

## Original Article

# Quantitative Analysis of Spontaneous Tongue Muscle Reinnervation after Long Nerve Resection for Hypoglossal Nerve Injury in Rats

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## Abstract

**Objectives:** This study aimed to investigate spontaneous tongue muscle reinnervation after long nerve resection for severe hypoglossal (XII) nerve injury in adult rats. **Methods:** The right XII nerve was exposed, and a 9-mm section was resected. The tongue was removed and cut coronally at 4, 8, and 12 weeks after the XII nerve resection. The sections were processed for immunohistochemical staining for vesicular acetylcholine transporter (VAcHT) to detect the regenerated right XII and the uninjured left XII nerve terminals. The numbers of VAcHT-positive nerve terminals were counted on the injured right and uninjured left sides, and the reinnervation rate, which is the percentages of the number of VAcHT-positive nerve terminals on the injured side relative to that on the uninjured side, was calculated. **Results:** The injured XII nerve gradually regenerated in the tongue after the nerve resection. The reinnervation rates in nerve-resected rats were significantly lower than those in normal rats, with mean reinnervation rates of 13.9%, 37.9%, and 41.8% at 4, 8, and 12 weeks after nerve resection, respectively. **Conclusion:** Spontaneous reinnervation occurred in the tongue muscle after the XII nerve injury with long nerve resection and injured nerve terminals regenerated approximately 40% of the normal value; this rate was considerably lower than that with the nerve transection.

**Keywords:** Hypoglossal nerve, Long resection, Spontaneous regeneration, Spontaneous reinnervation, Tongue muscle

## Introduction

Peripheral nerves generally regenerate after nerve injury. However, nerve grafting or bridging may be needed to cause nerve regeneration in severe injuries such as nerve resection<sup>1-3</sup>. Some studies revealed a critical nerve-gap length to enable spontaneous neural regeneration and the occurrence of nerve regeneration at less than the critical length in the sciatic nerve injury model of rats<sup>4-6</sup>. Recently, we revealed a spontaneous neural regeneration

in the hypoglossal (XII) nerve injury model of rats after a relatively long nerve resection and an approximately 10.0 mm critical nerve-gap length for the regeneration in the XII nerve-resected rats<sup>7,8</sup>. In these studies, we counted the regenerated XII neurons in the XII nucleus retrogradely labeled by tracer injections. The XII nerve exclusively provides motor innervation to the tongue muscle, so the XII nerve injury model is an informative model for craniofacial neuromuscular interactions. By expanding the subjects from neuronal regeneration to motor axon terminal regeneration, we thought that neuromuscular function could be shown more directly. Accordingly, we aimed to determine the actual amount of regenerated nerve terminals in the tongue muscles after the XII nerve resection. We employed immunohistochemical procedures with anti-vesicular acetylcholine transporter (VAcHT), which is a neurotransmitter transporter localized in small synaptic vesicles in cholinergic nerve terminals and has previously been used as a marker of motor reinnervation<sup>9</sup>, to detect the regenerated nerve terminals in the tongue<sup>10,11</sup>.

The authors have no conflict of interest.

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## Materials and Methods

### Animals

A total of 32 adult female Wistar rats (12-week-old, 160–200 g body weight; Japan SLC, Shizuoka, Japan) were used in this study. Rats were randomly divided into control (n = 6), nerve resection (n = 20), and nerve transection (n = 6) groups. The nerve resection group comprised 20 rats: 1 sacrificed at 3 days, 6 at 4 weeks, 6 at 8 weeks, 6 at 12 weeks, and 1 that subsequently underwent contralateral resection 12 weeks after the initial surgery. They were housed under standard laboratory conditions in a 12-h light/dark cycle and allowed free access to food and water.

### Unilateral hypoglossal (XII) nerve injuries

Preoperatively, rats were anesthetized via an intraperitoneal injection of a mixture of medetomidine (0.375 mg/kg), midazolam (2.0 mg/kg), and butorphanol (2.5 mg/kg). Atipamezole hydrochloride (0.75 mg/kg) was intraperitoneally injected to reverse the effects of medetomidine. In the nerve-resected rats, main trunk before the bifurcation of the right XII nerve was resected approximately 9 mm in length. The resection length was measured by using a digital caliper. In one rat of the nerve-resected rats, the left XII nerve was similarly resected 12 weeks after the initial right XII nerve resection. In the nerve-transected rats, the right XII nerve was sharply cut at the center of the trunk. After the transection, the epineurium was sutured at two opposing sites of the sectioned nerve to maintain continuous proximal and distal stump attachment at the nerve-sectioned site. In the control rats, the right XII nerve was only exposed with no nerve damage. Rats after surgery were allowed to recover under a heat lamp and returned to their home cages upon full recovery. They did not appear to be in pain with the postoperative analgesic effect of butorphanol and supplemental analgesia was not given. Rats were monitored for weight loss or feeding difficulties, given the potential for tongue dysfunction after the XII nerve injury.

