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## A simple method for reducing autotomy in rats after peripheral nerve lesions

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Experiments using peripheral nerve lesions (crush or transection) in rats to study repair processes are hampered by the tendency for the animals to attack the limb in which the peripheral nerves are damaged (autotomy). In this paper we describe a simple method which significantly reduces the incidence of autotomy after peripheral nerve lesions. The method consists of painting the hind paws of operated rats with a commercially available non-toxic lotion, which is used to discourage nail-biting and thumb-sucking in humans. Although the method is not absolute, it was extremely beneficial in our experiments, since the number of animals that had to be taken out of the experiment due to severe autotomy was greatly reduced. We believe that this method may prove to be as beneficial to other investigators who are using experimental peripheral nerve lesions to study the regenerative aspects of the nervous system.

### Introduction

Experimental peripheral nerve lesions (crush or transection) in rats provide a very useful model in which repair processes can be studied extensively. One of the major disadvantages of this experimental model is the tendency of the animals to attack the limb in which the peripheral nerves have been damaged. This phenomenon was described by Wall et al., 1979 and called "autotomy".

Autotomy starts with nibbling of toe nails and then extends progressively to the toes and feet. Subsequently infection and oedema may start in the attacked regions. Therefore, in most cases

where autotomy occurs, the hindlimb can no longer serve as the site at which nerve repair can be studied. This is because most methods used for studying the recovery process, such as sensory and motor recovery by means of foot flick (De Koning et al., 1986), walking pattern (De Medinacelli et al., 1982) and electrophysiological methods for the estimation of nerve conduction velocities (De Koning and Gispen, 1987), all depend on the presence of an intact hind paw.

We have encountered this problem in a preliminary experiment in which the recovery of tibial nerve was studied after an autograft. In this experiment a number of rats had to be killed because the hind paws were in extremely bad condition as a result of advanced autotomy.

In this paper we describe a simple method which significantly reduces the incidence of autotomy after a peripheral nerve lesion. The method consists of painting of the hind paws of operated

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animals with a non-toxic lotion, which is commercially available for human use to discourage nail-biting and thumb-sucking. Treatment was initiated immediately after experimental lesions were performed and repeated daily or every other day until recovery was complete.

## Materials and methods

Experiments were carried out on male Wistar albino rats. Surgery was carried out under aseptic conditions. The rats were anaesthetized with 30 mg/kg sodium pentothal i.p. All animals were housed in plastic cages on deep sawdust litter to avoid accidental damage to the hind paws.

In the preliminary experiment which was used to study the recovery of the tibial nerve after an autograft, a 5-mm portion from the left tibial nerve was removed and grafted on to the right tibial nerve. Peroneal and the sural nerves were removed from both sides. None of the animals were painted with the lotion at any time during the whole period of the experiment.

In the subsequent experiment, the surgical procedure was as above, but the hind paws of animals were painted with the lotion (Nail Bite: active ingredient Bitrex, i.e. benzyldiethyl[2,6-xylyl-carbamoylethyl] ammonium benzoate; J. Pickles and Sons, U.K.) every other day starting on the day of the surgery.

In both of the experiments the animals were followed until the sensory recovery on the grafted side was complete as assessed by the foot flick test (De Koning et al., 1986). The number of animals that had to be sacrificed because of severe autotomy was recorded.

A third experiment to assess systematically the effectiveness of this non-toxic lotion in the prevention of autotomy was carried out. Sciatic nerves on both sides were transected and a 3 mm portion was removed, to prevent regeneration. In one group the hind paws were painted every day with the lotion (Byte-X: active ingredient Bitrex; Byte-X Copr., New York, NY, U.S.A.) starting on the day of the surgery. The other group received no lotion. The animals were examined 5 days per

TABLE I

### INCIDENCE OF SEVERE AUTOTOMY IN RATS FROM TWO TIBIAL NERVE GRAFT EXPERIMENTS

In the 2nd experiment the hind feet of the rats were painted with Nail Bite from the day of surgery until all rats had recovered normal sensitivity to a noxious stimulus applied to the foot.

| Treatment           | No. rats | No. affected | % affected |
|---------------------|----------|--------------|------------|
| None                | 11       | 3            | 27         |
| Painted with lotion | 32       | 2            | 6          |

week, and the number of animals in which there were signs of toe-nibbling was recorded.

## Results

In the original graft experiment, in which no precautions were taken, autotomy severe enough to necessitate removal of the rat from the study had occurred in 3 animals out of 11 by 60 days after graft surgery, which is the first day on which all animals were responsive to the minimum stimulus (100 mA) with the foot-flick apparatus. In the second graft experiment, the hind paws were regularly painted with Nail Bite and only 2 out of a total of 32 animals had severe autotomy on the day on which all animals responded to the minimum stimulus (100 mA). The difference be-

