치의과학석사 학위논문

The change of taste bud morphology and taste cognition after bilateral hypoglossal neurectomy

양측 설하신경 절단술에 따른 미뢰 형태와 미각인지 변화

2020년 2월

서울대학교 대학원

치의과학과 구강악안면외과학 전공

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2019년 10월

서울대학교 대학원
치의과학과 구강악안면외과학 전공
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박상윤의 치의과학석사 학위논문을 인준함
2019 년 12 월

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Abstract

The change of taste bud morphology and taste cognition after bilateral hypoglossal neurectomy

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Objective

The taste nerves, including the chorda tympani, glossopharyngeal, and vagus nerves, have a significant influence on the maintenance of the structures of taste buds and taste cells. Previous studies have suggested that degeneration in the taste bud occurs following transection of the chorda tympani nerve, and other research studies indicate that there is a neural connection between the lingual and hypoglossal nerves. The purpose of this study is to investigate whether the hypoglossal nerve has a role in taste bud structure and taste cognition.

Materials & Methods

Twelve male Sprague-Dawley rats were randomly divided into two groups and underwent neurectomy of the hypoglossal nerve (neurectomy group: n = 6; control sham group: n = 6). Clinical observation of the tongue morphology and scanning electron microscopic comparisons of the fungiform papillae number per mm2 and size of fungiform papillae were completed after two weeks postneurectomy, and results were compared between two groups. Histological changes in taste buds after 2 weeks of neurectomy in fungiform papillae were evaluated with hematoxylin and eosin (H&E) staining. Immunofluorescence study using α-gustducin, a taste type II cell marker, was performed to compare taste cell type II expressions for fungiform and circumvallate papillae between the two groups. A sucrose preference test was used to examine changes in taste cognition. Two bottles (5% sucrose water/water) were installed equally in a single cage, and the intake of each bottle was measured between bottles, time points, and groups.

Results

In the neurectomy group, the number and size of fungiform papillae decreased with tongue muscle atrophy (neurectomy group: 2.389 ± 0.459 vs. control group: 3.833 ± 0.464 for fungiform papilla number/mm2; $p < 0.05$ and neurectomy:

7249.699 ± 182.463 vs. control: 14306.888 ± 66.894 for fungiform papilla size (μm2); $p < 0.05$). During the histological examination, the shape of the taste cells in the neurectomy group appeared irregular and reduced in size, unlike in the control (sham) group, and the expression of taste type II cells in fungiform papillae also decreased relative to that in the control (sham) group as revealed by immunofluorescence examination. Per the sucrose preference test, a decrease in sucrose preference in the neurectomy group had occurred compared to that in the control (sham) group (before neurectomy: 13.0 ± 4.89 g vs. after 2 weeks of neurectomy: 7.5 ± 2.85 g for 2.5-h intake; $p < 0.05$ and before neurectomy: 107.2 ± 20.50 g vs. after 2 weeks of neurectomy: 87.2 ± 33.66 g for 24-h intake; $p < 0.05$). At autopsy, swelling of the digestive system was consistently observed in the neurectomy group.

Conclusion

The number of fungiform papillae decreased with atrophy of the tongue muscle following bilateral hypoglossal neurectomy. Also, the procedure facilitated taste cell degeneration and decreased taste perception.

Key word: Hypoglossal nerve, taste bud, neurectomy, fungiform papilla, taste cognition

Student number: 2016-23817

Table of Contents

List of figures

List of graphs

Introduction

There are four types of papillae on the tongue: fungiform, circumvallate, foliate, and filiform papillae. Taste buds, cells of epithelial origin that repeat the process of continuous apoptosis and regeneration[1–4], are found in all types of papillae, except the filiform papilla[5,6]. In humans, stimulation of taste buds on the tongue is transmitted through the seventh cranial nerve (chorda tympani) and the ninth cranial nerve (glossopharyngeal) to be recognized as taste in the brain[2,3]. The taste nerves have a significant influence on the maintenance of the structure of taste buds. Previously, reports have shown that the degeneration of taste nerves and taste cells occurs in cases when the gustatory nerve was removed from the seventh cranial nerve[7]. Guagliardo and Hill[7] reported that histological degeneration and reductions in the number and size of taste buds could be seen 15 days after transection of the chorda tympani nerve in rats. However, the mechanism behind how some of the taste buds remained normal could not be explained.

The taste sensory nerves carry the taste cognition, and the motor nerves and muscles control the movement of the tongue independently of each other. It is believed that the neuromotor control of all of the intrinsic muscles of the tongue is derived from myotomes supplied by the hypoglossal nerve alone. However, Mu and Sanders[8] suggested that the lingual nerve is distributed in the superior longitudinal muscle fascicle and communicates significantly with the hypoglossal nerve. Saigusa et al.[9] indicated that some motor roots of the trigeminal nerve had nerve branches in the superior longitudinal and inferior longitudinal muscles and that the posterior branch of the lingual nerve communicated with the hypoglossal nerve in a cadaver anatomical study. Further, it has been documented that the

lingual nerves showed neuromotor control to some extent in the muscles of some tongues, and extra-lingual connections of the lingual and hypoglossal nerves were reported. In human neonates, rhythmic movement of the tongue was observed with sucrose, NaCl, HCI, and quinine. Stimuli and responses to taste may also affect hypoglossal nerve response and tongue movement, suggesting that the gustatory-hypoglossal reflex is a highly organized neural network[10]. This experimental hypothesis was based on previous findings of a neural connection between the lingual and hypoglossal nerves and the fact that taste cells degenerate when the taste nerves are damaged. Clinically, the case presented by Shwikh and al-Atrakchi[11] showed muscle atrophy, tongue excursion, and taste dysfunction after damage to the hypoglossal nerve due to trauma. The purpose of this study is to investigate whether diminishing the tongue's motor capacity by introducing damage to the hypoglossal nerve could affect taste cognition along with provoke structural and morphological changes in the taste buds.

Materials & Methods

Animals

Male Sprague-Dawley rats aged eight weeks and weighing 250 to 300 g were purchased from Orient Bio (Gapyeong, Republic of Korea). The animals were housed at Seoul National University School of Dentistry in a specific pathogen-free room of an experimental animal facility with the temperature maintained at 21°C ± 1°C, humidity at 55%, and a 13.5:10.5 light:dark cycle that began at 07:30. Normal diet (Purina Rodent Chow; Purina Co., St. Louis, MO, USA) and filtered water were provided ad libitum. Prior to experimentation, the animals were quarantined in a cage for a week. The study was approved by the Animal Care and Use Committee of Seoul National University, Korea (SNU-180328-1), under the same animal and laboratory protocol[12].

Surgical procedure

Twelve male Sprague-Dawley rats were randomly divided into two groups and underwent neurectomy of the hypoglossal nerve. Rats were anesthetized via an intraperitoneal injection with a 4:1 mixture of ketamine hydrochloride (100 mg/kg, Ketara®; Yuhan, Seoul, Korea) and xylazine hydrochloride (25 mg/kg, Rumpun®; Bayer, Leverkusen, Germany). The bilateral hypoglossal nerves were exposed by a ventromedial incision made in the neck of the rat and resected in the neurectomy group. Next, the medial and lateral branches of the hypoglossal nerve were identified in the neurectomy group, and neurectomy of the bilateral hypoglossal

nerve was performed as shown in Figure 1. Surgeries in the control (sham) group were performed in the same manner, but the nerves were not resected. All animals were sacrificed two weeks later. Samples of the tongue tissue were collected with decapitation of the rats. All animal tests were conducted in accordance with established animal testing ethics and methods.

Retrograde neuronal labeling

Retrograde neuronal labeling with 0.2% 1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate (Dil) ($C_{59}H_{97}ClN_2O_4$; Molecular Probes, Eugene, OR, USA) and the macrophage marker ionized calcium-binding adapter molecule 1 (Iba1) was used to verify the correct neurectomy of the hypoglossal nerve. Subsequently, it was confirmed that neurectomy surgery of the hypoglossal nerve was conducted as planned by staining the retrograde tracer on the affected area of the rat brain stained with Dil and Iba1 (Figure 2).