### Tissue preparation

At 3 days postoperatively, one rat in the resection group was euthanized with anesthetic overdose and transcardially perfused with fixative solution containing 4% paraformaldehyde in 0.1-M phosphate buffer. At 4, 8, and 12 weeks postoperatively, the rats in the resection group were similarly perfused (4, 8, and 12 weeks; n = 6 per group). The other rat received the left XII nerve resection 12 weeks after the initial right XII nerve resection was also perfused 3 days after the left XII nerve resection. The tongue was harvested and post-fixed overnight in the fixative solution. They were soaked in 30% phosphate-buffered sucrose for 2 days and serially cut into 50- $\mu$ m thick sections in the frontal plane at 1200- $\mu$ m intervals (every 24<sup>th</sup> section; in total, 14–15 sections were acquired per rat) with a freezing microtome. At 12 weeks postoperatively, the same procedures were performed in the control and nerve transection groups.

### Immunohistochemistry for vesicular acetylcholine transporter (VACHT)

To detect nerve terminals, the series of frozen sections from the tongue were immunostained for VACHT. The sections were immersed in 0.3% H<sub>2</sub>O<sub>2</sub> for 30 min to suppress endogenous peroxidase activity and in phosphate-buffered saline containing 0.3% Triton X-100 for 1 h. They were then incubated overnight with goat anti-VACHT antibody (1:4000; ABN100; Merck, Darmstadt, Germany; RRID: AB\_2630394) at room temperature. After washing, the sections were incubated with biotinylated anti-goat immunoglobulin (1:500; EO466; Dako, Glostrup, Denmark; RRID: AB\_3676677) for 2 h and then with streptavidin-peroxidase (1:500; PO397; Dako) for 2 h. The Metal Enhanced DAB Substrate Kit (Thermo Fisher Scientific Inc., Pierce Biotechnology, Rockford, IL) was used to visualize the peroxidase reaction product. The sections were rinsed, mounted on coated slides, air-dried, dehydrated, and cover-slipped using Entellan New (Merck).

### Quantification of vesicular acetylcholine transporter (VACHT)-positive nerve terminals

The immunostained sections were observed using a BZ-9000 microscope (Keyence, Osaka, Japan). To view the images of thick samples, Z-stack images were captured at 10- $\mu$ m intervals in the control, nerve-transected, and nerve-resected rats. The VACHT-positive nerve terminals were counted manually by a single observer according to the Z-stack images from all optical planes on both sides of the middle section of the tongue. The entire right or left side was included in the counting area and the whole tongue musculature was treated as one compartment. The number of VACHT-positive nerve terminals in the tongue was identified for the injured right and uninjured left sides, and the reinnervation rate of injured right XII nerves was calculated as the percentage of the number of VACHT-positive nerve terminals on the injured right side relative to those on the uninjured left side (%).

