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The role of the neuroma in autotomy following sciatic nerve section in rats

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Summary A delay in the formation of the terminal neuroma following sciatic nerve section in rats was obtained by means of free nerve grafts sutured to the proximal stump of the sectioned sciatic nerve branches. The automutilating behaviour in these animals was statistically compared with that which follows single sciatic section and sciatic section plus end-to-end suture. The results showed that in animals with grafted nerve stumps, autotomy begins significantly later than in those with single sciatic section. However, when the self-mutilation started, it followed the same increasing evolution in both groups. These results suggest that autotomy after a nerve section is behaviour related to the aparition and nature of the terminal neuroma.

Key words: Neuropathic pain; Neuroma; Anaesthesia dolorosa; Autotomy; Animal pain

Introduction

In 1979, Wall et al. [14,17] proposed that the behavioural alteration occurring in some species of rodents after sciatic nerve section is due to a chronic pain condition: anaesthesia dolorosa. According to their conclusions, the external manifestation of discomfort in the animal is expressed by behaviour characterized by a more or less extensive automutilation of the denervated limb, provoked by disaesthetic and, perhaps, painful sensations. This behaviour is called autotomy and it can be measured by the extent of self-mutilation. The pathophysiology was attributed to the spontaneous and abnormal activity generated in regenerative axonal sprouts caught in the neuroma of the sectioned nerve [5,10,14,17,18].

Later studies have questioned the value of this animal model of chronic pain. Sweet [12] and Rodin and Kruger [8] suggested that autotomy is an example of animal behaviour exclusively applied to eliminate the denervated limb which is anaesthetized and, consequently, useless. They rejected self-mutilation as an expression of pain.

The hypothesis that relates autotomy with pain seems to be the one most accepted. However, recent investigations on neurotoxic drugs (capsaicin) associated with sciatic nerve section in rats have produced contradictory interpretations about the cause of this pain [6–9]. Consequently, the intrinsic value of the neuroma as the origin of impulses which produce autotomy is questioned, and the main role in the automutilating behaviour is attributed to the activity of the deafferented posterior horn neurones [3,7,9].

Our present aim is to study the role of certain kinds of neuroma, not subjected to the effects of drugs, in the event of autotomy appearing and evolving following nerve section.

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Material and method

Sixty adult male Wistar rats were operated on. Three experimental groups were formed, each containing 20 animals. The right sciatic nerve was exposed in all animals by means of a posterior approach in the thigh. On exposing the trunk of the nerve under the surgical microscope, the 3 main sciatic branches, tibial, peroneal and sural, were isolated.

In the first group (SS), used as a control, every branch was sectioned 3 mm down the ramification. No ligation or any kind of protective material was applied to the proximal end of the sectioned nerve.

In the second group (SSS), the branches were sectioned 3 mm from their origin and an end-to-end suture immediately performed between the corresponding stumps using 2 stitches of 8-0 silk.

In the third group (SSG), the same operation was carried out as in the second group but, immediately afterwards, a new section was performed — located exactly 7 mm below the end-to-end suture. In this way a free graft was placed on the proximal end of each sectioned branch, with the intention of producing the same anaesthesia in the denervated limb as in the other groups, and of retarding the formation of the terminal neuroma.

A member of the team, who was not aware of the kind of operation performed on each animal, observed the animals daily during the first 3 weeks, registering the changes in behaviour, the alterations in sensitivity of the limb and the existence and intensity of self-mutilation when present. Wall's scale [17] was used for this last purpose. From the third week onwards, the animals were examined every 2 days with a total follow-up of 10 weeks.

At the end of the observation period, the animals were reoperated on to observe the conditions of the neuroma. Under light anaesthesia, slight pressure was exerted with a microforceps on the different parts of the exposed nerve, including the suture lines and the distal free end of the grafts (group SSG) and the sectioned nerves (group SS). The purpose of this was to provoke an acute painful reaction, the intensity of which was subjectively estimated. To obtain the same pressure in all cases, a small block was put between the branches of the microforceps.

All the recommendations for study of chronic animal pain, established by the IASP, were followed.

The parameters for statistic study were the arithmetic mean and standard error for the first day of autotomy and the autotomy score observed in each daily examination. These parameters were submitted to the Mann and Wihtney U test and considered as statistically significant when $P < 0.05$.

Results

Onset of autotomy

The day of operation was considered as day 0, and the day on which at least a score of 1 was reached was recorded as the day of onset of autotomy.