Clinical and microscopic examination

Morphological changes of the tongue dorsal surface in the control (sham) and neurectomy groups were observed by clinical and microscopic examinations. During the clinical examination, counting of fungiform papilla per 1 cm^2 in the neurectomy group was performed at certain time intervals from prior to surgery to two weeks postoperation (Day 1,3,7,14). Scanning electron microscopy (SEM) images were also used to compare the tongue dorsal surface and fungiform papillae. Separately, the number of fungiform papillae per 1 mm^2 was compared across the groups at two weeks after the surgery using SEM. Finally, papilla size was

compared across groups by using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Histomorphometric evaluation

Two weeks after the surgery, one died during the observation period without reason, a total of 11 rats (neurectomy group: n = 5; sham group: n = 6) were overdosed with anesthesia and transcardially perfused first with 100 mL of heparinized isotonic 1× phosphate-buffered saline (Maxim Biotech, Inc., Rockville, MD, USA) and then 400 mL of ice-cold 4% paraformaldehyde (Merck Millipore Co., Darmstadt, Germany) in 1× phosphate-buffered saline. The sampled tissues were stained with H&E for examinations of the tongue muscle and fungiform papilla under a light microscope.

Immunofluorescence evaluation

An affinity-purified rabbit polyclonal antibody [Gα gust(I-20); Santa Cruz Biotechnology, Dallas, TX, USA] that reacts with rat α-gustducin was used[13]. Fungiform papilla and circumvallate papilla were obtained by performing coronal sectioning of the tongue. The 7-μm-thick sections were cut and subsequently deparaffinized in xylene and rehydrated in ethanol by standard protocols. The sections were incubated overnight at 41°C with the primary antibody, anti-α-gustducin (Santa Cruz Biotechnology Inc., Dallas, TX, USA), diluted at 1:200. A horseradish peroxidase–labeled goat anti-rabbit IgG secondary antibody (Zhongshan, Beijing, China), diluted at 1:100, was applied for 20 to 30 min. Sections were developed by reaction in 0.05% 3,3-diaminobenzidine

tetrahydrochloride (Zhongshan, Beijing, China) in 0.05 M of Tris buffer (pH: 7.6) and hydrogen peroxide. Sections were then rinsed for 3 to 5 min in distilled water, counterstained with hematoxylin, dehydrated through a graded series of ethanol, cleared in xylene, and mounted with neutral gum. According to a method previously described[13], an immunohistochemistry experiment protocol was performed to verify whether the expression of α-gustducin reaction differs between the two groups observed. All images were collected using an Olympus Fluoview confocal laser scanning microscope (Olympus, Tokyo, Japan).

Sucrose preference test

Taste perception was analyzed using a two-bottle test, where one bottle contained 5% sucrose water and the other contained water only[3]. These bottles were equally installed in the animal cage (single cage), and the animals were trained to consume a small amount to test their preference regarding 5% sucrose water one week before the surgery. Sucrose preference was measured by comparing the difference between the intakes of water and 5% sucrose water at 2.5 and 24 h after the two bottles were installed.

Statistical analysis

All data were analyzed with both one- and two-way analyses of variance (ANOVAs) using the StatView software (Abacus, Berkeley, CA, USA). A *p*-value less than 0.05 was considered statistically significant, and all values were presented with standard deviations.

Results

Clinical and microscopic examination

At Two weeks after neurectomy, upon visual inspection, muscle atrophy of the tongue was clearly observed in the neurectomy group. A deep groove on the midline of the tongue and symmetrical horizontal grooves on each side of the tongue were seen (Figure 3). In addition, the number of fungiform papillae had decreased together with the occurrence of changes in their arrangement. Overall, the number of fungiform papillae per cm^2 tended to decrease following the surgery, with findings at postoperative days 7 and 14 being statistically significant, as shown in Graph 1 (Day 0: 0.405 ± 0.062, Day 7: 0.224 ± 0.043, Day 14: 0.200 ± 0.062; $p < 0.001$, one-way ANOVA).

SEM analysis revealed that the fungiform papillae in the neurectomy group decreased in number and size and were irregular in shape, unlike the smooth and round papillae visible in the control (sham) group (Figure 4). The change in the number of fungiform papillae per mm^2 in the neurectomy group was statistically significantly different as shown in Graph 2 (neurectomy group: 2.389 ± 0.459 vs. control group: 3.833 ± 0.464; $p = 0.0186$). On average, the size of the fungiform papillae in the neurectomy group significantly decreased to about 7,250 μm^2, which is about a twofold decrease relative to that in the control (sham) group as shown in Graph 3 (neurectomy group: 7249.699 ± 182.463 μm^2 vs. control group: 14306.888 ± 66.894 μm^2; $p < 0.001$).

Histomorphometric evaluation

Following H&E staining of the tongue, no histological change could be seen in the coronal section of the tongue, except for the groove on the surface of the neurectomy group (Figure 5). The taste cells of the neurectomy group did not present a well-defined circular shape like that seen in the control (sham) group but rather showed a distorted irregular shape. It can be assumed from this that morphological changes or degeneration occurred among the taste cells in the neurectomy group (Figure 6).

Immunofluorescence evaluation

Considering α-gustducin staining of the fungiform papillae of taste cells in the neurectomy group, the expression of α-gustducin in the taste buds of fungiform papillae significantly decreased when compared with that in the control (sham) group (Figure 7-A). This indicates that the type II cells among the taste cells in the neurectomy group decreased. However, the degree of α-gustducin expression in the circumvallate papillae was not significantly different between the two groups (Figure 7-B).

Sucrose preference test

A total of two experiments using the two-bottle test were carried out. The same training was conducted for sucrose water preoperatively. The first experiment was designed to compare the intake of water and 5% sucrose water for one week from

nine days after the surgery. The results showed clear differences in the preference between 5% sucrose water and water in the control (sham) group. In the neurectomy group, sucrose water intake was higher than water intake but not to a significantly different degree (Graph 4).

The measured value in the second experiment compared the intake of water and 5% sucrose water for 2.5 and over 24 h between the control and neurectomy groups. There were three measurement intervals: before the surgery, one week after the surgery, and two weeks after the surgery. The results showed a statistically significant difference in water intake for 2.5 h in the neurectomy group before and after one and two weeks. In addition, sucrose water intake was significantly decreased before the surgery and two weeks after the surgery. At one week after the surgery, sucrose intake in the neurectomy group was lower than that in the control (sham) group to a degree that was statistically significant in Graph 5 (sucrose water intake before surgery: 20.6 ± 6.2 g vs. 2 weeks after surgery: 7.5 ± 6.8 g in the neurectomy group, mean difference: 13.2 ± 7.6 g; $p < 0.05$) (neurectomy group: 15.3 ± 8.6 g vs. control group: 29.9 ± 12.4 g at one week after surgery; $p < 0.05$).

For 24 h of intake, sucrose water intake in the neurectomy group achieved a statistically significant decrease between preoperation and one week postoperation. At one week after the surgery, the neurectomy group showed statistically significantly less sucrose intake than that in the control (sham) group as shown in Graph 6 (sucrose water intake before surgery vs. one week after surgery mean difference: 46.7 g in the neurectomy group; $p < 0.05$) (sucrose water intake in the

neurectomy group: 60.5 ± 40.0 g vs. in the control group: 124.8 ± 51.4 g at one week after surgery; $p < 0.05$).

Gastrointestinal swelling

A significant amount of gastrointestinal swelling in the neurectomy group was observed unexpectedly. This was noted in all rats of the neurectomy group (Figure 8). By contrast, the gastrointestinal organs of the control (sham) group were normal.

Discussion

In the present experiments, hypoglossal neurectomy caused a decrease in the number and size of fungiform papillae as well as muscle atrophy as expected. Histologically, the atrophy and degeneration of taste cells were observed during microscopic visualization, and the degeneration of taste type II cells in fungiform papillae was also observed by the immunofluorescence of α-gustducin. A taste cognition test was also conducted to indirectly check whether the taste function was actually reduced, and the preference for sucrose water also decreased. These results correspond to previously proposed hypotheses. In addition, in circumvallate papillae, changes in taste type II cells were not significant per the immunofluorescence of α-gustducin. This finding may be based on the connection between the hypoglossal and lingual nerves that regulate the fungiform papilla as noted in the hypothesis of the present experiment. However, the circumvallate papillae are regulated by the glossopharyngeal nerve for taste. The results obtained indicate that this experiment is similar but vary in degree relative to that in the experiment of Guagliardo and Hill[7] in which the chorda tympani was transected and degeneration of the taste cells was observed.