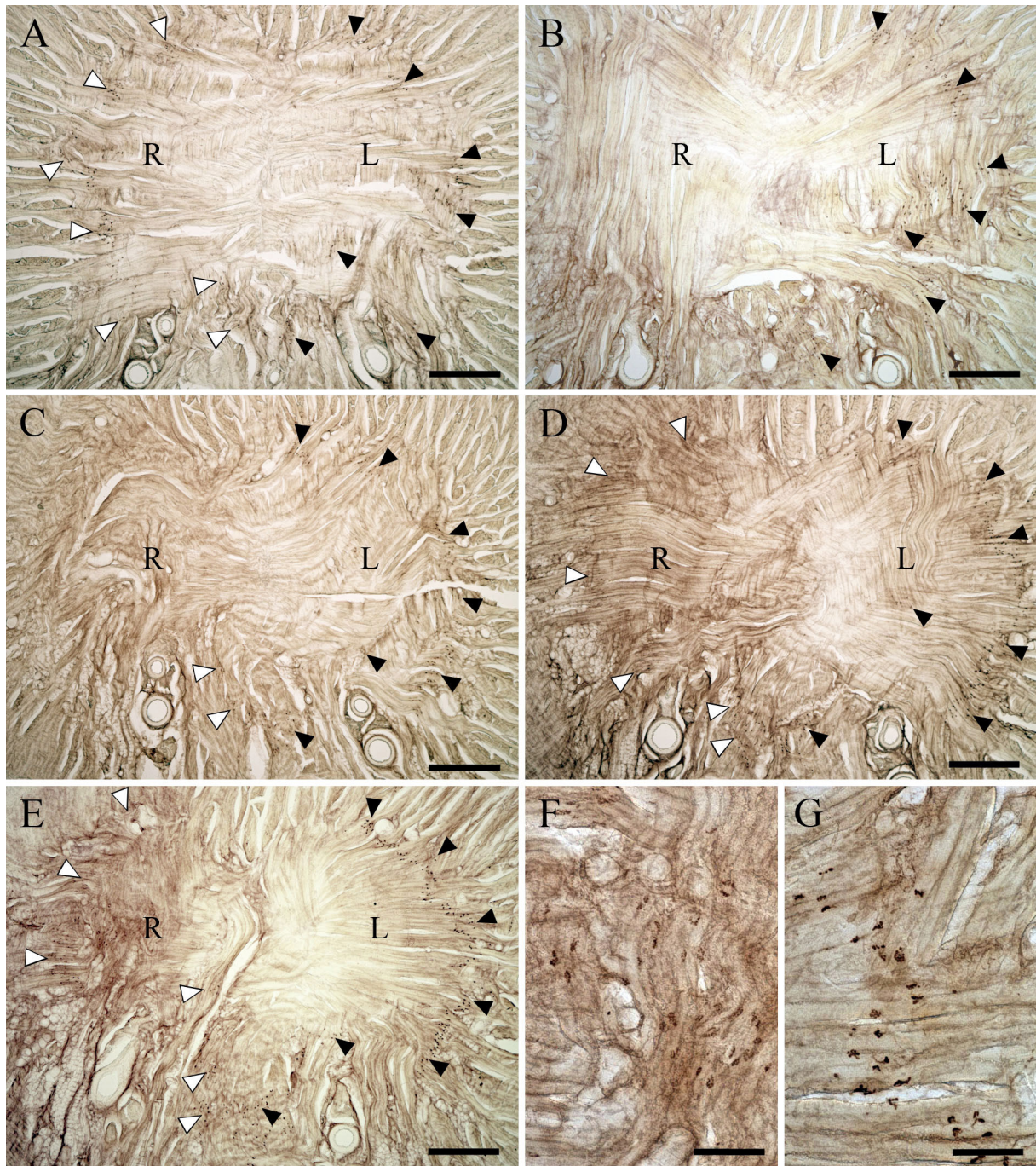
## Statistical Analysis

Statistical analyses were performed using Statcel 3 software (version 3; OMS Publisher, Tokorozawa, Japan). Data are presented as means  $\pm$  standard deviation. Statistical significance of the mean value obtained from the experimental groups was assessed with a one-way analysis of variance followed by the Tukey–Kramer test, with *P*-values of <0.05 indicating statistical significance.

