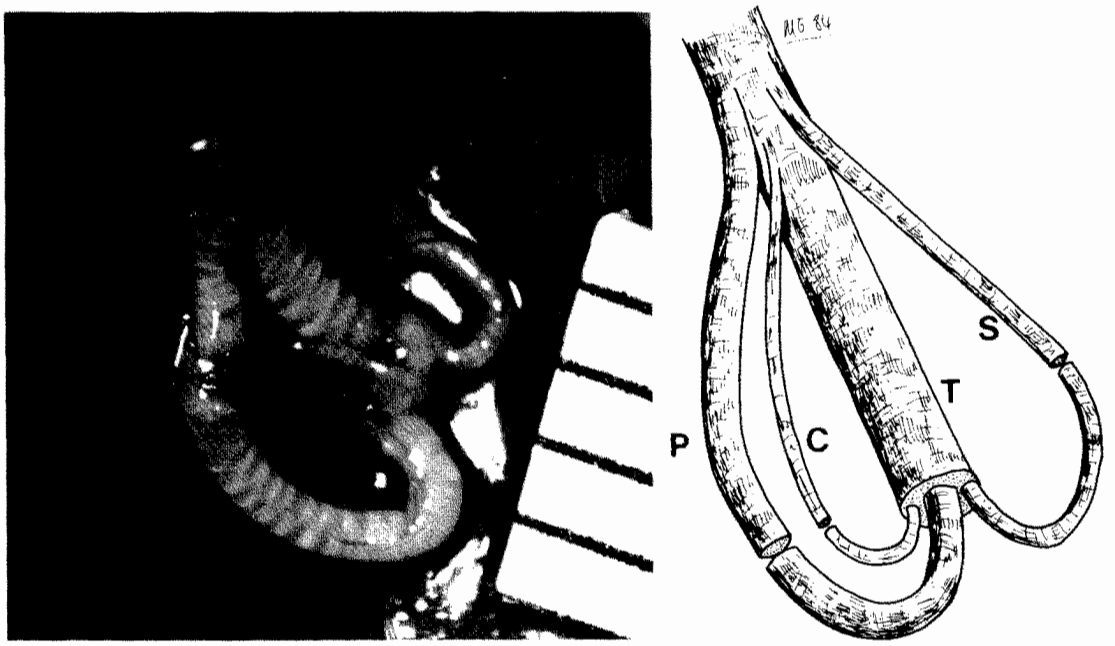

FIG. 1. Surgical technique of centrocentral anastomosis. *Left:* Postoperative photograph of the completed anastomosis operation taken through the surgical microscope (measure is in millimeters). *Right:* Sketch of the surgical technique. The cutaneous (C), sural (S), and peroneal (P) branches of the sciatic nerve are anastomosed to the tibial branch (T). A nerve graft, taken from the anastomosed branch, is interposed.

resected before the anastomosis procedure. In Group IV, a simple resection of the terminal neuroma formation was made in 20 rats 5 days after the left sciatic nerve was sectioned. No anastomosis was performed. Group V included 10 sham-treated rats. In this group, fascicular dissection of the left sciatic nerve was performed using a microsurgical technique. Nothing else was done.

In all animals, the surgical wounds were closed in layers, and no antibiotic agents or topical or parenteral drugs were administered. All animals used in the experiment were housed under standard colony conditions with regard to day-night cycle, feeding, temperature, and boxes. The observation period was of 10 weeks' duration. The animals were examined every 48 hours by a member of the investigation team who was unaware of the animal's group of origin. The experiment was conducted within the guidelines established by the International Association for the Study of Pain (IASP) for research experiments in chronic animal models for pain study.[7]

The extent of autotomy was quantified in accordance with the scale proposed by Wall, *et al.*:[23] one point was scored for the loss of one or more nails; a further point for each half digit lost; an additional point for autotomy of the metatarsal area; and another for autotomy of the tarsus. The maximum score possible was 13 points. The parameters used in the experiment were the time of onset of autotomy, taken as when the animal's first point was observed, and the time course of autotomy, taken as the number of points scored on the autotomy scale during the observation period. Statistical evaluation was then carried out using the Student t-test, the Mann-Whitney test, and the chi-square test with Yates' correction, with a significance value of $p < 0.05$.