An observational phenomenon evaluation after hypoglossal neurectomy was mainly performed in this experiment. Further investigations of the mechanism of taste cell degeneration and taste perception function deterioration are needed. Even though the hypothesis is supported, the hypoglossal nerve is not directly involved in taste but rather may be involved in the histological and structural maintenance of

taste cells and papillae. This may be related to decreased blood flow and growth factors caused by tongue muscle disuse atrophy because of a decrease in motor function. On the other hand, it may be assumed that influencing factors such as brain-derived nerve factor may affect the degeneration of taste cells and the suppression of regeneration because of effects stemming from the brain after neurectomy[14,15]. It is necessary to clarify this phenomenon through further studies, however, before attempting to draw conclusions. Considering the experimentation methods, as seen in the study of Boughter et al.[16], quantification through the serial sectioning of taste papilla was not performed in this experiment. Through serial sectioning, it is necessary to observe the complete morphological changes of taste buds and to confirm the degeneration trend of entire taste cells through markers acting on taste cell types I, II, and III other than α-gustducin[2]. In this experiment, α-gustducin was used to assess only the change in type II cells involved with regard to sweetness with sucrose preference testing. The bitter taste preference test (three-bottle test) should be used in further studies to observe whether the preference for bitter taste increases after neurectomy. The reason why neurectomy was performed bilaterally in this experiment was to compare the effect of neurectomy of the hypoglossal nerve between groups and to observe the change in taste cognition more clearly. In experiments with unilaterality, there are some aspects that make it difficult to compare taste cognition deviations by group. In future experiments, it may be necessary to establish a unilateral neurectomy group and to observe whether there is a difference in the neurectomy site histologically and whether there is a decrease in taste cognition. Taste cells, taste buds, and papillae are cells of epithelial origin. As observed in this experiment, morphological changes are

important, but finally from a neuroanatomy perspective, it is necessary to observe the degeneration or changes in the taste nerves. As noted in Mu's research[8], the hypothesis needs to be established by observing changes in the hypoglossal, lingual, and glossopharyngeal nerves through staining and observation of the nerve fiber.

As a new finding of this experiment, edema of the digestive system was observed in all neurectomy group rats and was first thought to be caused by swallowing disorders due to bilateral hypoglossal nerve damage. This suggests that bilateral damage of the hypoglossal nerve may be related to other functions of the digestive system. It is known to cause digestive system disorders in nonobstructive reflux disorders[17]. However, it is unknown whether such reactions will occur in humans because of the difference in the upright position and esophagus and airway structures. Clinically, there are many cases of unilateral hypoglossal damage, which makes it difficult to understand the results of bilateral damage.

Conclusion

In a rat animal model, bilateral hypoglossal neurectomy caused atrophy in the tongue muscles. As hypothesized in the present experiment, the number and size of fungiform papilla were reduced. Histologically, the taste cells of taste buds were distorted and irregular in shape, and the expression of α-gustducin immunofluorescence decreased after neurectomy of the hypoglossal nerve. This means that the number of taste type II cells decreased. Sucrose water preference testing also revealed a decrease in taste change after neurectomy of the hypoglossal nerve. In conclusion, damage to the hypoglossal nerve caused a degenerative change in taste cells and affected taste cognition.

References

1. Chaudhari N, Roper SD. The cell biology of taste. J Cell Biol. 2010 Aug 9;190(3):285-96.

2. Breslin PA, Spector AC. Mammalian taste perception. Curr Biol. 2008 Feb 26;18(4):R148-55.

3. Barlow LA. Progress and renewal in gustation: new insights into taste bud development. Development. 2015 Nov 1;142(21):3620-9. doi: 10.1242/dev.120394.

4. Smith DV, Margolskee RF. Making sense of taste. Sci Am. 2001 Mar;284(3):32-9.

5. DeVere R. Disorders of taste and smell. Continuum (Minneap Minn). 2017 Apr;23(2, Selected Topics in Outpatient Neurology):421-446.

6. Feng P, Huang L, Wang H, et al. Taste bud homeostasis in health, disease, and aging. Chem Senses. 2014 Jan;39(1):3-16. doi: 10.1093/chemse/bjt059. Epub 2013 Nov 28.

7. Guagliardo NA, Hill DL. Fungiform taste bud degeneration in C57BL/6J mice following chorda-lingual nerve transection. J Comp Neurol. 2007 Sep 10;504(2):206-16.

8. Mu L, Sanders I. Human tongue neuroanatomy: Nerve supply and motor endplates. Clin Anat. 2010 Oct;23(7):777-91. doi: 10.1002/ca.21011.

9. Saigusa H, Tanuma K, Niimi S, et al. Nerve fiber analysis for the lingual nerve of the human adult subjects. Surg Radiol Anat. 2006 Mar;28(1):59-65.

10. Yamamoto T, Fujiwara T, Kawamura Y, et al. Hypoglossal motor nerve activity elicited by taste and thermal stimuli applied to the tongue in rats. Brain Res. 1982 Apr 22;238(1):89-104.

11. Shweikh AM, al Atrakchi S. A rare case of hypoglossal nerve palsy complicating a head injury. J Accid Emerg Med. 1998 Nov;15(6):427-9.

12. Lee SH, Jin WP, Lee JH, et al. Recombinant human fibroblast growth factor-2 promotes nerve regeneration and functional recovery after mental nerve crush injury. Neural Regen Res. 2017 Apr;12(4):629-636.

13. Zhou LH, Liu XM, Liu GD, et al. Expression of alpha-gustducin in the circumvallate papillae of taste buds of diabetic rats. Acta Histochem. 2009;111(2):145-9.

14. Mistretta CM, Goosens KA, Reichardt LF, et al. Alterations in size, number, and morphology of gustatory papillae and taste buds in BDNF null mutant mice demonstrate neural dependence of developing taste organs. J Comp Neurol. 1999 Jun 21;409(1):13-24.

15. Meng L, Ohman-Gault L, Krimm RF, et al. Taste bud-derived BDNF is required to maintain normal amounts of innervation to adult taste buds. eNeuro. 2015 Dec 31;2(6). pii: ENEURO.0097-15.2015.

16. Boughter JD Jr, Pumplin DW, Smith DV, et al. Differential expression of alpha-gustducin in taste bud populations of the rat and hamster. J Neurosci. 1997 Apr 15;17(8):2852-8.

17. Cho YS, Choi MG, Park DH et al. A case of dysphagia accompanied with GERD refractory to medical treatment. Kor J Neurogastroenterol Motil 2002; 8(1): 47-52.

Figure legends

Figure 1. Intraoperative photographs showing the exposed hypoglossal nerve.

Figure 2. Retrograde neuronal labeling with Dil and Iba1 was used to confirm whether hypoglossal neurectomy was performed correctly.

Figure 3. Photographs showing the dorsal surface of the tongue (left: control group, right: neurectomy group). In the tongue of the neurectomy group, grooves in the vertical and horizontal directions were formed, and the tongue volume decreased overall relative to that in the control (sham) group.

Figure 4. SEM image of the dorsal plane of the tongue (upper: control (sham) group, lower: neurectomy group). Arrows indicate fungiform papilla. In the enlarged image, the surface of the fungiform papilla was reduced in the neurectomy group (right image).

Figure 5. H&E staining image of the dorsal plane of the tongue.

Figure 6. H&E staining image of fungiform papilla and taste cells (left: control group, right: neurectomy group). Irregular changes in shape in the neurectomy group can be clearly observed. The arrow indicates taste bud.

Figure 7

A. Immunofluorescence staining (α-gustducin) of fungiform papillae (upper: control group, lower: neurectomy group). Red color indicates that α-gustducin expression decreased in the neurectomy group.