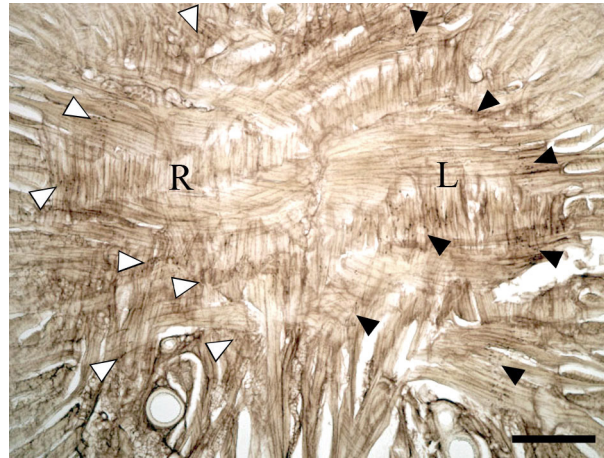
## Results

### Vesicular acetylcholine transporter (VACHT)-positive nerve terminals

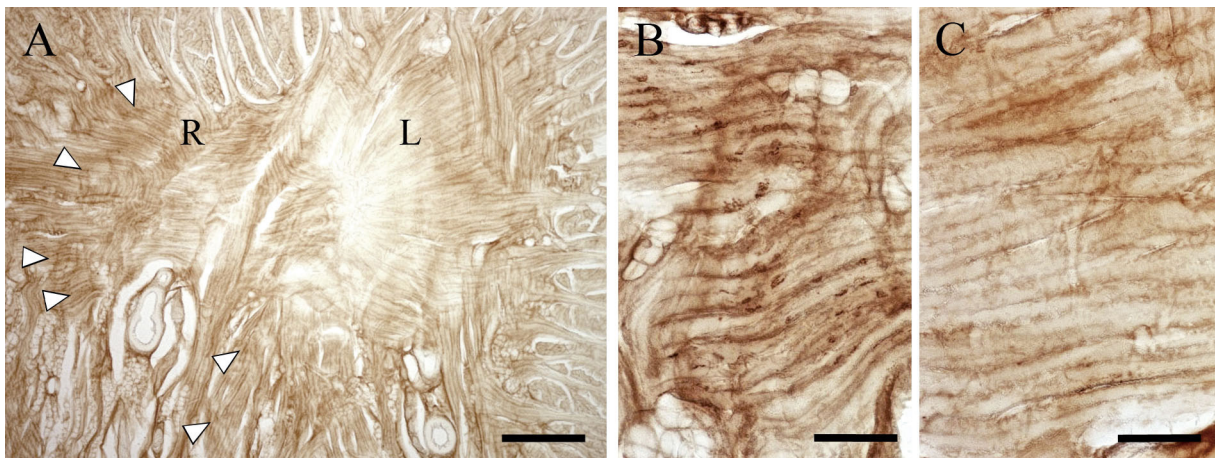
The VACHT-immunostaining detected the nerve terminals of the tongue muscle. Many VACHT-positive nerve terminals



**Figure 1.** Vesicular acetylcholine transporter (VACHT)-positive nerve terminals in the tongue. (A) Control rat. Many nerve terminals were observed on both sides, right (white arrowheads) and left (black arrowheads), symmetrically. (B) Hypoglossal nerve-resected rats at 3 days after nerve resection. Black arrowheads indicate the uninjured nerve terminals on the left side. (C–E) Hypoglossal nerve-resected rats at 4 (C), 8 (D), and 12 (E) weeks after nerve resection. White and black arrowheads indicate the regenerated nerve terminals on the injured right side and the uninjured nerve terminals on the left side, respectively. (F, G) Enlarged image of the VACHT-positive nerve terminals on the right (F) and left (G) sides from (E). Scale bars: 500  $\mu\text{m}$  (A–E), 100  $\mu\text{m}$  (F, G).



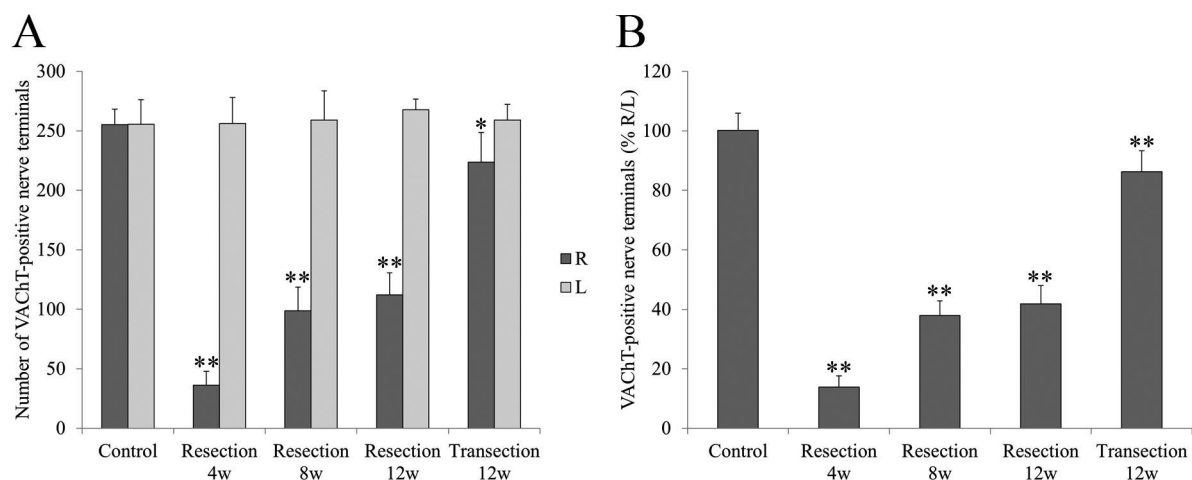
**Figure 2.** Vesicular acetylcholine transporter (VACHT)-positive nerve terminals in the tongue of hypoglossal nerve-transected rat at 12 weeks after nerve transection. White and black arrowheads indicate the regenerated nerve terminals on the injured right side and the uninjured nerve terminals on the left side, respectively. Scale bar: 500  $\mu$ m.



