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Morphologic and histologic outcomes of tongue reduction surgery in an animal model

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Abstract

Objective—Describe the effect of anterior tongue reduction surgery on tongue size, morphology and histology.

Design—Prospective experiment.

Materials—Twenty-two 12 week old Yucatan minipigs.

Methods—Six sibling pairs had tongue reduction (Group B) or sham surgery (Group A), and underwent euthanasia the day of surgery. Five sibling pairs had tongue reduction (Group D) or sham surgery (Group C), and were raised for 4 weeks. Data collected included: changes in tongue morphology, histology and animal response to surgery.

Results—All animals tolerated surgery and maintained their weight. Tongue size was uniformly reduced in all animals as compared to sham surgery. Tongue reduction was stable long-term in Group D. All animals had normal wound healing and neurovascular structure preservation. Fibrosis occurred at the repair site.

Conclusion—Midline tongue reduction resulted in uniform tongue reduction in all dimensions and volume, without damaging neurovascular structures. Localized fibrosis is a sequelae of healing.

Introduction

Tongue reduction surgery for non-neoplastic macroglossia is controversial as surgical indications and long-term efficacy are debated.[1-5] This type of surgery has been applied to dental malocclusion and syndromes associated with macroglossia, such as Beckwith-Wiedemann syndrome. More recently tongue reduction surgery has been used for sleep disordered breathing (SDB), commonly seen in Down's syndrome.[6,7] The controversy surrounding tongue reduction surgery is centered on whether techniques of open surgery are effective longterm. Macroglossia, associated with human disease, may lead to progressive tongue enlargement from several etiologies, making surgical results from tongue reduction surgery temporary.[6,8] For example some feel that macroglossia associated with Down syndrome and SDB is primarily due to hypotonia and not an actual increase in tongue size.[7] Obstructive SDB from the tongue base is not addressed by anterior tongue reduction surgery

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(i.e. anterior to the circumvallate papilla), but there are procedures both open and submucosal that can correct this problem.[6-9] In Beckwith-Wiedemann syndrome, it has been thought that the facial skeleton grows to accommodate the size of the tongue, so that tongue reduction may be unnecessary.[2,10] One thing that is important in tongue surgery is to maintain tongue shape and innervation.[1,3,8,9,11] To assess this in a standardized manner we report our findings in anterior tongue body reduction surgery in pigs. These animals have tongues of similar size to humans. In this study we evaluated the morphology and histology in this animal model following surgery.

Materials and Methods

Animals and surgery

Twenty-two 12 week old Yucatan minipigs were used for this study. “Acute” experimental groups consisted of 6 sibling pairs (3 in each gender), who had anterior wedge tongue reduction (group B) or sham surgery (group A), and underwent euthanasia the day of surgery. All animals received tongue and dental impressions before the surgery. “Chronic” experimental groups consisted of 5 sibling pairs (3 males and 2 females), who received the same tongue reduction (group D) or sham surgery (group C) under aseptic conditions. Animals in these groups were raised for 4 weeks after surgery before the euthanasia. The sample sizes of 5-6 in each group were determined by the fact that with the estimated 20-30% coefficients of variation in measured parameters and 1.3 to 1.5 fold ratio of mean between treatment groups, a sample size 4-6 should be adequate for 80% power and 5% significant level.[12] Tongue and dental impressions were taken before surgery and 1-2 days before the euthanasia. Body weights were monitored once a week during the entire experimental period. All procedures were approved by University of Washington IACUC.

With the animal under general anesthesia, local infiltration of the tongue with 1%lidocaine with 1:100,000 epinephrine was performed, incisions through tongue tissue were created with micro-needle electrocautery (ValleyLab SSE2, Colorado Biomedicals, Evergreen, CO), hemostasis was obtained with the same instrument. The surgical defect was closed in layers using 5-0 Monocryl (Ethicon Inc., Somerville, NJ). The amount of tongue resected is depicted (Fig 1). Sham surgery was done by making an incision 2-3 mm into the tongue surface in the same pattern as used for tongue reduction surgery. This incision was closed with the same suture.