The values obtained were: group SS (n = 20), $\bar{x} = 3.65$, S.D. = ±3.38; group SSG (n = 20), $\bar{x} = 13.35$, S.D. = ±12.58; group SSS (n = 15), $\bar{x} = 8.13$, S.D. = ±5.36. Five animals in this last group were removed from the total, because when they were reoperated at the end of the experiment, the end-to-end suture appeared broken and the nerve ends were separated, and only a fibrous tract remained between them. In this small group of 5 animals the mean onset of autotomy was: $\bar{x} = 5.4$, S.D. = ±3.07.

The statistical analysis showed that the differences between group SS and group SSS were statistically significant ($P < 0.01$) and those between group SS and group SSG were also statistically significant ($P < 0.005$). No differences were found between group SSS and group SSG.

Evolution of autotomy

Fig. 1 shows the evolution in the degree of autotomy for each group during the entire observation period.

It is interesting to note that the autotomy observed initially in group SSS was practically arrested by the third week and its score became significantly lower than that of the other groups from the fourth week onwards.

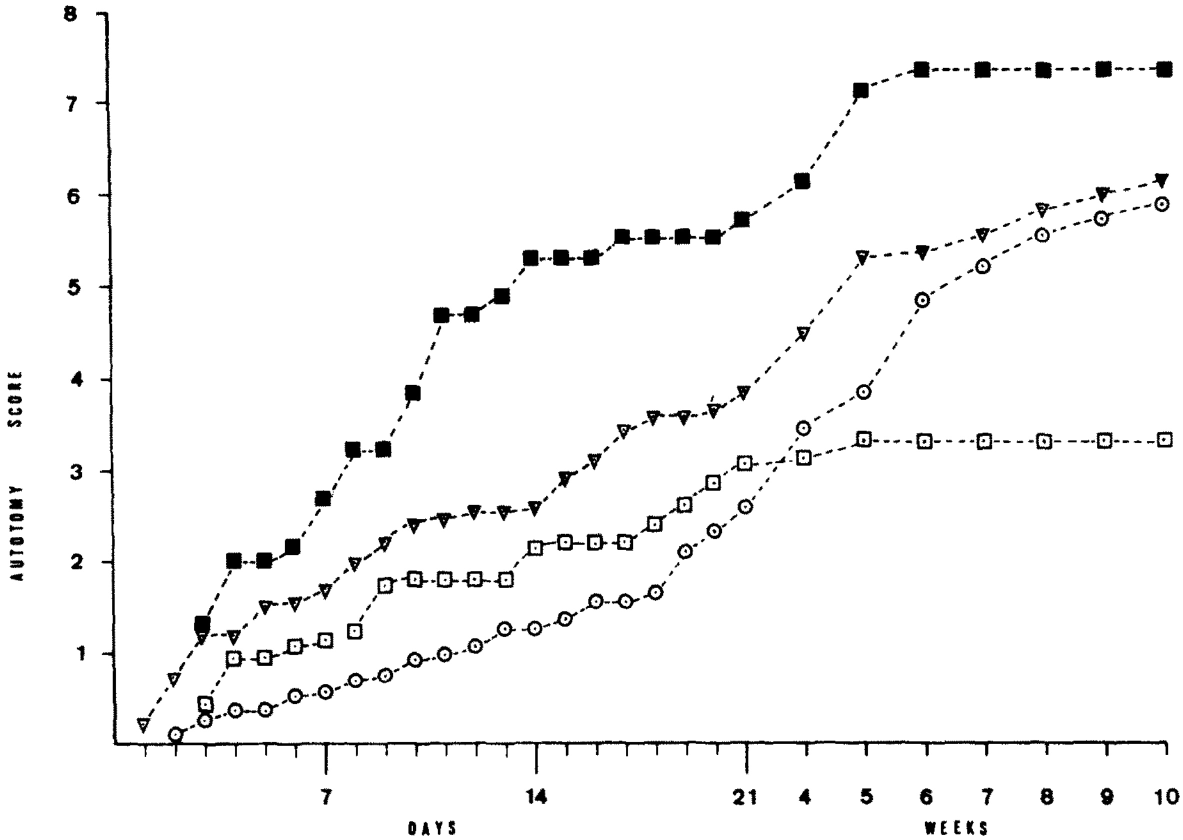

Fig. 1. The graph shows the average autotomy score obtained daily during the first 3 weeks and weekly until the tenth week. Triangles = single sciatic section (SS); circles = sciatic section plus free nerve graft (SSG); empty squares = sciatic section plus end-to-end suture (SSS); black squares = sciatic section plus failed end-to-end suture.

