Effects of multiple nerve injuries on functional recovery of the sciatic nerve

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Abbreviations: AchRs, acetylcholine receptors; GT, greater trochanter; SEM, standard error of the mean; SSI, static sciatic index

Abstract

  The effects of repeated crush injuries of peripheral nerve remains in poorly understood. In this study, the rats were divided on five groups (control, single crush, double crush on once a week, triple crush on once a week, triple crush on every four weeks) and analyzed to investigate the influence on motor function by repeated nerve crush injuries. Additionally, the muscle fiber diameter and reinnervation of the tibialis anterior muscle were measured and comparisons were made with two groups, single crush group and triple crush group. The five groups had their respective pattern of dysfunction and restoration. The motor function in four groups except the group of triple crush on once a week eventually recovered to normal range. The group of triple crush on once a week was not obtained the full restoration of the motor function. The motor function of double and triple crush groups on once a week were significantly low in comparison with control group and it took longer to obtain the full restoration. In analysis of the muscle, the width of muscle fiber in single crush group was clearly wider and the muscle fiber reinnervation in single crush group was significantly faster than in triple crush group.

  In the rats with repeated sciatic nerve crush injuries at short intervals before recovering motor function, the function could not recover completely because of delay of the reinnervation and the atrophy of the muscles.

Keyword: Sciatic nerve; Motor function; Repeated nerve crush; Foot print; Reinnervation; Adult rat.

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Introduction

The crush of the sciatic nerve is commonly used experimental model in peripheral nerve research and it is generally agreed that recuperation after peripheral nerve crush is observed between the 16th and 25th days (Guth, 1956; Berenberg et al., 1977; Hasegawa, 1978; Devor et al., 1979; de Medinaceli et al., 1982). The axonal regeneration occurs approximately 1-2mm per day after the crush and finally the motor function fully recovers from the injury (Sunderland, 1991; Hadlock et al., 2005), whereas nerve section or resection is not obtain full recovery of motor function (Ducker et al., 1969; Ballantyne and Campbell, 1973). The study for the sciatic nerve injury by single crush is performed enough, but there are few reports about repeated crush injuries of sciatic nerve. Thus, it is unclear how the motor function is restored by repeated crush injuries of sciatic nerve.

The present study was undertaken to investigate the influence on motor function, thickness of the muscle fiber, and muscle fiber reinnervation by repeated sciatic nerve crush of adult rats. The motor function of the lower limb was assessed by the static sciatic index (SSI) for sciatic function (Bervar, 2000). In addition, we measured a diameter of the muscular fiber, and assessed a quantification of muscle fiber reinnervation by immunohistochemistry using synaptophysin, a transmembrane protein in synaptic vesicles, and α-bungarotoxin, acetylcholine receptor antagonist (Rupert and John, 1999). They have been widely used for detecting the components of neuromuscular junction, nerve terminals and acetylcholine receptors (AchRs) (Gould et al., 1987; Chang, 1999). We evaluated nervous regeneration by these investigations.

Experimental Procedures

Animals

The experiments were carried out on adult female Wistar 124 rats (220-260g body

weight: Japan SLC Inc., Hamamatsu, Shizuoka, Japan). All surgical manipulations were performed under general anesthesia. Rats were anesthetized by an intraperitoneal injection of a mixture of ketamine hydrochloride (50mg/kg) and medetomidine (10mg/kg). To reverse the anesthesia, atipamezole hydrochloride (2mg/kg, intraperitoneal) was injected. All procedures were conducted in adherence to 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. Every effort was made to minimize animal suffering and pain, as well as the number of animals used necessary to produce reliable scientific data.

Crush on the sciatic nerve and classification of the rats

Under general anesthesia, the unilateral left sciatic nerve of the rats was exposed just behind the greater trochanter (GT). The nerve expose to distal was performed by width of 15mm (Figure1). One time nerve crush was carried out for 180 seconds with fine forceps. The animals were divided into five groups (Figure 2). Rats in A group (n=5), consisted of surgical control group, received only neurolysis not to damage the nerve. Rats in B group (n=52) received one crush at a position of GT level (single nerve crush). Rats in C group (n=6), received the first crush at a position of 7.5mm distal of GT level, and the second crush at a position of GT level 1 week later (double nerve crush). Rats in D group (n=55) and E (n=6) groups received the first crush at a position of 15mm distal of GT level, the second crush at a position of 7.5mm distal of GT level and the third crush at a position of GT level at 1 week (D group) or 4 weeks (E group) intervals (triple nerve crush). In the nerve-crushed groups, the final crush was designed to perform at a position of GT level of each animal. After surgical procedures, the animals were kept 3-4 rats per cage ($26 \times 42 \times 18$ cm^3) under standard laboratory conditions with a 12-h light/dark cycle, and room temperature was maintained at 22°C.