B. Immunofluorescence staining (α-gustducin) of circumvallate papillae (upper: control group, lower: neurectomy group).

The difference between A and B indicates that the type II cells of the taste cells in the neurectomy group decreased in the fungiform papilla but not in the circumvallate papillae.

Figure 8. Swelling of the gastrointestinal system in the neurectomy group was seen during autopsy unexpectedly. This was found in all rats of the neurectomy group but not in any in the control (sham) group.

Graph legends

Graph 1. Calculation of fungiform papilla count/cm^2 in the neurectomy group. (Day 0: 0.405 ± 0.062, Day 7: 0.224 ± 0.043, Day 14: 0.200 ± 0.062; $p < 0.001$).

The number of fungiform papillae per cm^2 tended to decrease following the surgery, with findings on postoperative days 7 and 14 being statistically significant.

Graph 2. Comparison of the number of fungiform papilla/mm^2 (neurectomy group: 2.389 ± 0.459 vs. control group: 3.833 ± 0.464; $p < 0.05$). The change in the number of fungiform papillae per mm^2 in the neurectomy group was statistically significantly different from that in the control (sham) group.

Graph 3. Comparison of the size of fungiform papilla (μm^2) (neurectomy group: 7249.699 ± 182.463 vs. control group: 14306.888 ± 66.894; $p < 0.001$). The size of the fungiform papillae in the neurectomy group significantly decreased by about twofold as compared with that in the control (sham) group.

Graph 4. One-hour intake during the two-bottle test (g) (from nine days after neurectomy; error bars are standard error of the mean). In the neurectomy group, sucrose water intake was decreased more than before surgery as compared with that in the control (sham) group.

Graph 5. Two-and-half–hour intake during the two-bottle test (g). Neurectomy group sucrose water intake before surgery: 20.6 ± 6.2 g vs. 2 weeks after surgery: 7.5 ± 6.8 g, mean difference: 13.2 ± 7.6 g; $p < 0.05$. Neurectomy group: 15.3 ± 8.6 g vs. control group: 29.9 ± 12.4 g at one week after surgery; $p < 0.05$. Data are presented as means ± standard deviations.

Graph 6. Twenty-four–hour intake during the two-bottle test (g). Neurectomy group sucrose water intake before surgery vs. one week after surgery mean difference: 46.7 g; $p < 0.05$. Sucrose water intake in the neurectomy group: 60.5 ± 40.0 g vs. in the control (sham) group: 124.8 ± 51.4 g at one week after surgery; $p < 0.05$.

Figure 1.

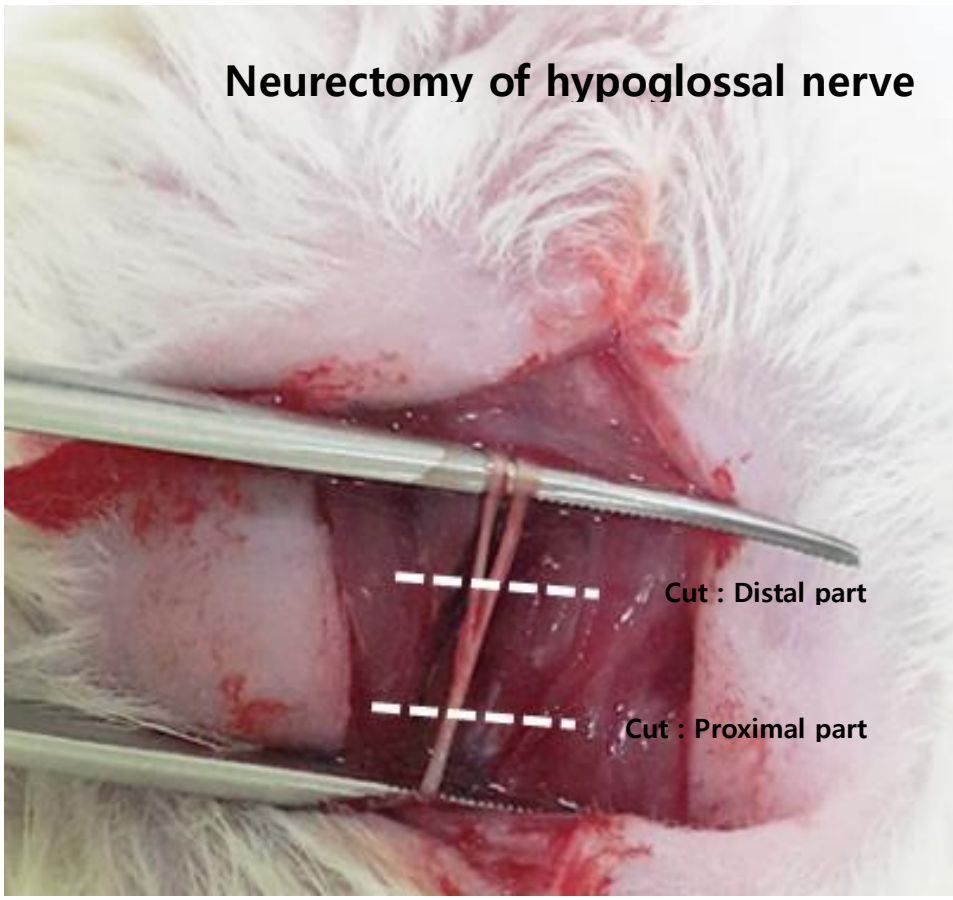
Neurectomy of hypoglossal nerve
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Figure 2.

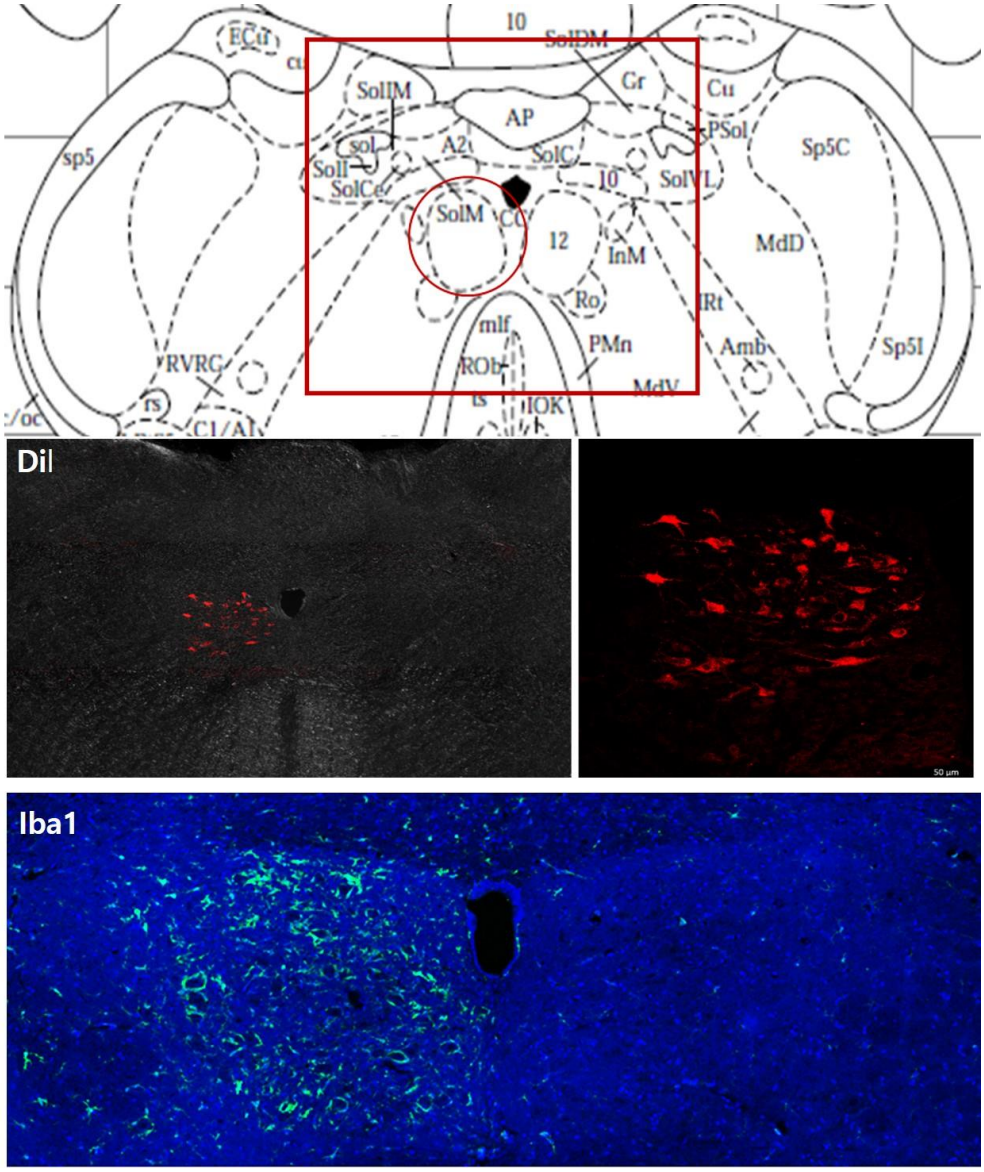
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Figure 3.