At the end of the observation period, the proximal stump of the sciatic nerve was microsurgically exposed in all animals to evaluate its size and relationship to surrounding tissues. The stump was removed from all animals and stored in 10% formalin for routine histological study. The animals were then sacrificed by means of a lethal dose of sodium thiopental.

Results

There were no surgical complications. After surgery, the affected paw remained extended and was used irregularly in walking. Throughout the observation period, the area innervated by the sectioned nerves did not respond to pinprick. Muscular atrophy occurred in the denervated paws. Trophic changes were observed in the denervated paws, including temporary edema, desquamation, and hypertrophy of the claws. The latter change was noted regularly in the nonautotomized paws. The contralateral limb remained normal in all animals. In the sham-treated group (Group V) no neurological deficiencies, trophic changes, or autotomy were observed. None of the animals died during the observation period. One of the control rats (Group I) reached the maximum autotomy score at the 8th week

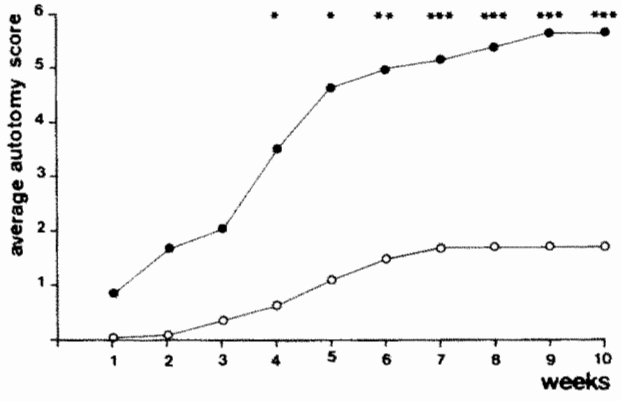

FIG. 2. Graph showing the average autotomy score in the control *(solid circles)* and freshly performed centrocentral anastomosis *(open circles)* groups. Significant differences are apparent between the mean values from the 4th week on (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

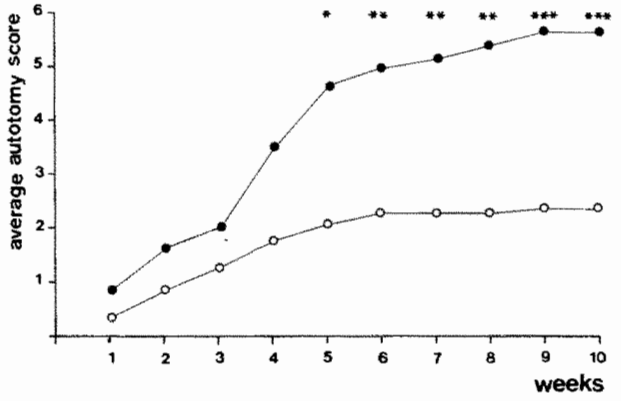

FIG. 3. Graph showing the average autotomy score in the control *(solid circles)* and delayed centrocentral anastomosis *(open circles)* groups. Significant differences are apparent between the mean values from the 5th week on (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

TABLE 1

*Onset of autotomy and average autotomy scores**

Autotomy Data	Group I	Group II	Group III	Group IV
day of onset	15.1 ± 12.3	21.6 ± 8.2	11.2 ± 6	8.3 ± 9.9
score				
1 wk	0.9 ± 1.8	0.1 ± 0.2	0.4 ± 0.5	0.6 ± 0.5
2 wks	1.7 ± 2.4	0.1 ± 0.3	0.9 ± 0.3	2.0 ± 2.5
3 wks	2.1 ± 2.4	0.4 ± 0.5	1.3 ± 1.3	2.2 ± 2.7
4 wks	3.5 ± 2.9	0.7 ± 1.1	1.8 ± 1.6	3.2 ± 2.8
5 wks	4.7 ± 2.8	1.1 ± 1.3	2.1 ± 1.7	4.8 ± 4.1
6 wks	5.1 ± 2.7	1.5 ± 1.8	2.3 ± 1.7	4.8 ± 4.1
7 wks	5.2 ± 2.6	1.7 ± 2.1	2.3 ± 1.7	4.8 ± 4.1
8 wks	5.4 ± 2.5	1.7 ± 2.1	2.3 ± 1.7	4.9 ± 4.0
9 wks	5.7 ± 2.1	1.7 ± 2.1	2.4 ± 1.7	5.0 ± 3.9
10 wks	5.7 ± 2.1	1.7 ± 2.1	2.4 ± 1.7	5.0 ± 3.9

