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Effects of repeated nerve injuries at different time intervals on functional recovery and nerve innervation

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ABSTRACT

Effects of repeated nerve injuries on functional recovery and nerve innervation were examined in rodents. Crush injuries of the sciatic nerve were inflicted on adult rats and repeated twice or thrice at different time intervals of 1, 2, 3, and 4 weeks. Motor function was assessed by the static sciatic index at 1, 7, 14, 21, 28, 35, 42, 49, and 56 days after the final crush. The rates of nerve innervation of the tibialis anterior muscle, a main muscle innervated by the common peroneal nerve, were evaluated by the quantification of βIII-tubulin-positive nerve terminals and α-bungarotoxin-positive acetylcholine receptors 21 and 56 days after the final crush of triple nerve injuries at 1-, 2-, 3-, and 4-week intervals. Compared with single nerve crush injury, delayed recovery of motor function was observed in repeated crush injuries. In addition, recoveries in the triple crush groups were slower than those in the double crush groups. The rates of reinnervation were lower in the triple crush groups than in the single crush groups, both at 21 days (single: 59.7%; triple: 54.1%–56.1%) and 56 days (single: 88.8%; triple: 72.5%–83.0%) after the final crush, except in the groups with 1-week (triple: 73.8%) and 2-week (triple: 70.5%) intervals at 21 days after the final crush. We concluded that the recovery of motor function was delayed according to the number of repetitions of crush injuries, and that the rates of nerve innervation were still low in the triple crush groups 8 weeks after the final crush.

1. Introduction

There are a few reports regarding effects of repeated crush injuries on the sciatic nerve of rodents. Our previous report [1] showed that the recovery of motor function was delayed after repeated (2 and 3 times at 1-week intervals) crush injuries in rats. Recently, Sakuma et al. [2] reported that there was a gradual failure in the recovery of motor function after 3, 4, and 5 crushes at 1-week intervals in mice. However, to our knowledge, there is no literature describing effects of repeated nerve crush injuries at different time intervals on the recovery of motor function, although the time intervals between repeated injuries are diverse in the clinical cases.

After a single nerve crush of the sciatic nerve, regenerating axons begin to reach the tibialis anterior muscle, a main muscle innervated by the common peroneal nerve, between 2 and 3 weeks after the injury and almost reach the muscle by 4 weeks after the injury [1]. Therefore, in case of repeated nerve crush injuries at 1- and 2-week intervals, a second injury is supposed to be inflicted on the sciatic nerve whose regenerating axons are extending toward the target muscle. In the same sense, a second injury is supposed to be inflicted on the nerve whose regenerating axons are consisted of various stages of axons that have reached or are extending toward the muscle at 3-week intervals, while a second injury is supposed to be inflicted on the nerve whose regenerating axons have mostly reached the muscle at 4-week intervals.

Because the time interval between repeated injuries is an essential factor for the recovery of motor function in addition to the number of injuries, we examined in detail the effects of repeated nerve crush injuries on functional recovery at different numbers of repetitions (2 and 3 times) and at different time intervals (1-, 2-, 3-, and 4-week intervals). Motor function of the lower limb was assessed by the static sciatic index (SSI) for sciatic function. In addition, we evaluated muscle fiber reinnervation by immunohistochemically visualized βIII-tubulin, an axonal marker, and histochemically visualized α-bungarotoxin, an acetylcholine receptor antagonist.

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2. Materials and methods

2.1. Animals

Adult female Wistar rats (180–220 g body weight; Japan SLC Inc., Hamamatsu, Japan) were used for this study. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and protocols were approved by our Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and pain. Surgical procedures were performed under general anesthesia induced with an intraperitoneal injection of a mixture of pentobarbital (50 mg/kg) and medetomidine (10 mg/kg). To reverse the effect of anesthesia, atipamezole hydrochloride (2 mg/kg) was intraperitoneally injected.

2.2. Crushing of the sciatic nerve and classification of rats

The left sciatic nerve was exposed by a gluteal muscle-splitting incision. The nerve was completely crushed with a brain aneurysm clip (Sugita standard aneurysm clip; holding force, 147 g; Mizuho Ikakogyo Co. Ltd., Tokyo, Japan) for 180 s [3,4]. The animals were divided into 9 groups (Fig. 1). Rats in group 1c (single nerve crush, $n = 24$), i.e., the control group, received 1 nerve crush at the level of the greater trochanter (GT). Rats in groups 1w2c ($n = 6$), 2w2c ($n = 6$), 3w2c ($n = 6$), and 4w2c ($n = 6$), i.e., the double crush groups, received the first crush at a position 10 mm distal to GT and second crush at the level of GT at 1-, 2-, 3-, and 4-week intervals, respectively. Rats in groups 1w3c ($n = 18$), 2w3c ($n = 15$), 3w3c ($n = 16$), and 4w3c ($n = 18$), i.e., the triple crush groups, received the first crush at a position 20 mm distal to GT, second crush at a position 10 mm distal to GT, and third crush at the level of GT at 1-, 2-, 3-, and 4-week intervals, respectively. In all the groups and cases, the final crush was designed to be performed at the level of GT. After the surgery, the rats were housed under standard laboratory conditions with a 12-h light/dark cycle and a room temperature maintained of 22 °C. Food and water were supplied *ad libitum*.