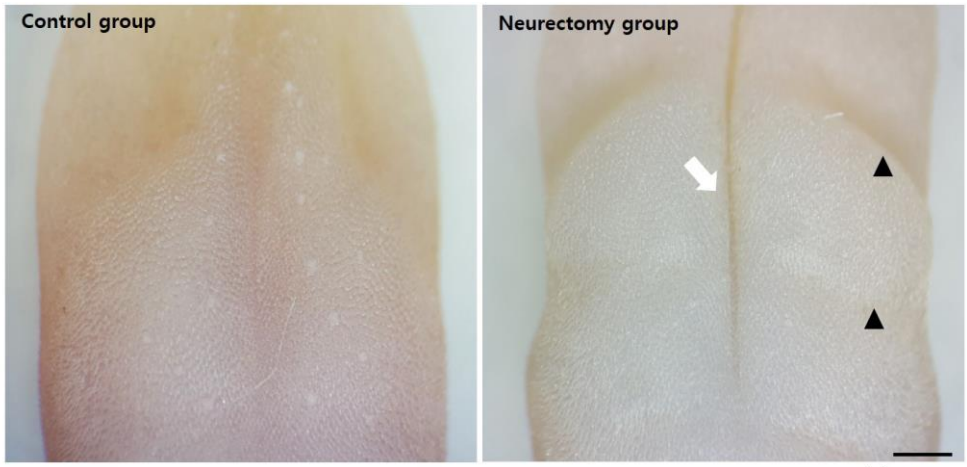


Figure 4.

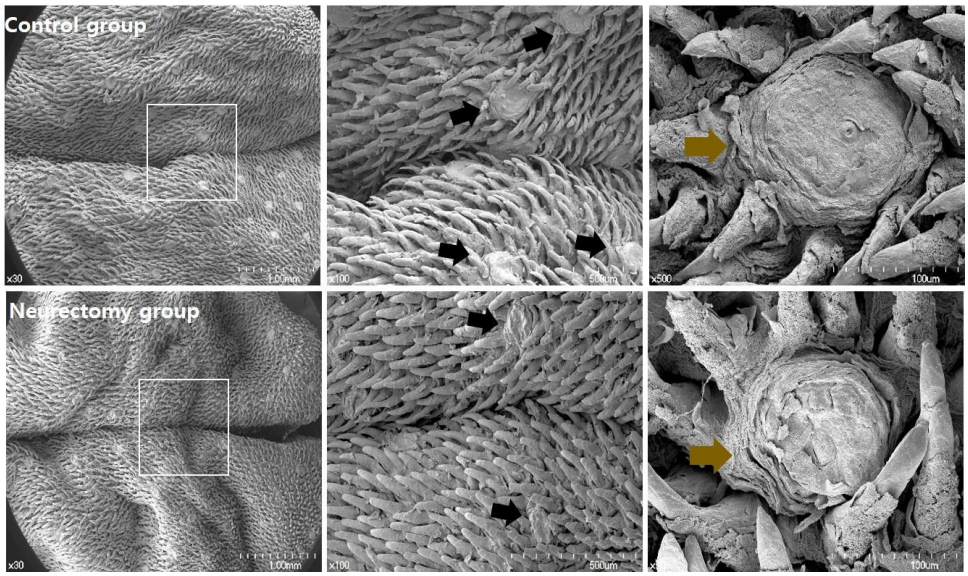


Figure 5.

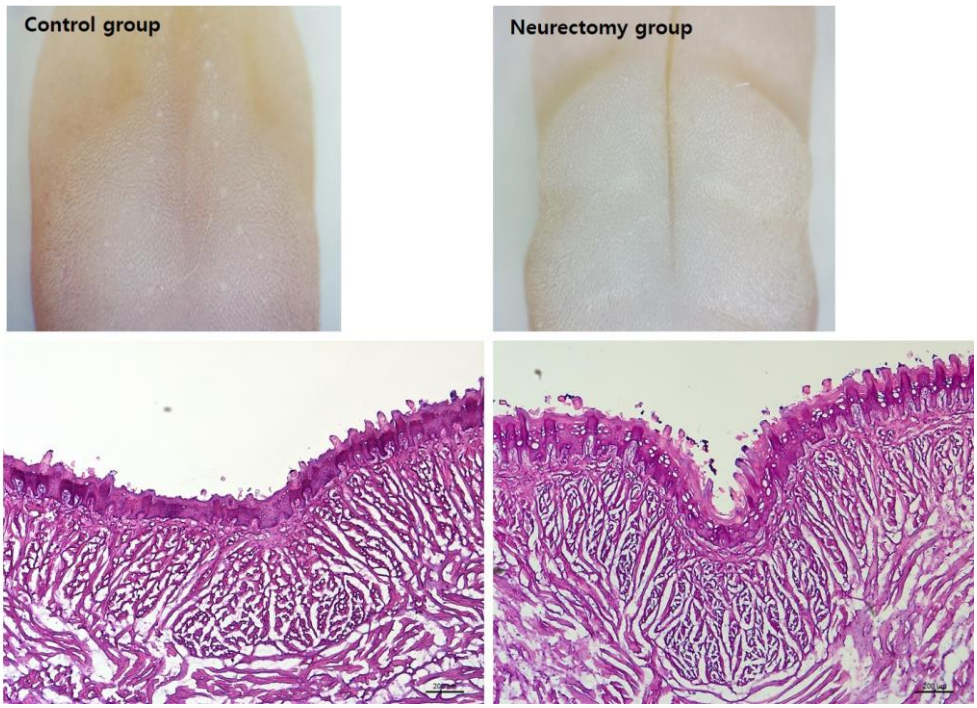


Figure 6

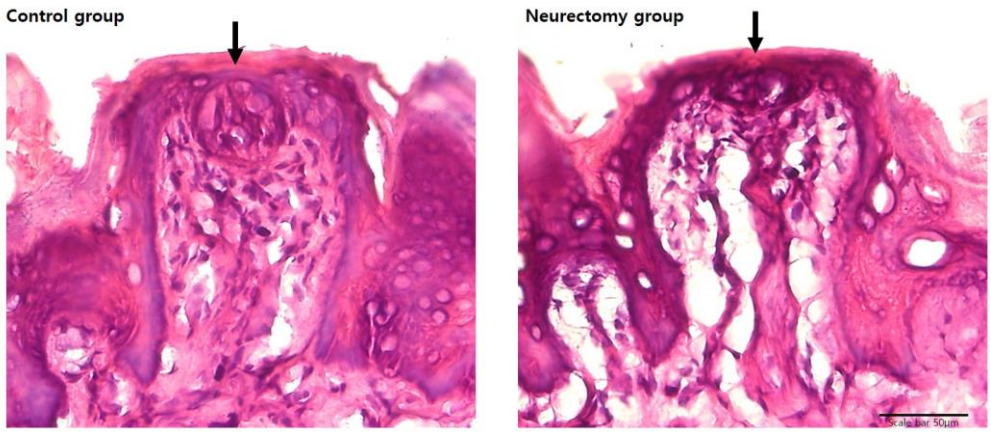


Figure 7-A.