They could move freely in the cage, and food and water were supplied ad libitum.

Functional assessment by static sciatic index (SSI)

To assess functional loss following crush to the sciatic nerve, we performed a conventional footprint analysis in this study according to previous publications (Bervar, 2000; Varejão et al., 2001a, b). Footprints were taken every week until 8 weeks after the last crush for all groups (A group, n=5; B group, n=5; C group, n=6; D group, n=8; E group, n=6) (Figure 2). Hind footprints were obtained by applying a quick-drying Indian ink to both hind feet and allowing the animal to walk freely in a dark box ($55\times40\times22cm^3$) with one side opened as an entry gate; thus tracks were left on the underlying paper. The two parameters of 1-5 toe spread and 2-4 toe spread lengths on both sides were measured manually by a ruler from three footprints, and their average values were obtained. On the basis of these values, the SSI was calculated in each animal from the control and nerve-crushed rats according to the formula defined by Bervar. When footprints were not obtained because of very severe motor dysfunction in the nerve-crushed rats, the SSI scores of the animals were regarded as -100.0.

Tissue preparation

At 2, 3 and 4 weeks after the last crush, rats in B (2 weeks, n=14; 3 weeks, n=16; 4 weeks, n=18) and D (2 weeks, n=14; 3 weeks, n=16; 4 weeks, n=16) groups were deeply anesthetized with sodium pentobarbital (80 mg/kg, intraperitoneal) and perfused through the heart with 400 ml of 4% paraformaldehyde in 0.1M phosphate buffer (Figure 2). Tibialis anterior muscles of the crush-injured side were removed, postfixed overnight in the same fixative and soaked in 30% phosphate-buffered sucrose for 2 days. Longitudinal sections of the tibialis anterior muscle were cut serially into 50 μm slices on a freezing microtome, and collected at 200 μm intervals. As the control,

rats in A group (n=5) were perfused with a fixative after the last assessment of the sciatic motor function by SSI, followed by removal and preparation of the 50 μm-thick frozen section of the tibialis anterior muscle in the same manner (Figure 2).

Measurement of muscle fiber diameter

Longitudinal sections of the tibialis anterior muscles were mounted on coated slides (Matsunami, Osaka, Japan) and stained with hematoxylin-eosin. The diameters of 50 muscle fibers in the thickest part near the middle of the muscle were measured using BIOREVO microscope BZ-9000 (Keyence, Osaka, Japan). The average of the muscle fiber diameters were calculated for each animal and group.

Neuromuscular junctions

Longitudinal sections of the tibialis anterior muscle were stained on a shaker at room temperature. The sections were immunostained for nerve terminals using anti-synaptophysin antibody and stained for acetylcholine receptors (AchRs) using α-bungarotoxin. The sections were immersed in 0.3% H_2O_2 for 30 min to suppress endogenous peroxidase activity, and for 2h in phosphate-buffered saline containing 0.3% Triton X-100. They were then incubated overnight with rabbit polyclonal anti-synaptophysin antibody (1:200; Zymed Laboratories, South San Francisco, CA) or biotin-conjugated α-bungarotoxin (1:200; Molecular Probes, Eugene, OR). For synaptophysin immunostaining, they were incubated with biotinylated anti-rabbit immunogloblins (1:500; Dako, Glostrup, Denmark) for 2h. After washing, they incubated with streptavidin-peroxidase (1:500; Dako) for 2h, and the peroxidase reaction product was visualized with the Metal Enhanced DAB Substrate Kit (Thermo Fisher Scientific Inc., Pierce Biotechnology, Rockford, IL, USA) (diaminobenzidine: 0.01%; H_2O_2: 0.0015%). The sections were rinsed, mounted on coated slides, air-dried,

dehydrated, and coverslipped with Entellan New (Merck, Darmstadt, Germany). To evaluate the neuromuscular junctions, the numbers of synaptophysin-positive nerve terminals and α-bungarotoxin-positive AchRs were counted in the serial sections. The most numerous five sections were selected and total numbers of synaptophysin-positive nerve terminals and α-bungarotoxin-positive AchRs were calculated for each animal. The ratios of the number of synaptophysin-positive nerve terminals to that of α-bungarotoxin-positive AchRs were used to determine the degree of reinnervation of the neuromuscular junctions. The average of the ratios was calculated for each group.

Statistical analysis

  Statistical analysis was carried out with SPSS software (version 8.0.1; SPSS Tokyo). Data were expressed as mean ± standard error of the mean (SEM), and the statistical significance of the means of the experimental groups was evaluated with the One-way factorial ANOVA with post-hoc test or paired t-test. P-values less than 0.05 were considered statistically significant.