**Figure 3.** Vesicular acetylcholine transporter (VACHT)-positive nerve terminals in the tongue. (A) Left hypoglossal nerve-resected rat 12 weeks after right hypoglossal nerve resection at 3 days after left nerve resection. White arrowheads indicate the regenerated nerve terminals on the right side. (B, C) Enlarged image of the VACHT-positive nerve terminals on the right (B) and left (C) sides from (A). Scale bars: 500  $\mu$ m (A), 100  $\mu$ m (B, C).

were observed on both sides of the tongue in the control rats (Figure 1A). In the nerve-resected rat, the VACHT-immunostaining did not detect any nerve terminals on the nerve-injured right side at 3 days postoperatively (Figure 1B). In the XII nerve-resected rats at 4 (Figure 1C), 8

(Figure 1D), and 12 (Figure 1E–G) weeks postoperatively, the VACHT-immunostaining detected the regenerated nerve terminals on the nerve-injured right side and uninjured nerve terminals on the left side. The number of VACHT-positive nerve terminals in the tongue on the injured right



**Figure 4.** Vesicular acetylcholine transporter (VACHT)-positive nerve terminals. (A) Total number of the VACHT-positive nerve terminals on the injured right and uninjured left sides in the control, nerve-resected, and nerve-transected rats.  $**P < 0.01$ ,  $*P < 0.05$  vs. control. Differences on the injured right side were significant between control and resection at 4 weeks ( $P < 0.01$ ); control and resection at 8 weeks ( $P < 0.01$ ); control and resection at 12 weeks ( $P < 0.01$ ); control and transection at 12 weeks ( $P < 0.05$ ), between resection at 4 weeks and resection at 8 weeks ( $P < 0.01$ ); resection at 4 weeks and resection at 12 weeks ( $P < 0.01$ ); resection at 4 weeks and transection at 12 weeks ( $P < 0.01$ ), between resection at 8 weeks and transection at 12 weeks ( $P < 0.01$ ), and between resection at 12 weeks and transection at 12 weeks ( $P < 0.01$ ). (B) VACHT-positive nerve terminals (% R/L) in the control, nerve-resected, and nerve-transected rats.  $**P < 0.01$  vs. control. The differences were significant between control and resection at 4 weeks ( $P < 0.01$ ); control and resection at 8 weeks ( $P < 0.01$ ); control and resection at 12 weeks ( $P < 0.01$ ); control and transection at 12 weeks ( $P < 0.01$ ), between resection at 4 weeks and resection at 8 weeks ( $P < 0.01$ ); resection at 4 weeks and resection at 12 weeks ( $P < 0.01$ ); resection at 4 weeks and transection at 12 weeks ( $P < 0.01$ ), between resection at 8 weeks and transection at 12 weeks ( $P < 0.01$ ), and between resection at 12 weeks and transection at 12 weeks ( $P < 0.01$ ).

side increased gradually after nerve resection and the regenerated axons grew through the tongue muscles. In the XII nerve-transected rats at 12 weeks postoperatively, the distribution of regenerated VACHT-positive nerve terminals in the tongue on the injured right side was comparable to the uninjured nerve terminals on the uninjured left side (Figure 2). In the rat that received the left XII nerve resection 12 weeks after the initial right XII nerve resection, the VACHT-positive nerve terminals were detected on the right but not on the left side 3 days after the left XII nerve resection (Figure 3).

#### Reinnervation rates

Figure 4 illustrates the measuring numbers of VACHT-positive nerve terminals and the calculated reinnervation rates of all groups. The statistical analyses were performed separately for the right and left sides, and separately again for the reinnervation rate (% R/L). All factor levels (control, resection 4w, resection 8w, resection 12w, transection 12w) were included in each ANOVA. The data met the assumption of ANOVA concerning normality and homogeneity of