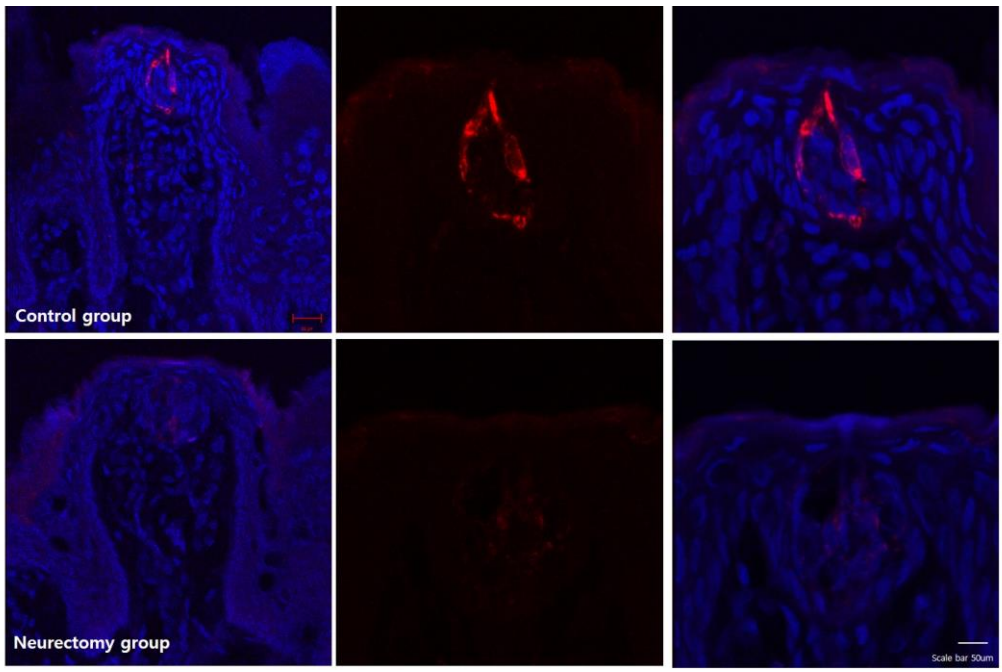


Figure 7-B

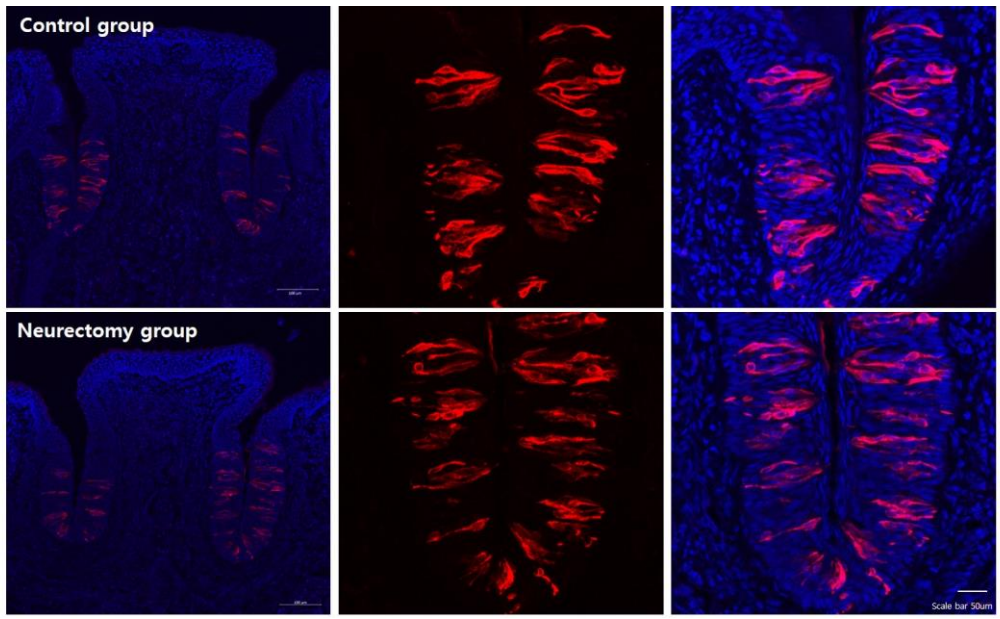
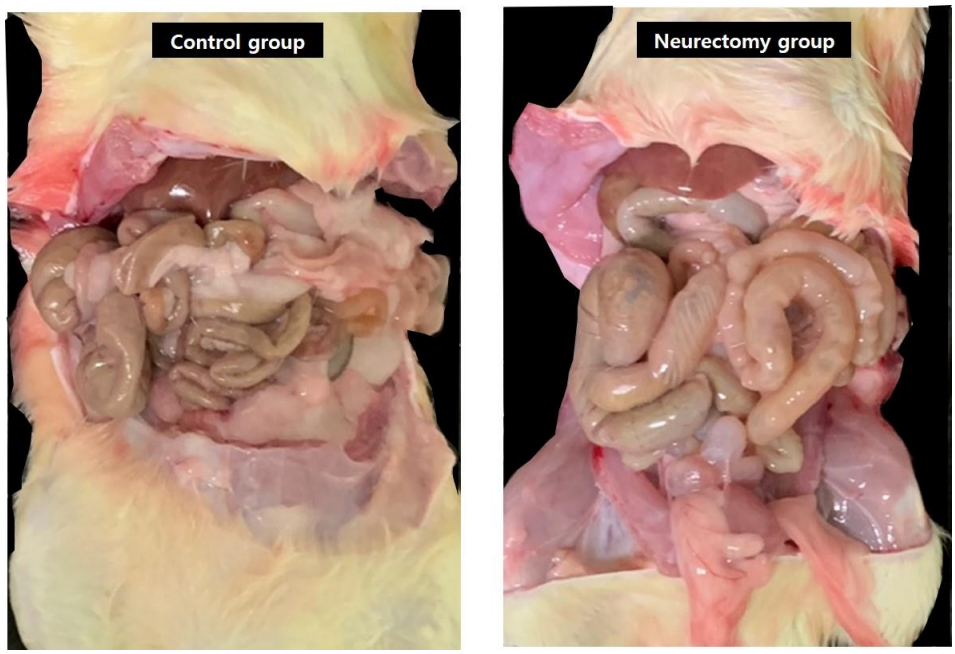
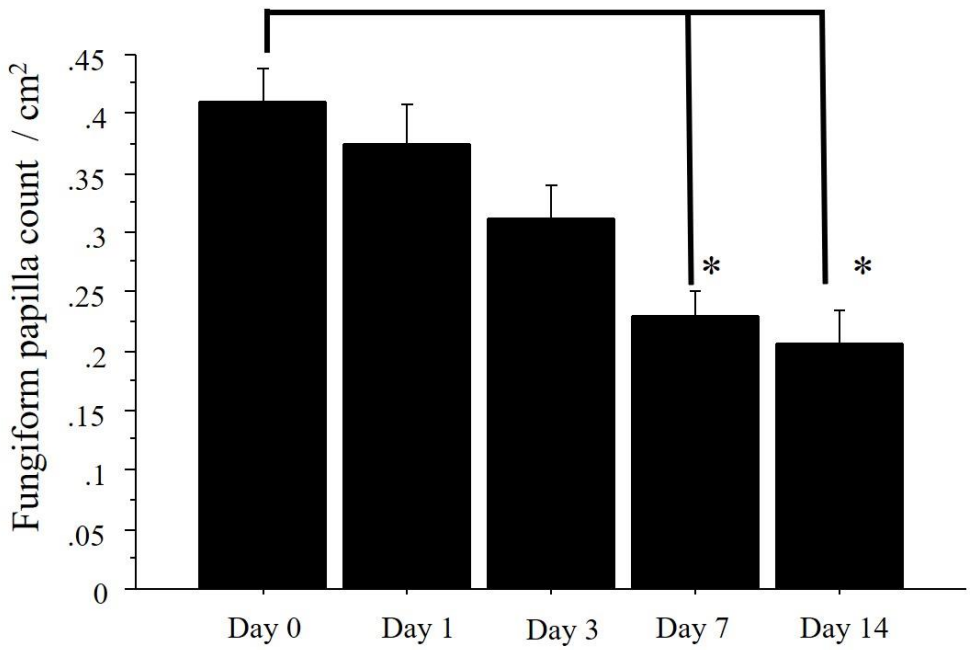