Results

SSI scores

  Figure 3a shows the change of SSI scores in A (control), B (single nerve crush), C (double nerve crush), D (triple nerve crush) groups until 8 weeks after the last crush and figure 3b shows representative footprints. The control rats in A group showed no gait disturbance and the SSI scores were above -20, normal motor function, over an 8-week period. Their SSI scores ranged from -15.6 to -4.1. On the first week, all rasts in the nerve-crushed groups showed very severe motor dysfunction. Their SSI scores were below -80 (B group, -95.4±6.8; C group, -96.1±9.7; D group, -94.5±11.1), and

some rats could not put the sole on the floor and kept pulling the foot while walking on the injured side. In the nerve-crushed groups, the SSI scores gradually recovered until the fourth weeks although the recovery speeds were different, slower in multiple crush groups. The SSI scores in B group were -37.3±14.2, -24.4±15.9 and -20.7±8.3 at 2, 3 and 4 weeks after the last crush, those in C group were -58.9±16.1, -42.0±7.7 and -31.0±9.0 and those in D group were -95.3±10.5, -47.1±13.8 and -46.0±11.0. In the latter half of the experiments, the SSI scores in B, C and D groups were -25.8±7.9, -28.2±4.3 and -47.0±11.0 at 5 weeks, -17.6±6.1, -20.5±9.4 and -39.0±9.9 at 6 weeks, -12.4±4.6, -18.8±8.1 and -33.1±7.4 at 7 weeks, -8.0±8.9, -14.7±5.6 and -34.1±10.4 at 8 weeks. The SSI scores in B and C groups recovered to a normal range (above -20) on the fourth and sixth weeks, but those in D group did not recover throughout the experimental period. Statistically significant differences compared with A (control) group were found at 1 week ($p<0.01$), 2 and 3 weeks ($p<0.05$) and 5 weeks ($p<0.01$) in B group and between 1 and 5 weeks ($p<0.01$) in C group, whereas those siginificant differences were found through for 8 weeks ($p<0.01$) in D group.

Different intervals in triple nerve crush

Figure 4 shows the change of SSI scores in D (triple nerve crush at 1 week intervals) and E (triple nerve crush at 4 weeks intervals) groups until 8 weeks after the last crush. The SSI scores in D group from 1 to 8 weeks after the last crush were -94.9±11.1, -95.3±10.5, -47.1±13.7, -46.0±11.0, -47.0±11.0, -39.0±9.9, -33.1±7.4 and -34.1±10.4, and those in E group were -84.7±9.0, -75.8±10.3, -60.3±13.6, -48.2±9.9, -33.3±11.1, -31.3±11.0, -24.6±6.5 and -18.6±10.2. The SSI scores recovered to a normal range on the eighth weeks in E groups, but those in D group did not recover through for 8 weeks. Statistically significant differences between these two groups were found at 1, 2, 5, 7 and 8 weeks ($p<0.05$).

9

Effects of multiple nerve crush injuries

Muscle fiber diameter

  Figure 5 shows the microscopic image of the tibialis anterior muscle fiber in B (single nerve crush) and D (triple nerve crush) groups 4 weeks after the last crush. The width of the muscle fiber in B group were clearly wider than those in D group. The muscle fiber diameters of the tibialis anterior muscle in B group were 16.2±0.5 μm, 17.9±0.7 μm and 19.9±1.1 μm at 2,3 and 4 weeks after the last crush, and those in D group were14.7±1.0 μm, 16.9±0.6 μm and 16.8±0.6 μm (Figure 6).   Statistically significant differences between B and D groups were found in all weeks (p<0.01). In A (control) group, the muscle fiber diameter was 20.4±0.3 μm.

Muscle fiber reinnervation

  Figure 7 shows neuromuscular junctions of the tibialis anterior muscle in B (single nerve crush) and D (triple nerve crush) groups at 2, 3 and 4 weeks after the last crush. Regenerating axons were detected with synaptophysin (Figure 7a-f) and the motor endplates were labeled with α-bungarotoxin (Figure 7g-l). The numbers of synaptophysin-positive nerve terminals gradually increased both in B (Figure 7a-c) and D (Figure 7d-f) groups, in other words, the muscle had become reinnervated. The numbers of α-bungarotoxin-positive AchRs did not change in all weeks. To evaluate the degree of reinnervation, the ratios of the number of synaptophysin-positive nerve terminals to that of α-bungarotoxin-positive AchRs were calculated. The ratios in B group were 0.0±0.0%, 73.7±11.3% and 93.6±11.7% at 2,3 and 4 weeks after the last crush, and those in D group were 2.9±4.6%, 53.4±12.5% and 83.6±10.0% (Figure 8). Statistically significant differences between B and D groups were found at 3 weeks after the last crush (p<0.01). In A (control) group, the ratio was 91.8±3.4%.