In all groups, measures of oral function and feeding behaviors were determined pre and postoperatively. This was done through previously described methods of studying muscle activities, tongue deformations, jaw movements and mechanical loading during feeding (ingestion, chewing and drinking), as well as under stimulated activations of hypoglossal nerves and individual tongue muscles, and the results were reported elsewhere.[13-15]

Morphologic measurements

For acute experiments (groups A and B), the actual linear, volumetric and weight changes in the entire tongue after the surgeries were measured postmortem using a digital caliper, water displacement approach, and a digital balance, respectively. Linear measures of tongue size were made according to the following tongue demarcation: tongue blade: from the tip to the lingual frenum; tongue body: from the lingual frenum to the circumvallate papillae; tongue base: from the circumvallate papillae to the end. The percentage of the loss of weight or volume was calculated using the following formula: (weight or volume of removed portion/ weight or volume of the tongue + weights or volumes of removed portion) × 100%.

For chronic experiments (groups C and D), the linear distance changes in the entire tongue were measured through longitudinal tongue casts in addition to the measurements from postmortem tongue specimens in the same manner as acute experimental animals. Complications related to this invasive procedure, in particular edema and infection, were documented twice a day for the first 5 days, and on a daily base thereafter.

By using SPSS statistical package (ver. 11.0, SPSS Inc. Chicago, IL), the original scales of these measurements were first checked for skewness values and their standard errors to estimate their distribution. These original scales were further transformed to their logarithms and then examined by parametric non-paired (comparison between the two groups at preor postoperative time points) and paired (comparison between preor postoperative time points for the groups C and D) t tests. Because all skewness values were smaller than twice their standard error, and no substantial differences were identified by comparing the t-test results on the transformed data and the original scale, the results of these t tests on the original scales were used for the present study as summarized in Tables 1 and 2.

Histologic evaluations

Six of 12 acute experimental pigs were subjected to Microfil (Flow Tech. Inc, Carver, MA) injection through carotid artery perfusion upon euthanasia. This pressured perfusion first used 0.9% saline, followed by MV-122 Microfil compounds which fills and opacifies all vessels with colored latex. After dissection (see below), the Microfil processed tongue was cleared by alchohol-methyl salicylate, then placed in glycerin with concentrations of 50%, 75%, 85% and 100% for 24 hours.

After euthanasia the remaining six acute animals had tongue excision as follows: the mucosa below the tongue was removed, and the transverse plane between the genioglossal and geniohyoid muscle defined, the posterior tongue was sectioned flush with the hyoid bone's superior surface and tongue specimen removed. These specimens had modified Sihler's staining. This technique renders large postmortem specimens translucent while staining the entire nerve supply. All peripheral nerves and the arrangement of individual muscles can be seen in their normal 3D position.[16,17] Fixed specimens were macerated in 5% KOH, decalcified and stained in Sihler's solution I and II, darkened in 0.05% lithium carbonate solution for 2 hours, destained in Sihler's solution I for 3-4 hours, and cleaned and preserved in 50% and 100% glycerin, respectively.

All 10 chronic study pigs received 0.9% saline perfusion, followed by diluted Prefer solution (Anatech Ltd, Battle Creek, MI). After 1-2 month fixation, the excised tongues were divided into 12 and 14 blocks for reduction and sham tongues, respectively (Fig 2). These blocks provided 3D views (sagittal, coronal and horizontal) in different regions of the tongue. These blocks were embedded in paraffin, and sectioned at a thickness of 10μm . These sections were stained with either hematoxylin & eosin (H&E) or Masson's trichrome.

Role of the funding source

The funding source had no involvement in the conception, design, data analysis, data interpretation and writing of this report.

Results

1. Surgical complications and general health

All acute experimental animals (groups A and B) were able to take and eat food immediately after the surgery. Certain behavior changes were noticed, such as using the mandible, rather than the anterior tongue, to take food into the oral cavity; a slightly distorted chewing rhythm;

and “inertial” chewing/swallowing (i.e. head moving and shaking while chewing and swallowing). No noticeable edema of the tongue was found after the surgery in both acute and chronic experimental animals. In groups C and D, local infection at the tongue incision was found in 3 pigs (2 in group D and 1 in group C), which resolved 7-10 days postoperatively with the application of Clavamax (50mg, Bid, Pfizer Animal Health, New York NY). Slight body weight drop occurred in group C at the first postoperative week. No significant difference was identified between the two groups at each time point (Supplemental Fig 1). All incisions were healed completely by 4 weeks postoperative. One reduction animal had slight tongue tip dehiscence.