variance. The mean percentage of the number of VACHT-positive nerve terminals on the right side relative to those on the left side in the control group was  $100.2\% \pm 5.8\%$  (right/left:  $255.3 \pm 12.9/255.5 \pm 20.8$ ). In the nerve resection groups, the mean reinnervation rates of injured right XII nerves at 4, 8, and 12 weeks postoperatively were  $13.9\% \pm 3.7\%$  (right/left:  $36.2 \pm 11.6/256.2 \pm 21.7$ ),  $37.9\% \pm 4.9\%$  (right/left:  $98.8 \pm 19.9/259.0 \pm 24.5$ ), and  $41.8\% \pm 6.2\%$  (right/left:  $112.2 \pm 18.5/267.8 \pm 8.8$ ), respectively. Conversely, the mean reinnervation rate was  $86.3\% \pm 7.0\%$  (right/left:  $223.7 \pm 24.9/259.0 \pm 13.2$ ) in the nerve transection group. Differences in the number of VACHT-positive nerve terminals among the groups were significant on the injured right side but not on the uninjured left side (Figure 4A). Compared with the control group, significant differences on the injured right side were observed in the nerve resection groups at 4 ( $P < 0.01$ ), 8 ( $P < 0.01$ ), and 12 ( $P < 0.01$ ) weeks as well as in the nerve transection group ( $P < 0.05$ ). Moreover, significant differences on the injured right side were observed between the nerve resection group at 4 weeks and the nerve resection group at 8 weeks ( $P < 0.01$ ) and 12 weeks ( $P < 0.01$ ), and

the nerve transection group ( $P < 0.01$ ); between the nerve resection group at 8 weeks and the nerve transection group ( $P < 0.01$ ); between the nerve resection group at 12 weeks and the nerve transection group ( $P < 0.01$ ). Differences in the reinnervation rates among the groups were significant (Figure 4B). Compared with the control group, significant differences were observed in the nerve resection groups at 4 ( $P < 0.01$ ), 8 ( $P < 0.01$ ), and 12 ( $P < 0.01$ ) weeks as well as in the nerve transection group ( $P < 0.01$ ). Moreover, significant differences were observed between the nerve resection group at 4 weeks and the nerve resection group at 8 weeks ( $P < 0.01$ ) and 12 weeks ( $P < 0.01$ ), and the nerve transection group ( $P < 0.01$ ); between the nerve resection group at 8 weeks and the nerve transection group ( $P < 0.01$ ); between the nerve resection group at 12 weeks and the nerve transection group ( $P < 0.01$ ).

## Discussion

Our recent research demonstrated a critical nerve-gap length to enable spontaneous neural regeneration after the XII nerve resection, with critical nerve-gap length of approximately 10.0 mm<sup>7,8</sup>. Nerve resection lengths in this study were shorter than the critical length; thus, spontaneous regeneration and reinnervation would occur in the tongue muscle. After the XII nerve resection, regenerated nerve fibers gradually elongated toward the tongue that was originally innervated by the XII neurons. In the rat that received the left XII nerve resection 12 weeks after the initial right XII nerve resection, the VAcHT-positive nerve terminals 3 days after the left XII nerve resection were detected on the right but not on the left side. On the left side, the VAcHT-positive nerve terminals disappeared by 3 days after the left XII nerve resection. These findings suggest that the regenerated terminals mainly originated from the injured ipsilateral XII nerve rather than from the intact contralateral side. However, we could not definitively rule out the contralateral reinnervation. There were specific tongue regions that received bilateral XII nerve innervation in neonatal rat<sup>11</sup>, and these regions might be involved in contralateral reinnervation, although the regions gradually decreased during development.

The XII nerve controls the muscles of the tongue. We used VAcHT to detect the regenerated motor nerve fibers after the XII nerve injuries. In the peripheral nervous system, VAcHT is localized also in the parasympathetic nerve fibers<sup>12</sup>. Although the parasympathetic nerve was not injured, the VAcHT-positive nerve terminals were not detected on the nerve-injured right side of the tongue in the XII nerve-resected rat at 3 days postoperatively; thus, the numbers of parasympathetic postganglionic fibers would be negligible in the tongue muscles, and it was considered that the number of VAcHT-positive nerve terminals was approximately equal to the number of motor nerve terminals.