Discussion

By our experiment, a motor function after single nerve crush became the normal within four weeks. The distance from greater trochanter to the tibialis anterior muscle was from 26.5 to 27.0mm in the rats which we used in this study. Therefore, the speed of the motor neuron recuperation after single nerve crush was approximately 1-2mm per day similar to the past reports (Sunderland, 1991; Hadlock et al., 2005). There are only a few reports about repeated crush injuries of peripheral nerve (Jenq and Coggeshall, 1987; Jenq et al., 1988), and no reports about functional assessment for repeated nerve crush. In the present study, we evaluated the repeated crush injuries of sciatic nerve compared to the single nerve crush.

Previous studies have shown that myelinated axons after triple nerve crush were much more increased than those after single nerve crush and nerve crush might be useful in conditions where it would be desirable to increase numbers of processes from surviving neurons, although they did not mentioned the association with the motor function (Jenq and Coggeshall, 1987; Jenq et al., 1988). In this study, we investigated the effect of repeated nerve crush on the motor function. With increased the number of nerve crush, motor dysfunction was severe through the recovery process. It seemed that the increase in number of myelinated axon by repeated nerve crush did not accord with the recovery of motor function. Much work needs to be done to evaluate the association between the number or/and the size of axon and motor function.

Additionally, a difference was found in functional recovery between triple crush on once a week and every four weeks. In this study, the crush injuries were performed from distal to proximal point of the sciatic nerve and the last crush points were unified at the position of GT level. When the last crush was performed in triple crush on every four weeks, it was thought that the first and second crush points (in other words scar points) of sciatic nerve had been already in healing condition and the axons had fully

11

regenerated. Therefore, it was thought that the regenerative axon extended to the muscles smoothly after the last crush. However, when the last crush was performed in triple crush on a once a week, it seemed that the first and second crush points of sciatic nerve remained injured and inhibited the axonal extension, and the regenerated axon did not reach the muscles in view of the axonal regenerative speed (Sunderland, 1991; Hadlock et al., 2005). Therefore it probably caused the delay and limitation of the functional recovery. Furthermore, as another cause, the joint contractures of lower extremity of the injured side might be caused because the rats in triple crush on once a week did not use a foot more than three weeks (Jenq et al., 1988).

We also performed assessment of muscle fiber diameter and reinnervation of the tibialis anterior muscle to support SSI score. In control rats, the muscle fiber diameter of tibialis anterior muscle was 20.4±0.3 μm, and the ratio of nerve terminals for AchRs was 91.8±3.4%. In single nerve-crushed rats, those at four weeks after the crush were 19.9±1.1 μm and 93.6±11.7% and there were no statistical significant differences comparison with control rats. In other words, muscle fiber diameter and reinnervation after single nerve crush also recovered to normal range at four weeks. However, the recovery of muscular diameter and reinnervation became slow after repeated nerve crush. In regard to the muscle diameter, it seemed that unused time had influence on the recovery of injured-side limb. Previous studies have shown that muscle atrophy, that is the decrease of muscular volume and muscle cell number, already occurs at the point of two weeks after injury by muscular denervation and immobilization (Cooper, 1972; Booth, 1978; Sakakima et al., 2004). About the delay of muscle reinnervation in repeated nerve crush, the cause is unclear although it might be related to the decrease of axonal regenerative speed or axonal transport. Regardless, these results seemed to be consistent with the fact that the recovery of motor function was late after repeated nerve crush.

There was one contradicting result between SSI score and nerve reinnervation. SSI scores presented with a tendency to recovery in two weeks after the last nerve crush, although muscle fiber reinnervation did not reproduce at this point. The SSI scores reflected all muscular functions of lower limbs (Bervar, 2000), but muscle fiber reinnervation was evaluated only in tibialis anterior muscle in this study. The distances from GT to the biceps muscle were from 15.0 to 15.5mm and clearly shorter than distance to tibialis anterior muscle (26.5-27.0mm). The SSI scores in two weeks after the last crush might reflect to the recovery of biceps muscle. Furthermore, the recovery of sensory nerve may be earlier than motor nerve (Guth, 1956; Berenberg et al., 1977). Additional works including histological evaluation of motor and sensory nerves may be needed to reveal the reason for the early recovery of motor function.

Conclusion

Multiple nerve crush injuries of sciatic nerve delayed muscle fiber recovery and nerve reinnervation, resulting in lower motor function.