* Values are means ± standard error of the means. Group I: control group, sciatic nerve section; Group II: immediate centrocentral anastomosis; Group III: delayed centrocentral anastomosis; Group IV: resection of neuroma.

and was immediately sacrificed, but its score of 13 points was included in the data.

Onset of Autotomy

Table 1 shows the mean day of onset of autotomy in the different experimental groups. On the day of onset of autotomy there were no noticeable differences. At the end of the observation period, the onset of autotomy in the denervated limb was seen in 100% of the control animals (Group I), 95% of 19 Group IV rats (neuroma resection), 60% of 12 Group II rats with immediate anastomosis ($p < 0.01$), and 100% of the Group III rats (delayed anastomosis).

Time Course of Autotomy

Table 1 presents the mean autotomy score for each experimental group for each week of the observation period. The mean autotomy score increased with time, stabilizing in the final weeks. If Groups I and II are compared, it can be seen that statistical differences became increasingly marked from the 4th week on (Fig. 2). When Groups I and III are compared, noticeable statistical differences are also seen from the 5th week on (Fig. 3). There are no statistical differences between the Group I and IV animals and between the Group III and IV animals. However, the mean autotomy scores of the Group IV rats are lower than in the control (Group I) animals but higher than in Group III animals.

Pathological Findings

Microsurgical observation of the proximal stump of the sciatic nerve at sacrifice showed that the end bulb was smaller in size and clearly defined in relation to the surrounding tissues in those specimens where centrocentral anastomosis was performed. Light microscopic study showed a typical terminal neuroma in Group I and IV animals. In these specimens, axon sprouts mixed with connective scar and muscle tissue could be seen (Fig. 4 *left*). The end portion of the sciatic nerves with anastomosis showed nerve fascicles surrounded by intact perineurium and, between the fascicles, fibrous connective tissue (Fig. 4 *right*). The fascicles exhibited a typical regeneration structure; however, in the rats with delayed anastomosis, some intrafascicular fibrosis was seen.

Discussion

The pathogenesis of pain caused by an injury to the peripheral nervous system has been attributed to central and peripheral factors.[13,17] Wall,[19] in a discussion of pain resulting from postamputation terminal neuroma, stated that peripheral factors should first be totally eliminated before considering central factors, and he focused his attention on the terminal neuroma. Although there are arguments for and against this theory,