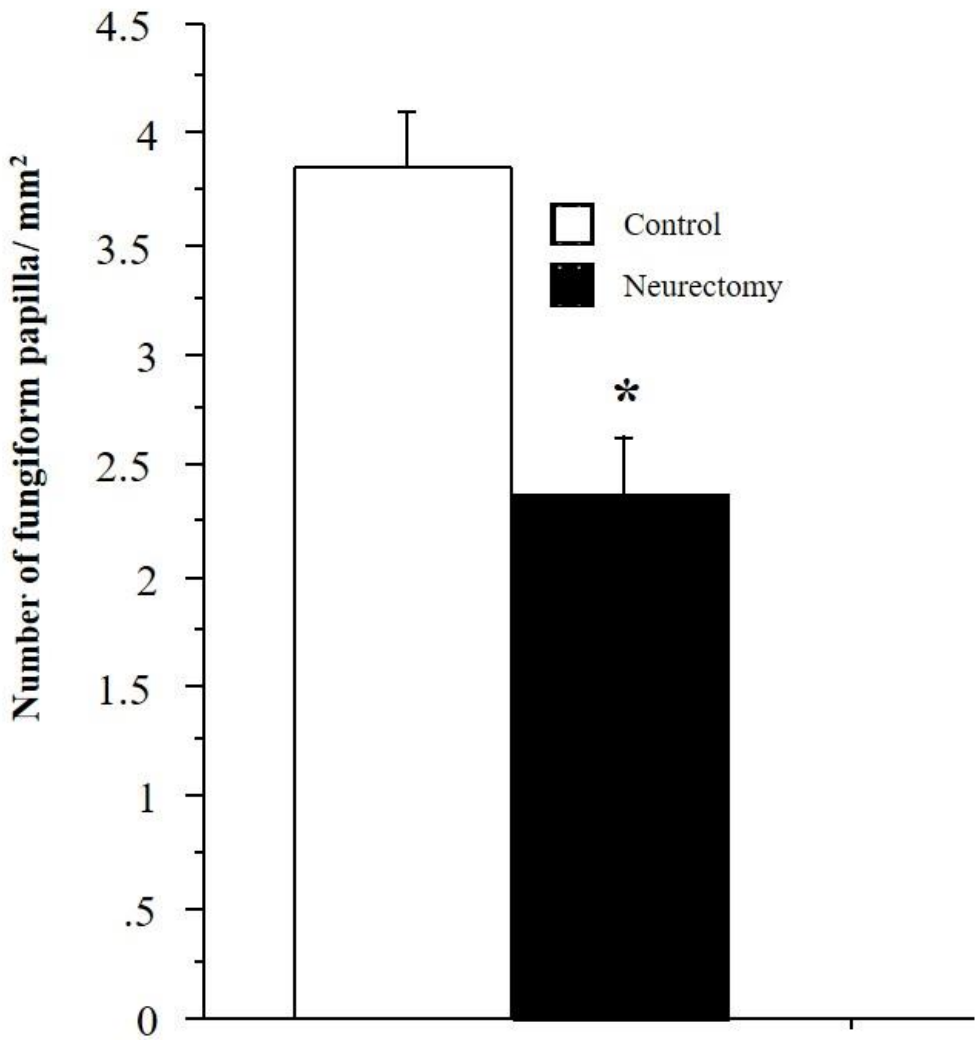
Figure 8.



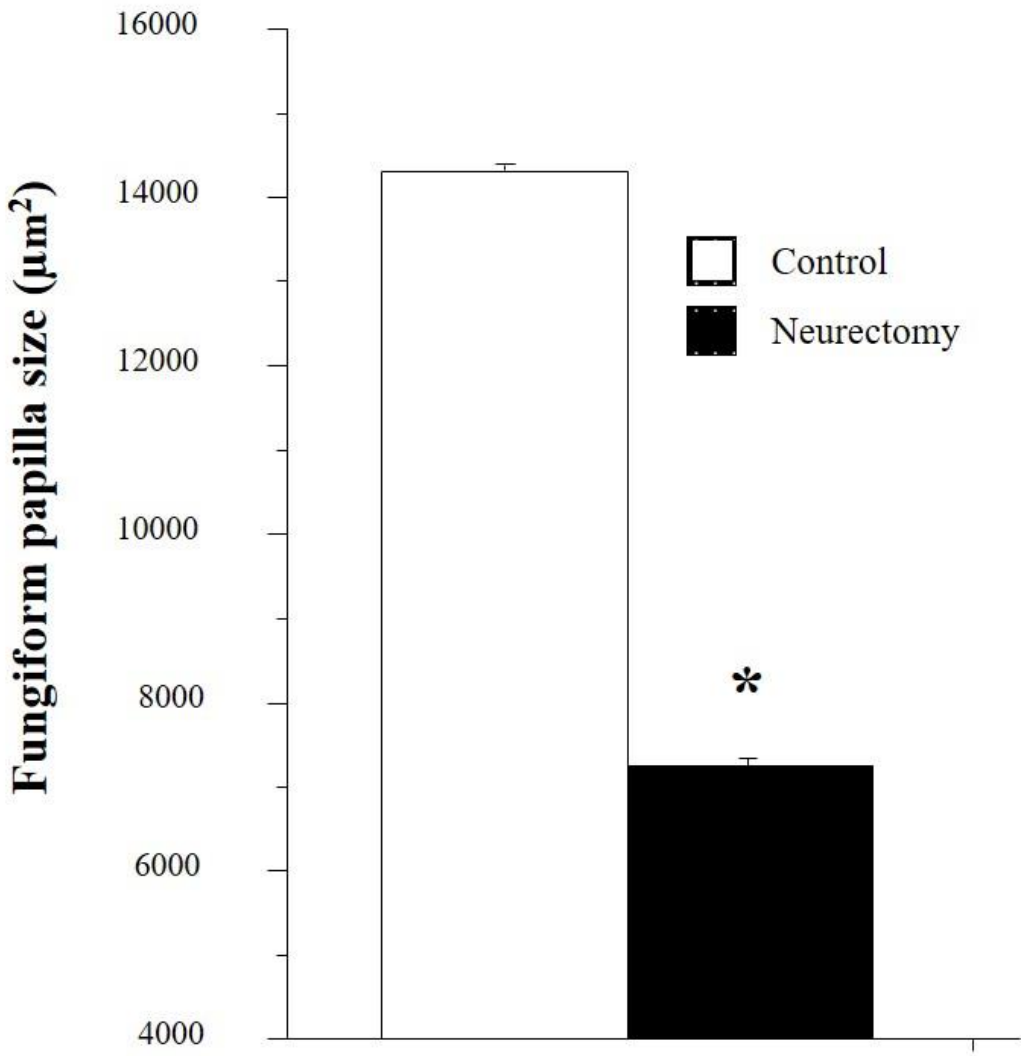
Graph 1.


Fungiform papilla count / cm²
.45
.4
.35
.3
.25
.2
.15
.1
.05
0
*
*
Day 0
Day 1
Day 3
Day 7
Day 14

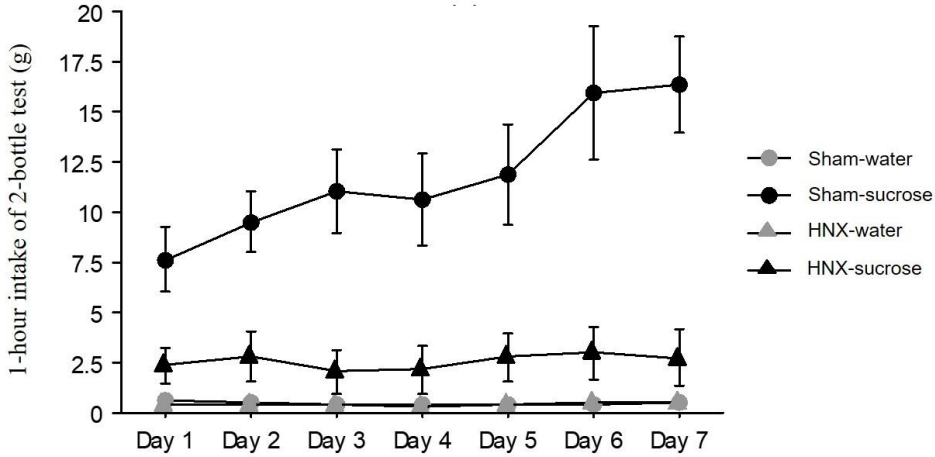
Graph 2.