Acknowledgements

References

Ballantyne JP, Campbell MJ (1973), Electrophysiological study after surgical repair of sectioned human peripheral nerves. J Neurol Neurosurg Psychiatry 36: 797-805.

Berenberg RA, Forman DS, Wood DK, DeSilva A, Demaree J (1977), Recovery of peripheral nerve function after axotomy: effect of triiodothyronine. Exp Neurol 57: 349-363.

Bervar M (2000), Video analysis of standing--an alternative footprint analysis to assess functional loss following injury to the rat sciatic nerve. J Neurosci Methods 30: 109-116.

Booth FW (1978), Regrowth of atrophied skeletal muscle in adult rats after ending immobilization. J Appl Physiol 44: 225-230.

Chang CC (1999), Looking back on the discovery of alpha-bungarotoxin. J Biomed Sci 6: 368-375.

Cooper RR (1972), Alterations during immobilization and regeneration of skeletal muscle in cats. J Bone Joint Surg Am 54: 919-953.

de Medinaceli L, Freed WJ, Wyatt RJ (1982), An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Exp Neurol 77: 634-643.

Devor M, Schonfeld D, Seltzer Z, Wall PD (1979), Two modes of cutaneous

reinnervation following peripheral nerve injury. J Comp Neurol 1: 211:220.

Ducker TB, Kempe LG, Hayes GJ (1969), The metabolic background for peripheral nerve surgery. J Neurosurg 36: 797-805.

Gould VE, Wiedenmann B, Lee I, Schwechheimer K, Dockhorn-Dworniczak B, Radosevich JA, Moll R, Franke WW (1987), Synaptophysin expression in neuroendocrine neoplasms as determined by immunocytochemistry. Am J Pathol 126: 243-257.

Guth L (1956), Regeneration in the mammalian peripheral nervous system. Physiol Rev 36: 441-478.

Hadlock TA, Heaton J, Cheney M, Mackinnon SE (2005), Functional recovery after facial and sciatic nerve crush injury in the rat. Arch Facial Plast Surg 7: 17-20.

Hasegawa K (1978), A new method of measuring functional recovery after crushing the peripheral nerves in unanesthetized and unrestrained rats. Experientia 15:272-273.

Jenq CB, Coggeshall RE (1987), Single vs multiple somatic nerve crushes in the rat. Brain Res 21: 250-258.

Jenq CB, Jenq LL, Bear HM (1988), Conditioning lesions of peripheral nerves change regenerated axon numbers. Brain Res 21: 63-69.

Rupert WD, John BH (1999), Nerve terminal damage by β-bungarotoxin. Its clinical

significance. Am J Pathol 154: 447-455.

Sakakima H, Yoshida Y, Sakae K, Morimoto N (2004), Different frequency treadmill running in immobilization-induced muscle atrophy and ankle joint contracture of rats. Scand J Med Sci Sports 14: 186-192.

Sunderland S (1991) Nerve Injuries and Their Repair. In: Axon regeneration and related changes, Churchill Livingstone, *pp* 91-113.

Varejão AS, Cabrita AM, Patrício JA, Bulas-Cruz J, Gabriel RC, Melo-Pinto P, Couto PA, Meek MF (2001a), Functional assessment of peripheral nerve recovery in the rat: gait kinematics. Microsurgery 21: 383-388.

Varejão AS, Meek MF, Ferreira AJ, Patrício JA, Cabrita AM (2001b), Functional evaluation of peripheral nerve regeneration in the rat: walking track analysis. J Neurosci Methods 15: 1-9.

Figure Legends

Figure 1 (a) Static nerve of left leg of an adult rat. (b) Extended image of the square in (a). An asterisk indicates the final crush point, GT level of the sciatic nerve. An arrow indicates the first (double crush group) or second (triple crush group) crush point, and an arrowhead indicates the first crush point in triple crush group.

Figure 2 Time-line diagram showing the experimental protocol used in A-E groups. Arrows indicates nerve crush times of B-E groups. Black arrow heads indicates functional assessment times of all groups. White arrow heads indicates measurement times of muscle fiber diameter and reinnervation of B and D groups.

Figure 3 (a) Comparison of the recovery determined by SSI in A (control), B (single nerve crush), C (double nerve crush) and D (triple nerve crush) groups. The graph shows function during the 8-week period in the experiments. SSI scores above the dotted line (-20) are considered normal function. Data are presented as mean ± SEM, and single and double asterisks show statistical significance compared with control (A) group (*p<0.05, **p<0.01). (b) Representative footprints of control (A), single nerve crush 1 week after the crush (B1W), single nerve crush 4 weeks after the crush (B4W) and triple nerve crush 4 weeks after the last crush (D4W) groups.