2. Immediate changes in tongue morphology

Tongues harvested from acute experimental animals show the tissue removed and the reduction in size in group B as compared to group A (Fig 3). The volume-reduced tongue was shortened and narrowed in the anterior 2/3. The entire lower dental arch is visible following the tongue reduction. The lengths, widths and thicknesses of the tongue blade, body and base, as well as entire tongue volumes and weights were directly measured and compared (Table 1). In Group B, surgery significantly reduced the lengths and widths of the tongue blade and body but not in the tongue base. Tongue blade thickness was also reduced in group B. These same animals also had a significant decrease in both weight and volume of the entire tongue. These measurements not only reveal that the tongue size was reduced consistently and uniformly in Group B, but it was accomplished with minimal postoperative edema in a short term (6-8 hours). Careful dissection and hemostasis with the microneedle seems to minimize potential edema.

3. Long-term changes in tongue morphology

Postmortem tongue morphology of chronic experimental animals demonstrates persistent size reduction in group D as compared to group C (Fig 4). Complete healing occurred in both groups. Direct measurements show reduction of length and width of the tongue blade and body were stable 4 weeks after the tongue reduction surgery, without evidence of tongue muscle hypertrophy (Table 1). It must be mentioned that all animals in this study were juvenile and rapidly growing. Therefore, growth was reflected in the increase of tongue width and thickness, rather than the length, and the reduction surgery did not negatively impact tongue growth (compare groups A and C, and groups B and D in Table 1).

Tongue cast measures in groups C and D demonstrated changes in tongue size four weeks after surgery (Table 2). In groups C the tongue’s anterior 2/3 (blade and body) had significant increases in length, width and thickness, as a result of growth. Linear reductions of the tongue size were only seen in length and width of the tongue blade in group D. In contrast, tongue body length, width and thickness increased at the 4 week measurement. These increases may reflect that factors stimulating normal tongue growth may override the surgical reduction of tongue size in this surgery.

4. Anatomic and Histologic findings

Whole-mount histology demonstrates preservation of tongue neurovascular bundles in both sham and reduction surgery. Postmortem whole tongue specimens processed by modified Sihler’s stain show the hypoglossal nerve with its’ lateral and medial branches, as well as the lingual nerve and its’ distal branches (Fig 5 and supplemental Fig 2). Microfil-vascular casting following reduction surgery reveals vascular preservation (Fig 6). Furthermore, coronal sections through the block12C (Fig 2) in reduction tongues show undisturbed neurovascular bundles located about 15mm inferior from the dorsal surface, similar to the sham tongue specimen (Fig 7 and supplemental Fig 3).

Complete external healing was seen after the surgery (Fig 4), but surgical site wound healing in group D did not demonstrate muscle fiber reconstitution as seen in group C. Trichrome-stained sagittal sections from the block 5S (Fig 2) in group C demonstrate collagen-rich underlying lamina propria and multiple transverse muscle fiber bundles interwoven with many vertical and a few longitudinal fibers. Collagen fibers were localized and organized in bundles (Supplemental Fig 4). In group D, disorganized collagen fibers were interwoven with a few sporadic muscle fibers, which lacked any detectable arrangement or orientation and had reduced myofiber perimysium, a typical sign of fibrosis (Fig 8). Coronal sections show scar tissue in the incision site in group C and in the reduction tongue surgical site in group D. Muscle fibers are reduced in number and size at the surgical site in group D as compared to group C.

Discussion

One of the goals of any tongue reduction procedure is to reduce tongue size while maintaining normal tongue function and shape.[1] Resection of the midline of the tongue in this animal model has demonstrated that masticatory and swallowing behavior changed after surgery, but that general health, tongue function and overall feeding function were not affected.[13,14] This is most likely due to preservation of tongue neurovascular structures. Lateral tongue resection always removes innervated tissue and puts these structures at risk more than midline resection, potentially compromising tongue function.[8] Preservation of neurovascular anatomy also helps wound healing. Midline tongue and/or lateral tongue reduction do not change the size of the tongue base, which must be treated in a different manner if it needs reduction.

Studies describing how tongue reduction surgery changes tongue shape and function have not been done. This study demonstrates that the surgery reduces tongue size in a predictable manner. The tongue did not hypertrophy in the four week period of observation after the surgical reduction; only normal growth was seen. When tongue surgery is done for macroglossia treatment, it has been hypothesized that hypertrophy of residual tongue tissue occurs, and reduction of tongue size is temporary. Our study does not demonstrate this occurrence in the growing pig. However, this information may not be applicable to macroglossia associated with human disease.