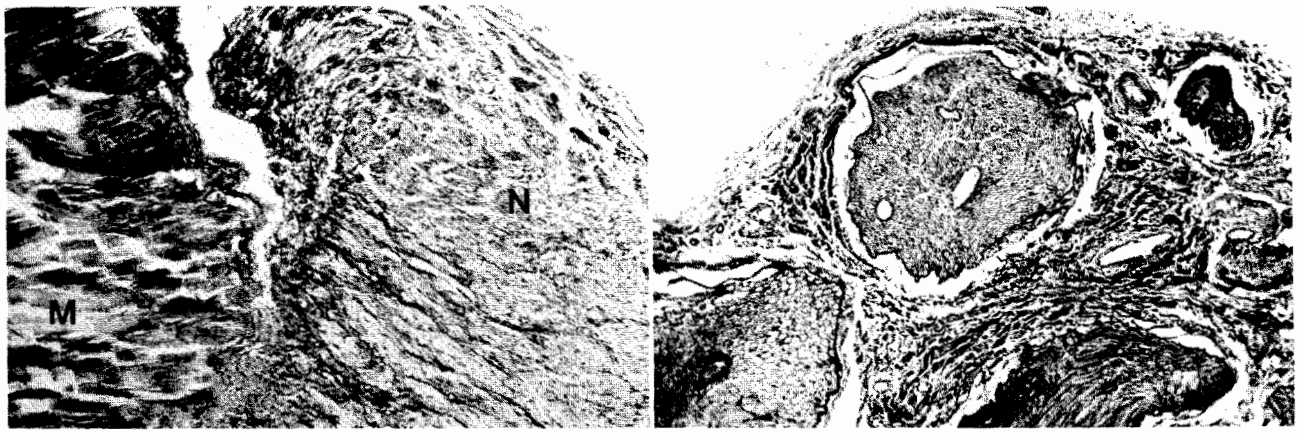

FIG. 4. Pathological findings. *Left:* Photomicrograph of the terminal neuroma that developed in rats with simple section of the sciatic nerve. There is a compact connective tissue (N) without fascicular structure and invasion of muscular bundles (M). H & E, × 14. *Right:* Photomicrograph of the end portion of a sciatic nerve with centrocentral anastomosis showing undamaged nerve fascicles, perineural fibrosis, and independence of the neighboring tissues. H & E, × 55.

the formation of the terminal neuroma must be a vital factor in the pain that may occur after the sectioning of a peripheral nerve trunk. However, other factors may be involved in other pain syndromes, such as phantom limb pain, causalgia, causalgic syndromes, or sympathetic reflex dystrophy.[13,17,18]

The autotomy that occurs in the rat following sciatic nerve section has also been related to the neuroma, which would be the source of abnormal discharges conveyed along sensitive afferent fibers. Wall and Devor[20] and Burchiel[5] have studied these discharges in the sciatic nerve and posterior roots in the rat following sciatic nerve section. They demonstrated that these discharges originate in the neuroma and the dorsal root ganglia, and that there is a relationship between the temporal distribution of the discharges and the latency and development of autotomy. Thus, autotomy would be the animal's behavioral response to the irregular discharges emitted from the neuroma, which would be directed to the sensitive area of the sectioned nerve. In the chronic pain animal model involving dorsal rhizotomy in the rat, autotomy of the denervated limb also occurs, but in this case the autotomy is related to epileptiform discharge activity from the dorsal horn neurons as a result of sudden deafferentation.[3]

Regeneration begins shortly after the transection of a peripheral nerve trunk, with the growth of numerous axon sprouts. If the axon sprouts do not find their way into endoneural distal tubes, some of them degenerate or reverse their course, but a large number reach the scar which has formed on the nerve ending, where they grow in an adverse environment. Wall and Gutnick[22] described aberrant properties in the axon sprouts of the neuroma: production of spontaneous and abnormal sensitive impulses; mechanosensitivity; adrenaline and noradrenaline sensitivity; and capacity for ephaptic conduction from one fiber to another. These properties have been demonstrated in experimental, clinical morphological, and neurophysiological studies,[2,8–10,20] and have been considered as the possible cause of pain in terminal neuroma.

There have been many attempts to prevent neuroma formation and to treat painful terminal neuromas, mainly for management of postamputation pain.[4,15–17] However, in most cases the results have been ineffective, as the neuroma inevitably regenerates and the pain recurs.

Centrocentral anastomosis reduces the size of experimental terminal neuroma that forms after sciatic nerve section in the rat. Furthermore, it noticeably reduces the autotomy score, whether the procedure is performed immediately after nerve section or (to a lesser degree) when following resection of a neuroma. The latency of onset of autotomy was the same in both of these experimental groups. These experimental results correspond with the clinical and experimental findings of Samii[14] and Lagarrigue, *et al.*,[11] who showed a reduction in neuroma size with axon sprouts into the interposed nerve graft and the disappearance of pain from painful postamputation neuromas and other kinds of terminal neuroma.

The results of centrocentral anastomosis can be explained if we consider that the axon sprouts which cross the perineural suture line penetrate into the nerve graft, where they grow freely, isolated and protected from the scar. If the graft is not interposed and the terminal stumps of the fascicles are sutured end-to-end, then the axon sprouts of each fascicle cannot penetrate into another because the endoneural tubes are full. This being the case, the axon sprouts penetrate into the neighboring connective perineural or scar tissue, and a true neuroma in continuity is formed. This surgical technique was initially used unsuccessfully in amputations in 1904 by Langley and Anderson,[12] who anasto-