Figure 4 Comparison of the recovery among triple nerve-crushed groups determined by SSI in D (every week) and E (every four weeks) groups. The graph shows function during the 8-week period in the experiments. SSI scores above the dotted line (-20) are considered normal function. Data are presented as mean ± SEM. *p<0.05.

Figure 5 Hematoxylin and eosin-stained longitudinal-sections of the tibialis anterior

muscle 4 weeks after the last crush. (a) B group (single nerve crush). (b) D group (triple nerve crush). Double-headed arrows show widths of the muscle fiber.

Figure 6 Muscle fiber diameters of the tibialis anterior muscle in B (single nerve crush) and D (triple nerve crush) groups at 2, 3 and 4 weeks after the last crush. Data are presented as mean ± SEM. **$p<0.01$.

**Figure 7** Neuromuscular junctions of the tibialis anterior muscle in B (single nerve crush) and D (triple nerve crush) groups. (a-f) Nerve terminals stained with synaptophysin in B (a-c) and D (d-f) groups at 2 (a and d), 3 (b and e) and 4 (c and f) weeks after the last crush. Arrows indicate the synaptophysin-positive nerve terminals. (g-l) AchRs stained with α-bungarotoxin in B (g-i) and D (j-l) groups at 2 (g and j), 3 (h and k) and 4 (i and l) weeks after the last crush. Numbers of synaptophysin-positive nerve terminals gradually increase in both groups, whereas those of α-bungarotoxin-poisitive AchRs are almost the same in all weeks.