Wound healing readily occurs in the oral cavity. Use of microneedle electrocautery has been described in other types of oral surgery and is associated with less tissue trauma. Our use of this device may have improved our outcomes due to the reduction in surgical trauma and theoretic improvement in wound healing. However, fibrosis without predominant myogenic regeneration is the major histologic consequence four weeks after tongue volume reduction. It has been claimed that electrocautery might cause more collateral tissue damage and late wound healing as compared to coblation in tongue surgery.[18] Therefore, different ablative devices may result in different histologic consequences in the tongue. A cohort animal study is ongoing to compare functional, morphological and histologic outcomes by using electrocautery, temperature-controlled radiofrequency ablation, and coblation on the tongue volume reduction surgery.

Conclusion

Tongue reduction surgery was well tolerated in this animal model and demonstrated the ability to uniformly reduce tongue weight and size using anterior midline tongue reduction. Tongue function was unaffected by this surgery, even though the length and width of the tongue were reduced. Histologic findings demonstrated that the neurovascular bundle of the tongue was untouched by midline tongue reduction surgery, and healing of the surgical site occurred without incident.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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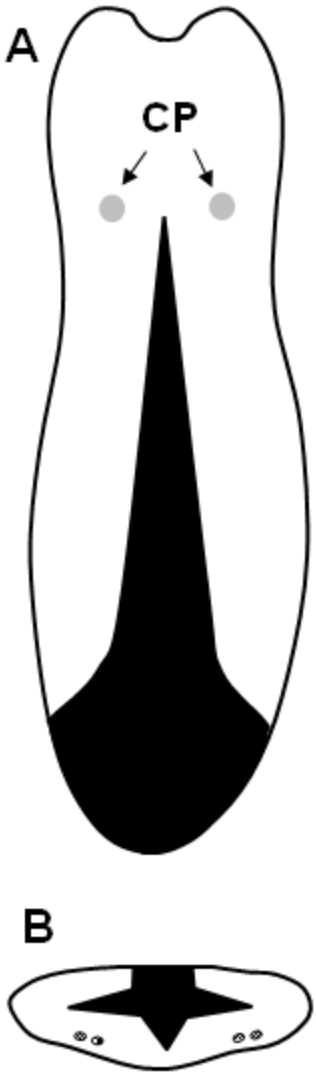

Figure 1.
Incision lines in dorsal (**A**) and cross-sectional (**B**) views for tongue volume reduction surgery. Black portions of diagram depict the tongue tissue resected from the circumvalate papilla (**CP**) anteriorly. Dots in B indicate neurovascular bundles.