There were significant differences ($P < 0.05$) between the scores of groups SS and SSG from the first until the twentieth day. From then on, the differences disappeared and the increases in the autotomy score of both groups were almost parallel, reaching the same value by the seventh week.

The 5 animals in which the suture was unsuccessful showed a specially high degree of autotomy (Fig. 1). By the second week this small group reached a score of more than 5 points which had not been reached by groups SS and SSG until the fifth week.

In order to facilitate comparison of the evolution of groups SS and SSG, we corrected, with a delay of 10 days, the evolution of the autotomy in group SSG. To do this we made the first day of group SS equivalent to the eleventh day of group SSG. In Fig. 2 it can be seen that these adjusted evolutions are almost identical.

On re-operation, the pressure on the free end of the graft sutured to the tibial branch provoked, in group SSG, an aversive motor reaction of the limb — similar to that produced by pressure on the distal end of the single sectioned tibial branch in group SS. This suggested that the regeneration into the graft finished in a typical neuroma at the distal end. A less intense reaction was produced when the pressure was applied to the end-to-end suture of the tibial branch in the SSS group and also on the suture line of the tibial branch in the

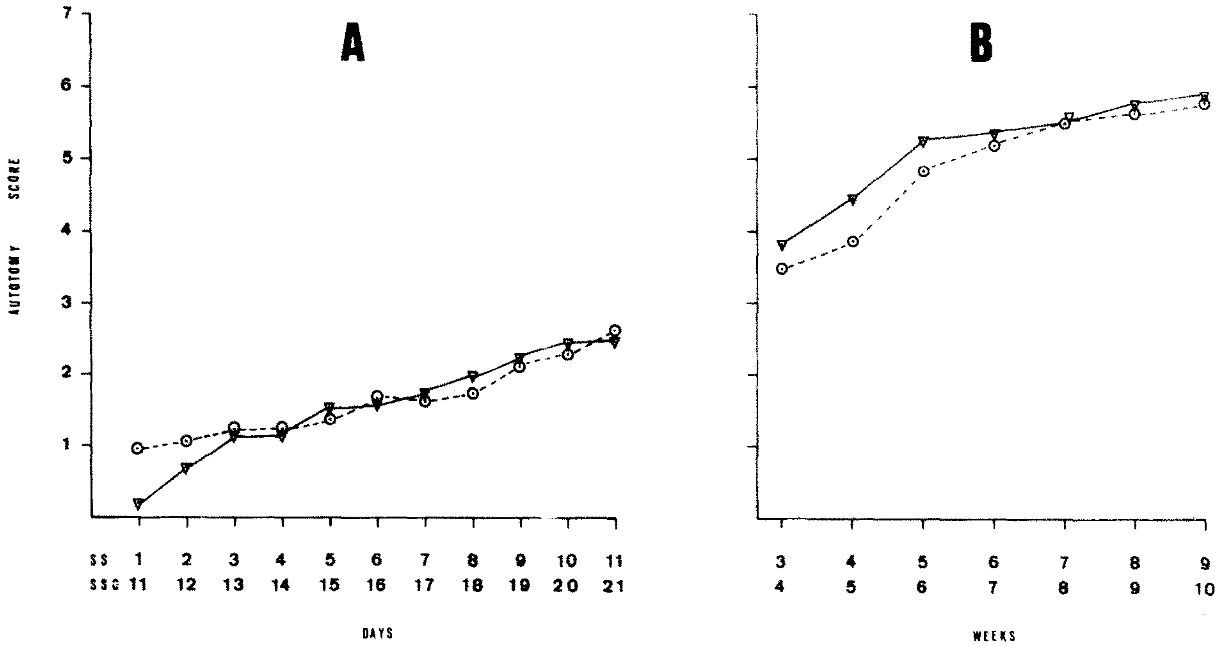

Fig. 2. This graph is based on data collected in Fig. 1. The first row of figures on the abscissa indicate, in A, the days and, in B, the weeks that correspond to the evolution of group SS. The lower row of figures indicates the days and weeks in the evolution of group SSG. In this way we can compare the evolution of the SSG group from its eleventh day with the evolution of the SS group from its first day. The graph shows that the evolution of autotomy in both groups is almost identical when this difference of 10 days is taken into account. Triangles = SS group; circles = SSG group.