Figure 8 Ratio of synaptophysin-positive nerve terminals to α-bungarotoxin-positive AchRs of the tibialis anterior muscle in B (single nerve crush) and D (triple nerve crush) groups at 2, 3 and 4 weeks after the last crush. Data are presented as mean ± SEM. **$p<0.01$.
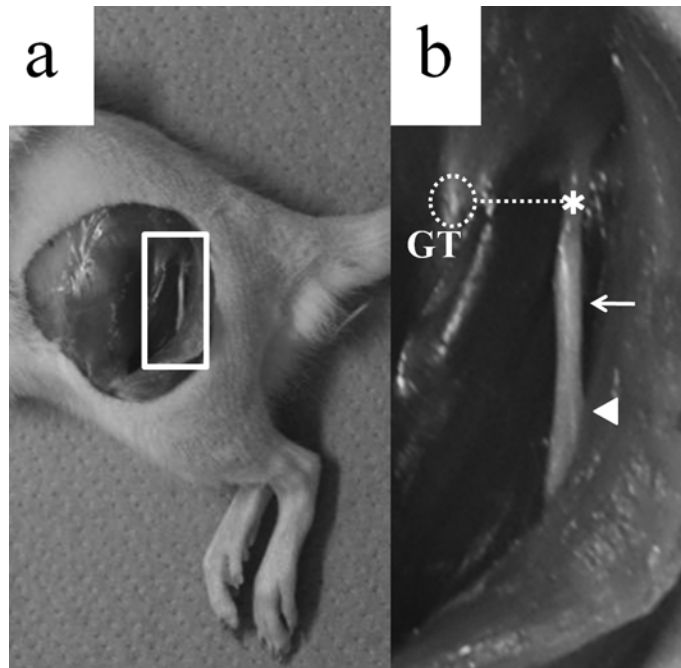
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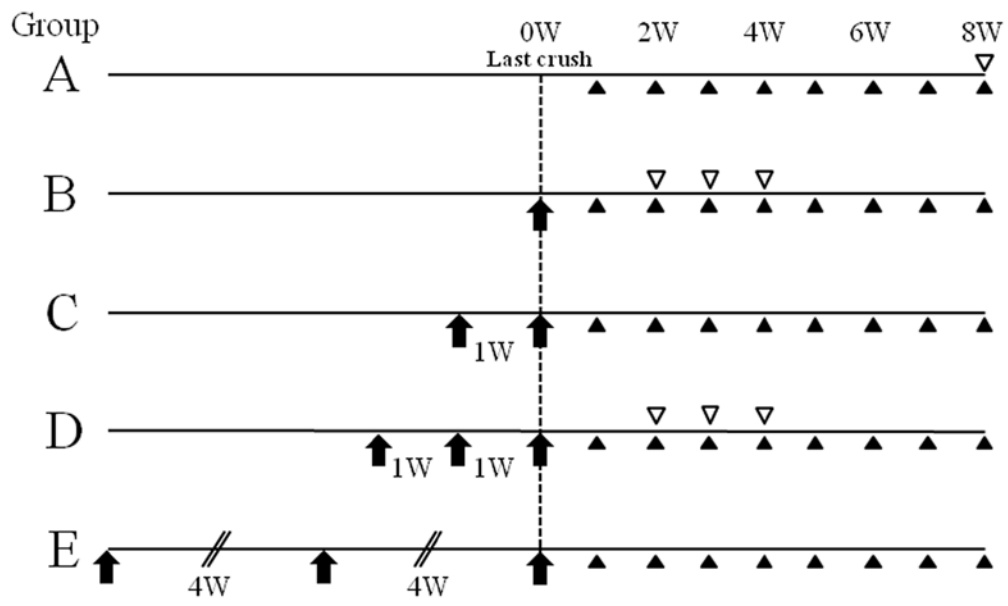