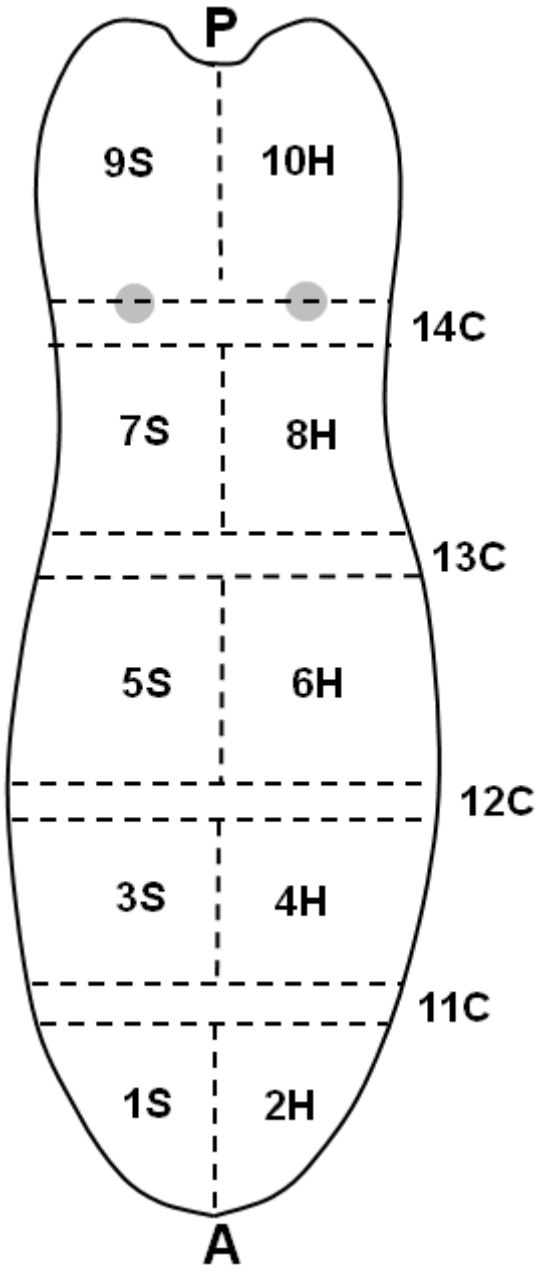

Figure 2.
Schematic of location for histologic sections of tongue specimens. **A**: anterior; **P**: posterior; **S**: sagittal; **C**: coronal; **H**: horizontal.

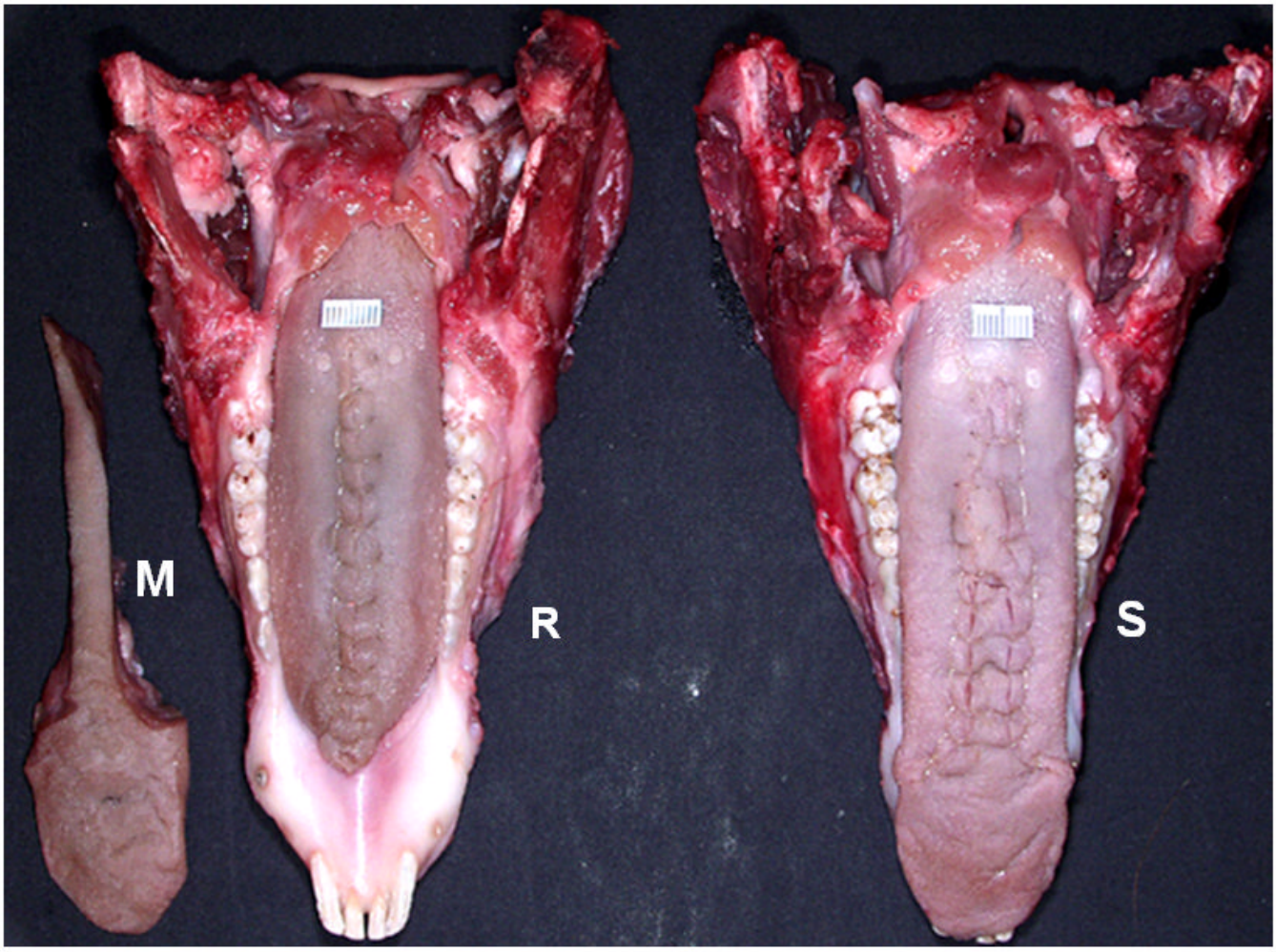

Figure 3.
Postmortem tongue specimens immediately after surgery. **R**: reduction tongue, groups B; **S**: sham tongue, groups A; **M**: removed tongue tissue, group B only.