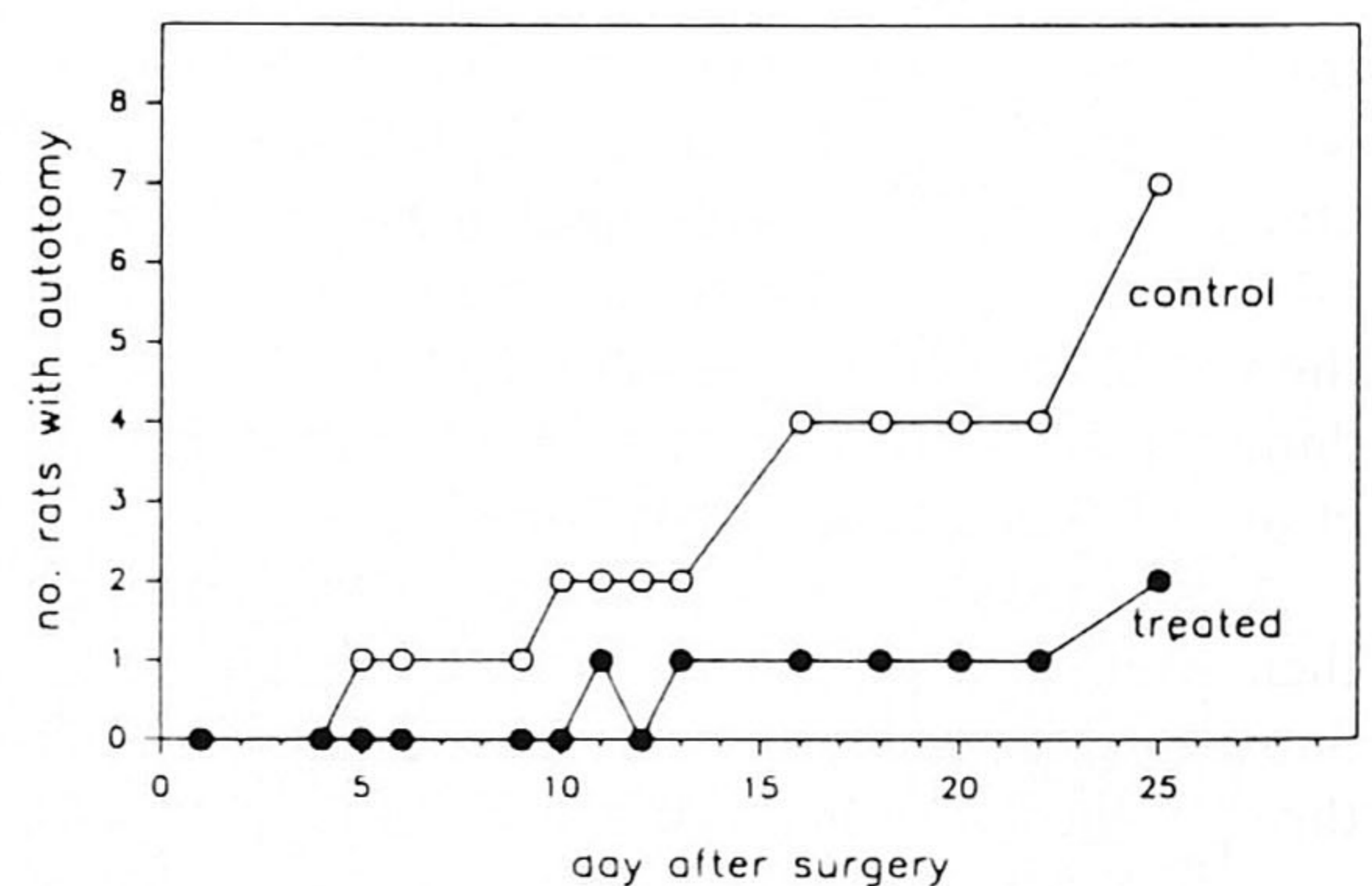


Fig. 1. Effect of Byte-X on the incidence of autotomy in rats after sciatic nerve transection. The hind paws of one group of 10 rats were painted every day with Byte-X from the time of sciatic nerve transection until the end of the experiment. The control group of 10 rats were untreated after surgery. All rats were examined 5 days a week for evidence of autotomy.

tween the painted and non-painted experiments (Table I) is significant ( $0.01 < P < 0.025$ ;  $\chi^2$  test).

In the second experiment the hind feet of the rats were painted with Nail Bite from the day of surgery until all rats had recovered normal sensitivity to a noxious stimulus applied to the foot.

In the third experiment, in which the sciatic nerve was transected, the incidence of some degree of autotomy was markedly lower in the group painted with the lotion Byte-X (Fig. 1). By the end of the experiment (25 days after surgery) some degree of autotomy was observed in 2/10 animals painted daily with the lotion and in 7/10 animals in the non-painted group. The difference between the painted and non-painted groups was statistically significant ( $0.01 < P < 0.025$ ;  $\chi^2$  test).

## Discussion

We were able to show that regular painting with commercially available non-toxic lotions, usually used to discourage nail-biting and thumb-sucking in humans, reduces the number of animals with autotomy after a peripheral nerve lesion. The reduction in the number of autotomic animals by the lotion improves the experimental model, by reducing the number of animals which have to be removed from the study owing to autotomy. While these studies were in progress, it came to our notice that other workers have used similar preparations (McDevitt et al., 1987) but the method is not generally known and no studies demonstrating systematically its effectiveness including that of McDevitt et al. (1987) have, to our knowledge, been published.

We observed that the prevention of autotomy using this method is not absolute. Although the

large decrease in the incidence suggests that the lotions taste unpleasant to rats, as they do to humans, occurrence of some degree of autotomy in the regularly painted groups suggests that autotomy does not simply result from the lack of sensation in the denervated hind limbs. Wall et al. (1979) suggested that pathological processes initiated by peripheral axon damage result in abnormal impulse discharges and may cause unusual, unpleasant and even painful sensations referred to the anaesthetic area. Thus the rats will attack the perceived source of discomfort in spite of an unpleasant taste.

Nonetheless, the method was extremely beneficial in our experiments and we believe that it may prove to be useful to other investigators who are using experimental peripheral nerve lesions to study the regenerative aspects of the nervous system.

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