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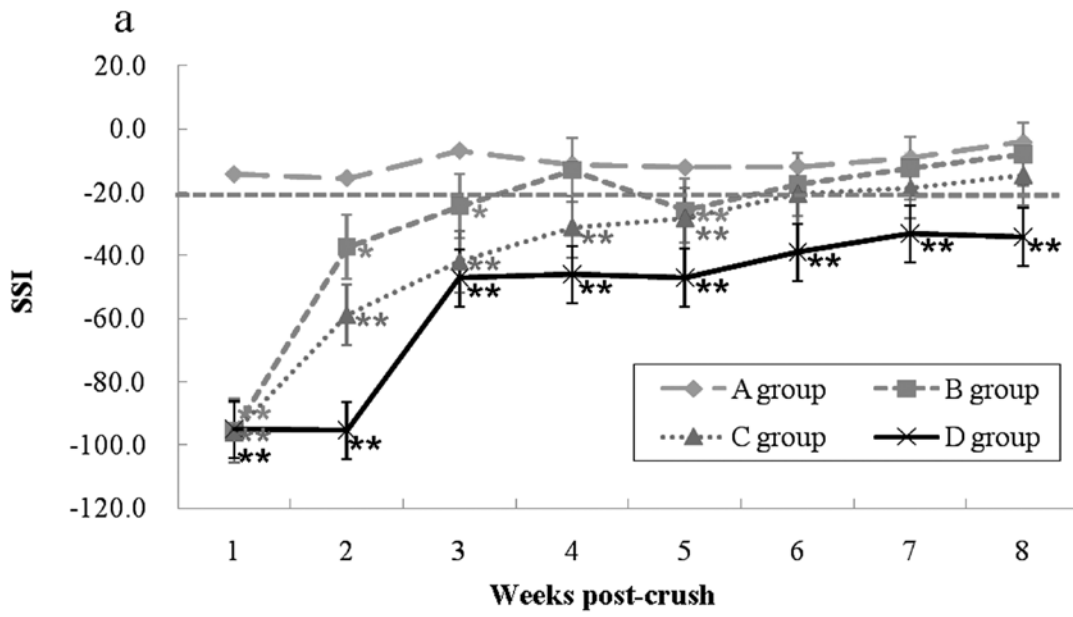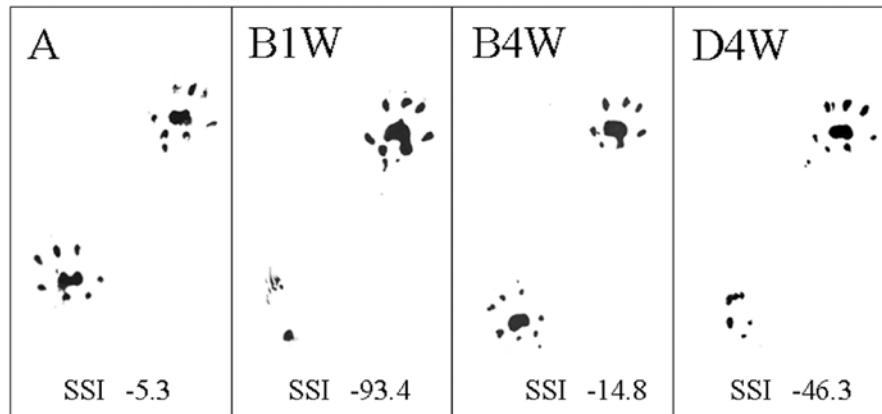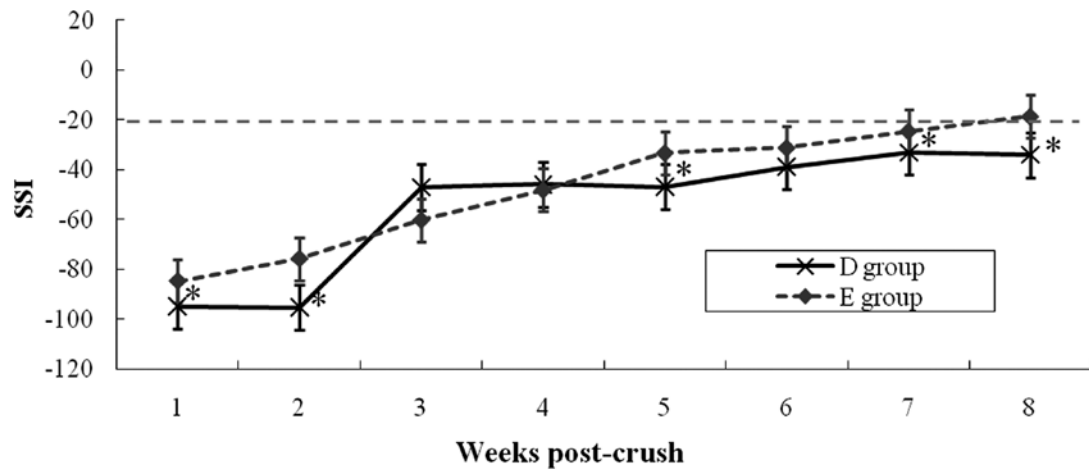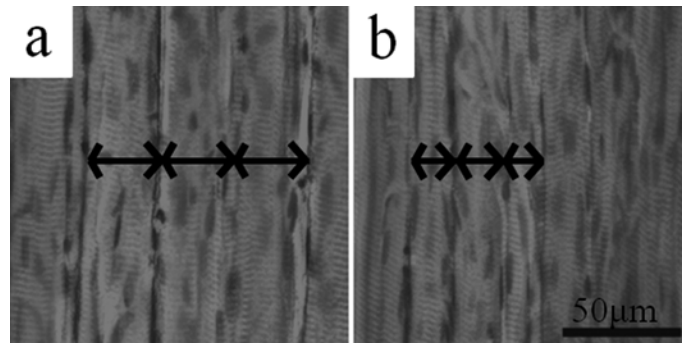
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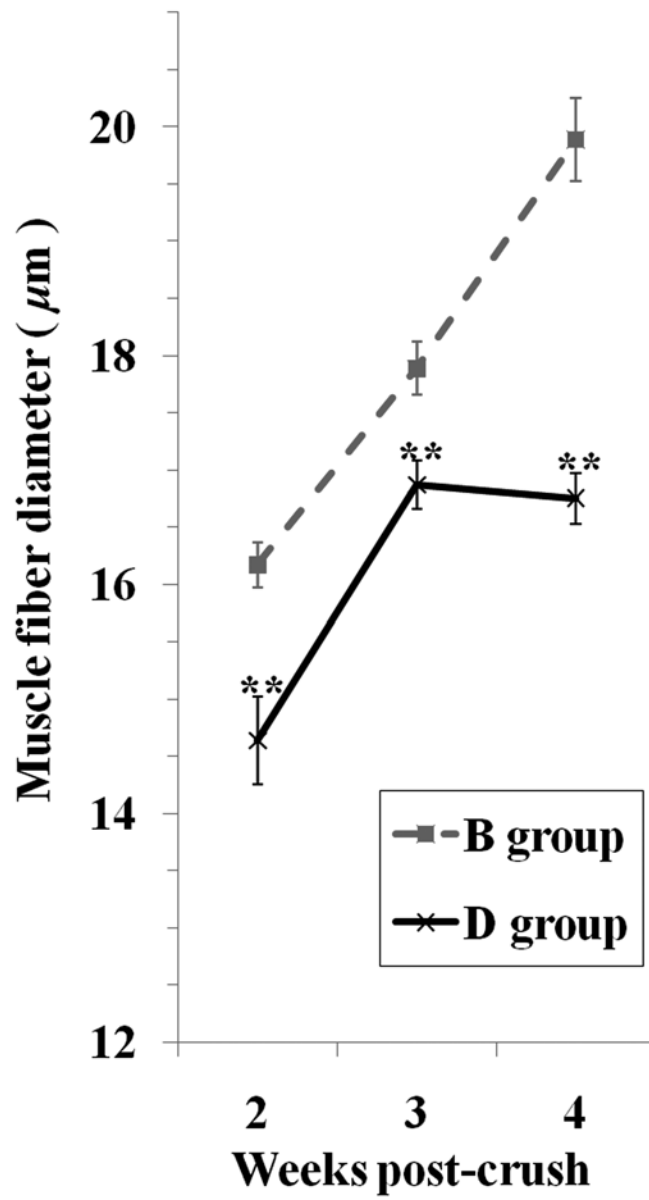
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