The injured XII nerve regenerated and reinnervated

the tongue muscle. We calculated the mean reinnervation rates as the percentage of the number of VAcHT-positive nerve terminals on the injured right side relative to those on the uninjured left side. Because the counts were not based on stereological methods, we could not estimate the absolute terminal number and density. The findings in this study were based on within-methods across groups rather than absolute terminal number or density. Moreover, we could not completely rule out the possibility of expectation bias because the counts were not performed in a blinded fashion. The reinnervation rates of injured right XII nerves in the nerve-resected rats gradually increased, and those rates were 13.9%, 37.9%, and 41.8% in the nerve resection group at 4, 8, and 12 weeks postoperatively. The reinnervation rates from 4 to 8 weeks after nerve resection greatly increased, with a significant difference between these two groups ( $P < 0.01$ ). However, only a slight increase was observed in the reinnervation rates from 8 to 12 weeks postoperatively. No significant difference was found between the reinnervation rates at 8 and 12 weeks in the nerve resection groups. The capabilities of spontaneous regeneration might be limited to nerve reinnervation in the tongue muscle after the XII nerve resection. Under the conditions of this study (adult female rats, 9-mm gap, 12-week follow-up), reinnervation reached approximately 40% of the contralateral side and appeared to plateau by 8–12 weeks. Longer follow-up might clarify whether there is any further late increase. Conversely, the regenerative rate of injured right XII nerves in the nerve transection group was 86.3%, with more than twice the difference in the reinnervation rate of nerve-resected rats vs. nerve-transected rats. A previous study reported a 46.9% reinnervation rate at 4 weeks after the XII nerve transection<sup>9</sup>; however, they had investigated only 4 weeks postoperatively. The regeneration of the injured nerve fibers to the tongue muscle may progress further between 4 and 8 weeks after the nerve transection. Based on the injury type, different limitations of spontaneous regeneration are considered to exist<sup>13–15</sup>.

The tongues did not move on the nerve-injured side because of the denervation after the unilateral XII nerve injuries<sup>16,17</sup>. Our recent study revealed spontaneous nerve regeneration in rats with shorter resection length than the critical length<sup>7</sup>, and those nerve-regenerated rats demonstrated functional recovery 12 weeks after the XII nerve resection in the tongue deviation assessment<sup>18</sup>. In this study, the XII nerve resection was shorter than the critical length and the reinnervation rates gradually increased during the experiment; therefore, motor paralysis of the tongue might be better as the regeneration proceeded, although we did not evaluate actual motor function in this experiment.

Functional recovery was not obtained even with less than the critical nerve resection length in the sciatic nerve-resected rats<sup>4–6</sup>; thus, a difference may exist between the sciatic and XII nerves in spontaneous regeneration after

nerve resection. Saliva, which fills the mouth and contains several cell growth factors<sup>9</sup>, might promote the functional recovery after the XII nerve resection because the XII nerve runs under the tongue. Maybe it is related that the XII nerve is a purely motor nerve different from the sciatic nerve.

In this study, the reinnervation rates of injured right XII nerves in the rats with 9-mm-length nerve resection was approximately 40%. Based on our previous findings<sup>7</sup>, we speculate that approximately 40% of the nerve terminals of the normal value in the tongue could contribute to some degree of functional recovery after the XII nerve resection. Moreover, the nerve-transected rats demonstrated functional full recovery 12 weeks after the XII nerve transection and exhibited no tongue deviation by the same assessment method<sup>7</sup>. We suggest the hypothesis inferred from comparison with previous behavioral data<sup>7</sup> that full functionality in the nerve-transected rats could be obtained by approximately 85% of the nerve terminals of the normal value in the tongue. Future experiments are warranted to reveal the minimum rate of reinnervation required for enough functional recovery after the XII nerve injuries.

## Conclusion

In this study, we revealed spontaneous reinnervation in the tongue muscle after the XII nerve injury with long nerve resection in adult rats. The regenerated XII nerve elongated in the tongue and the injured nerve terminals regenerated approximately 40% of the normal value; this rate was considerably lower than that with the nerve transection.

### Ethics approval

All animal experiments were performed in accordance with ARRIVE guidelines and approved by the Animal Care and Use Committee of Shinshu University (approval nos. O21117 and O24079).

### Authors' Contributions

N.F. and T.M. designed the study; N.F., N.S., A.N, Y.I., and A.K. performed experiments, analyzed data, prepared figures, and drafted manuscript; N.F. and T.M. edited and revised manuscript; N.F., N.S., A.N, Y.I., A.K., and T.M. interpreted results of experiments and approved final version of manuscript.