2.3. Functional assessment by SSI

We performed a conventional footprint analysis according to previous studies [1,3,5–7] to assess functional loss after the sciatic nerve crush. In brief, footprints of all the groups were obtained at 1, 7, 14, 21, 28, 35, 42, 49, and 56 days after the final crush (1c, $n = 13$; 1w2c, $n = 6$; 2w2c, $n = 6$; 3w2c, $n = 6$; 4w2c, $n = 6$; 1w3c, $n = 7$; 2w3c, $n = 6$; 3w3c, $n = 7$; 4w3c, $n = 7$) (Fig. 1). Hind footprints were obtained by applying quick-drying Indian ink to the hind feet and allowing the animals to walk freely in a box (70.0 × 33.5 × 22.0 cm); thus, tracks were left on the underlying paper. Two parameters, 1–5 toe spread and 2–4 toe spread lengths of both feet, were manually measured with a ruler, and SSI was calculated according to the formula defined by Bervar [5]. When footprints could not be obtained because of very severe motor dysfunction, the SSI score was considered as −100.0. SSI scores were calculated for each animal from 15 sets of footprints, and the trimmed mean (i.e., the average with the top 3 and the bottom 3 scores excluded) was considered the best estimate for each animal.

2.4. Tissue preparation

At 21 days after the single (1c, $n = 11$) or third nerve crush (1w3c, $n = 11$; 2w3c, $n = 9$; 3w3c, $n = 9$; 4w3c, $n = 11$) and at 56

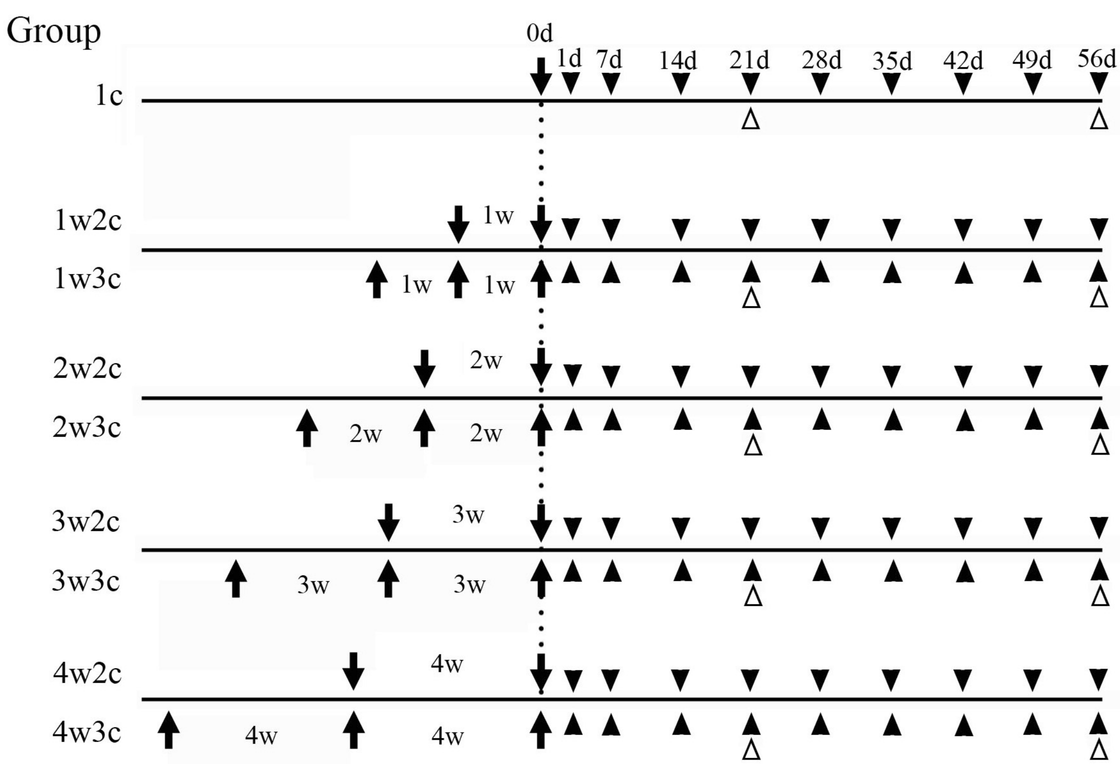

Fig. 1. Schematic drawing showing the experimental paradigm (1c: single nerve crush; 1w2c: double nerve crush with a 1-week interval; 1w3c: triple nerve crush with 1-week intervals; 2w2c: double nerve crush with a 2-week interval; 2w3c: triple nerve crush with 2-week intervals; 3w2c: double nerve crush with a 3-week interval; 3w3c: triple nerve crush with 3-week intervals; 4w2c: double nerve crush with a 4-week interval; 4w3c: triple nerve crush with 4-week intervals). Arrows indicate the time of nerve crush and the dotted line indicates the time of the final crush. Black and white arrowheads indicate the times of functional assessment and histological examination of the tibialis anterior muscle, respectively.

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