Number of fungiform papilla/ mm²
4.5
4
3.5
3
2.5
2
1.5
1
.5
0
Control
Neurectomy
*

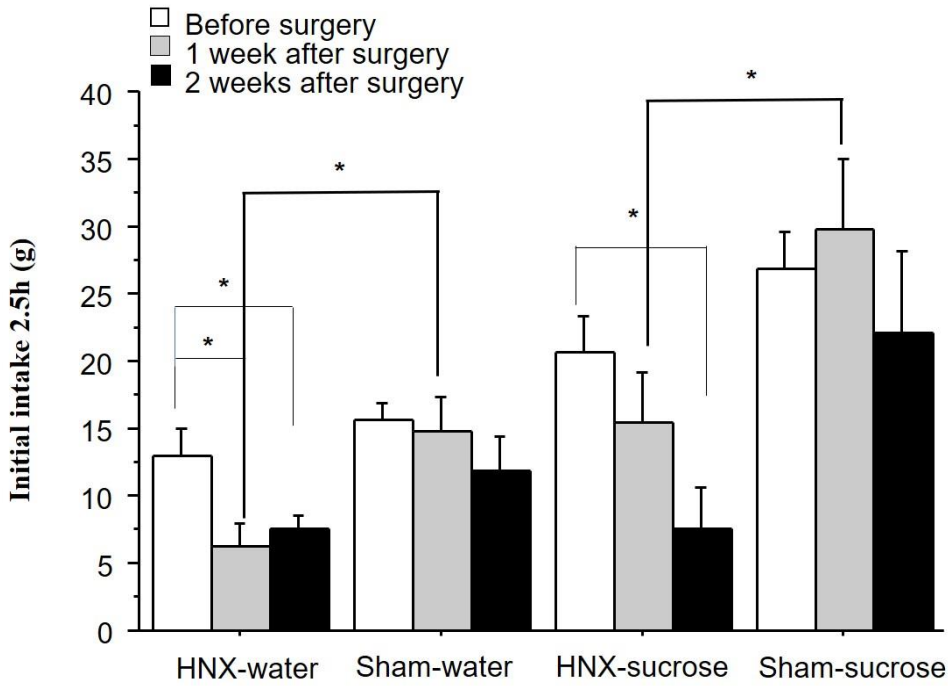
Graph 3.


Fungiform papilla size (μm²)
16000
14000
12000
10000
8000
6000
4000
Control
Neurectomy
*

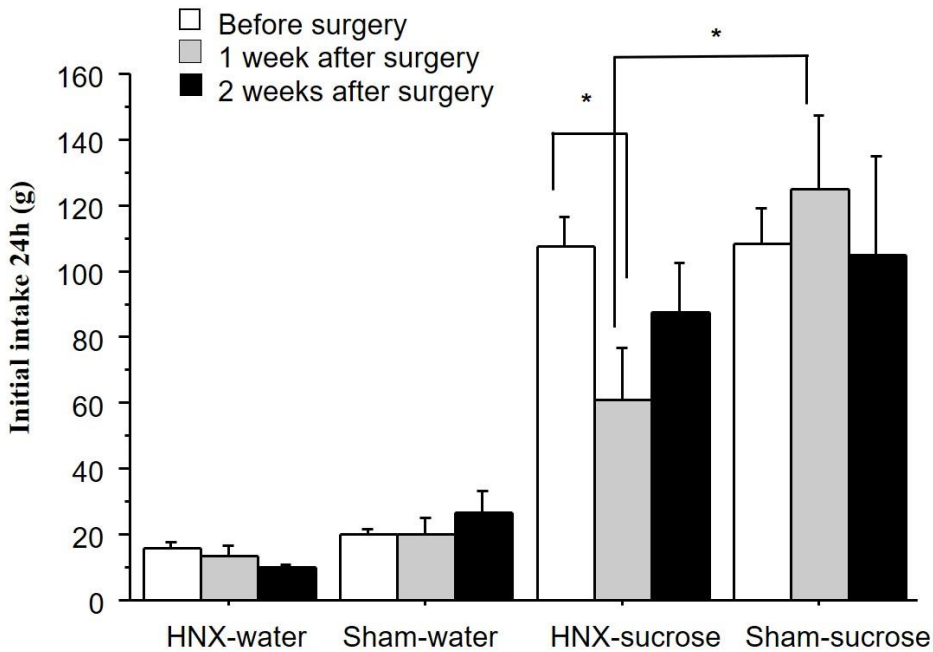
Graph 4.


1-hour intake of 2-bottle test (g)
20
17.5
15
12.5
10
7.5
5
2.5
0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 7
Sham-water
Sham-sucrose
HNX-water
HNX-sucrose

Graph 5.


Before surgery
1 week after surgery
2 weeks after surgery
Initial intake 2.5h (g)
0
5
10
15
20
25
30
35
40
*
*
*
*
*
HNX-water
Sham-water
HNX-sucrose
Sham-sucrose

Graph 6.


Before surgery
1 week after surgery
2 weeks after surgery
Initial intake 24h (g)
160
140
120
100
80
60
40
20
0
*
*
HNX-water
Sham-water
HNX-sucrose
Sham-sucrose

국문초록

양측 설하신경 절단술에 따른 미뢰 형태와 미각인지 변화

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(지도교수 이 종 호)

목적

고삭신경, 구인두신경과 미주신경과 같은 미각신경들은 미뢰와 미각신경을 유지하는 중요한 기능을 가지고 있다. 이전 연구들에서 고삭신경 절단 후 미뢰가 퇴화되는 현상과 설신경과 설하신경 간의 신경학적 연결이 있음이 보고되었다. 본 연구의 목적은 설하신경 손상 혹은 혀 운동능력 저하가 미각세포의 변화와 미각인지에 영향을 미치는지 파악하고자 하였다.

실험 방법 및 재료

백서를 임의로 두 군으로 나누어 신경절단군과 대조군으로 각각 6마리씩 나누어 2번에 걸쳐 실험을 진행하였다. 임상 및 현미경적 관찰로 버섯유두의 수와 형태를 관찰하였으며, 조직학적 변화를 관찰하기 위해 H&E 염색한 후 현미경 관찰을 진행하였다. 또한 미각신경 타입 II의 표지자인 α-gustducin을 이용한 면역형광검사를 시행하여 두 군에서 발현 정도를 비교하였다. 그리고 단맛에 대한 선호도를 조사하여 미각인지

변화를 관찰하였다.

결과

신경절단 실험군에서 혀의 위축성 변화와 함께 버섯유두의 수와 크기 모두 통계적으로 유의하게 감소하는 결과를 보였다. 조직학적 검사에서는 미각신경의 모양이 실험군에서는 대조군과는 달리 불규칙한 형태로 변화하였으며, 크기 또한 감소하는 경향을 보였다. 면역형광검사에서는 미각신경 타입2가 실험군에서 발현이 감소하는 경향을 관찰되었다. 신경절단 실험군에서 단맛에 대한 선호도가 감소하였다. 또한 백서의 부검과정에서 신경절단 실험군에서 모두 위장관의 부종이 관찰되었다.

결론

백서 동물실험에서 양측성 설하신경 절단은 혀의 위축과 함께 버섯유두의 수와 크기를 감소시켰다. 또한 설하신경의 절단은 미각신경 퇴화와 미각인지 저하를 유발하였다.

주요어 : 설하신경, 미뢰, 신경절단, 버섯유두, 미각인지
학번 : 2016-23817