SSG group. The most intense reaction was produced when pressure was executed on the proximal end of the failed suture in the SSS group.

Discussion

At the present time there are 2 explanations regarding the self-mutilation appearing in some animal species as a consequence of sciatic nerve section. The first of these is that autotomy is considered as animal behaviour directed to eliminate the denervated limb which has become useless [8,12]. The second, which is more generally accepted, is that self-mutilation is attributed to an abnormal feeling of disaesthetic character with painful connotations, due to neuronal modifications after the nerve section [3,5,10,13,14,17,18]. In this second case, the initial theory of Wall placed the origin of the unpleasant sensation in the spontaneous abnormal activity of the nerve fibres growing at the neuroma [14–17]. Nevertheless, this interpretation has recently been modified, to adapt it to the experimental findings obtained from the sequential or simultaneous application of neurotoxic drugs and nerve section. New ideas suggest that the autotomy following sciatic section is produced as a response to the hyperactivity of the deafferented posterior horn neurones, thus questioning the role of the neuroma [3,7,9].

Within this context, we must now consider 2 facts. Firstly, we observed significant differences in autophagic behaviour in animals with the same denervated area. This finding contradicts the theory which interprets self-mutilation as conduct directed exclusively to eliminate the insensitive and useless limb. If the latter were the cause of autotomy, any difference in conduct would be in the onset or in the evolution of the self-mutilation of the limb in which the anesthesia and the inutility are the same in all groups of animals. Therefore, we believe that autotomy is provoked chiefly by active sensorial stimuli and that self-mutilation does not reflect single systematic behaviour of the animal with an insensitive limb.

Secondly, we think that the stimuli provoking autotomy are related to the nature of the neuroma. Wall et al. [14] observed that the sciatic nerve ligation before the section produced an autotomy in the rat which starts later and follows a less intense evolution than that which follows after nerve section and encapsulated proximal nerve stump. These differences were attributed to the structural and histological differences in the neuromas. For the same reasons we explain the premature and intense autotomy shown by our control group in which the sciatic nerve was not ligated before or after the section. The free axonal sprouts should be exposed to an abnormal chemical excitation related to the strange tissular area where they grow. Also, the mechanical excitement of these axonal tips would be great because they grow in direct contact with active adjacent muscles, whose contractions mobilize them, thus exciting their intense mechanosensitivity [1,2,4,5,10,18]. In the same way, the extension of the proximal stump of a sectioned nerve, by means of a free nerve graft, should delay the formation of a typical terminal neuroma. In this case the majority of regenerative axons grow into tissue favourable to regeneration and, even though they should conserve their spontaneous activity, they are temporarily less exposed to chemical and mechanical stimuli [11]. The delayed onset of autotomy in this group and its slow evolution in the initial phases could thus be explained.

In group SSS, after the end-to-end suture of the sectioned nerve, many regenerative axons penetrate into the distal end where they also find a favourable tissular area for growth. But in this case, the suture is probably submitted to the traction produced by the dragging weight of the paralysed limb when the animal moves. Traction increases the mechanical stimuli and also the fibrous tissue at the suture which hinders the normal growth of the regenerative axons, provoking the escape of many of them and producing a lateral neuroma [11]. Consequently, in these animals it should be relatively easy to arrive at the critical number of abnormal impulses to start autotomy. After 3 weeks the suture is strengthened and the traction effect is weakened, and the regenerative fibres remain definitively free of their mechanical stimulation. Therefore, the autotomy decreases until it disappears although the recovery of sensitivity of the limb is possibly not complete. This hypothesis has been confirmed in the 5 animals in which the end-to-end suture failed because of excessive traction with a remaining fibrous tract between both stumps. In these cases the mechanical factor and the histological fibrous reaction at the proximal end should be so intense that they create the conditions for the most active self-mutilation observed.

In the SSG group, the distal end of the graft is free and, therefore, the suture will not be submitted to traction from the distal extremity, as occurs in the SSS group.

We have no electrophysiological proof of this speculative interpretation, but the results obtained from the pressure on some parts of the nerves, on re-operation, seem to confirm it.

The rhythm of the automutilation with sudden and sporadic attacks, as described by Coderre et al. [3], is in accordance with the irregular and accidental mechanical stimuli to which we attributed the autotomy in our different groups.

In conclusion, our findings lead us to believe that self-mutilation is a behaviour chiefly generated in the neuroma. The existence and characteristics of the neuroma as a source of centripetal abnormal impulses were demonstrated more than 10 years ago [15,16].

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