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## References

- Jiang X, Lim SH, Mao HQ, Chew SY. Current applications and future perspectives of artificial nerve conduits. *Exp Neurol* 2010;223:86–101.
- Siemionow M, Bozkurt M, Zor F. Regeneration and repair of peripheral nerves with different biomaterials: review. *Microsurgery* 2010;30:574–88.
- Hattori T, Matsuyama Y, Sakai Y, Ishiguro N, Hirata H, Nakamura R. Chondroitinase ABC enhances axonal regeneration across nerve gaps. *J Clin Neurosci* 2008;15:185–91.
- DeLeonibus A, Rezaei M, Fahradyan V, Silver J, Rampazzo A, Bassiri Gharb B. A meta-analysis of functional outcomes in rat sciatic nerve injury models. *Microsurgery* 2021;41:286–95.
- Kaplan HM, Mishra P, Kohn J. The overwhelming use of rat models in nerve regeneration research may compromise designs of nerve guidance conduits for humans. *J Mater Sci Mater Med* 2015;26:226.
- Dinh P, Hazel A, Palispis W, Suryadevara S, Gupta R. Functional assessment after sciatic nerve injury in a rat model. *Microsurgery* 2009;29:644–9.
- Ichinose Y, Nagira A, Sumitomo N, Kakegawa A, Fukushima N. Spontaneous regeneration after resection of various lengths of hypoglossal nerve in rats. *J Oral Biosci* 2024;66:582–6.
- Fukushima N, Sumitomo N, Nagira A, Ichinose Y, Kakegawa A. Spontaneous regeneration after hypoglossal nerve injury with long nerve resection in rats. *Neurophysiology* 2022;54:143–7.
- Maeda M, Ohba N, Nakagomi S, Suzuki Y, Kiryu-Seo S, Namikawa K, Kondoh W, Tanaka A, Kiyama H. Vesicular acetylcholine transporter can be a morphological marker for the reinnervation to muscle of regenerating motor axons. *Neurosci Res* 2004;48:305–14.
- Fukushima N, Karasawa M, Yokouchi K, Sumitomo N, Kawagishi K, Moriizumi T. Stereological assessment of the total number of hypoglossal neurons after repeated crush injuries to the hypoglossal nerve in adult rats. *Neurol Res* 2017;39:183–8.
- Fukushima N, Yokouchi K, Kawagishi K, Karasawa M, Kuroiwa M, Moriizumi T. Two specific tongue regions receive bilateral hypoglossal innervation: A study in neonatal rat pups. *Arch Oral Biol* 2015;60:432–8.
- Bründl M, Garreis F, Schicht M, Dietrich J, Paulsen F. Characterization of the innervation of the meibomian glands in humans, rats and mice. *Ann Anat* 2021;233:151609.
- McGraw TS, Mickle JP, Shaw G, Streit WJ. Axonally transported peripheral signals regulate alpha-internexin expression in regenerating motoneurons. *J Neurosci* 2002;22:4955–63.
- Atsumi Y, Imai T, Matsumoto K, Sakuda M, Maeda T, Kurisu K, Wakisaka S. Effects of different types of injury to the inferior alveolar nerve on the behavior of Schwann cells during the regeneration of periodontal nerve fibers of rat incisor. *Arch Histol Cytol* 2000;63:43–54.
- Borke RC, Curtis M, Ginsberg C. Choline acetyltransferase and calcitonin gene-related peptide immunoreactivity in motoneurons after different types of nerve injury. *J Neurocytol* 1993;22:141–53.

16. Fukuyama T, Yokouchi K, Fukushima N, Kawagishi K, Kakegawa A, Moriizumi T. Differential effects of hypoglossal and facial nerve injuries on survival and growth of rats at different developmental stages. *Int J Dev Neurosci* 2006;24:307–17.
17. Fuller DD, Williams JS, Janssen PL, Fregosi RF. Effect of co-activation of tongue protruder and retractor muscles on tongue movements and pharyngeal airflow mechanics in the rat. *J Physiol* 1999;519:601–13.
18. Evgenieva E, Schweigert P, Guntinas-Lichius O, Pavlov S, Grosheva M, Angelova S, Streppel M, Irintchev A, Skouras E, Kuerten S, Sinis N, Dunlop S, Radeva V, Angelov DN. Manual stimulation of the suprahyoid-sublingual region diminishes polynervation of the motor endplates and improves recovery of function after hypoglossal nerve injury in rats. *Neurorehabil Neural Repair* 2008;22:754–68.
19. Saruta J, To M, Sakaguchi W, Kondo Y, Tsukinoki K. Brain-derived neurotrophic factor is related to stress and chewing in saliva and salivary glands. *Jpn Dent Sci Rev* 2020;56:43–9.