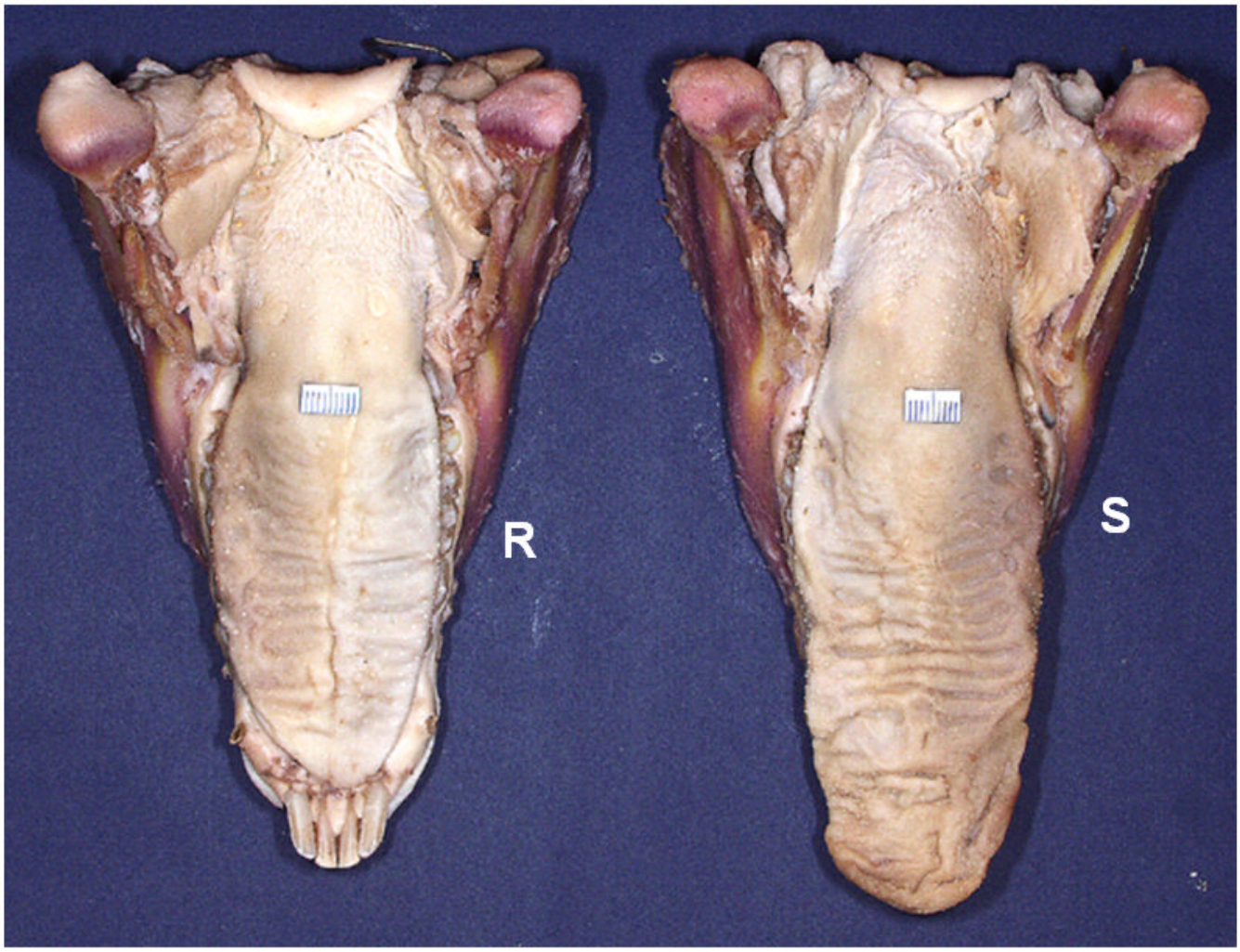

Figure 4.
Postmortem tongue specimens 4 weeks after surgery. **R**: reduction tongue, groups D; **S**: sham tongue, groups C.

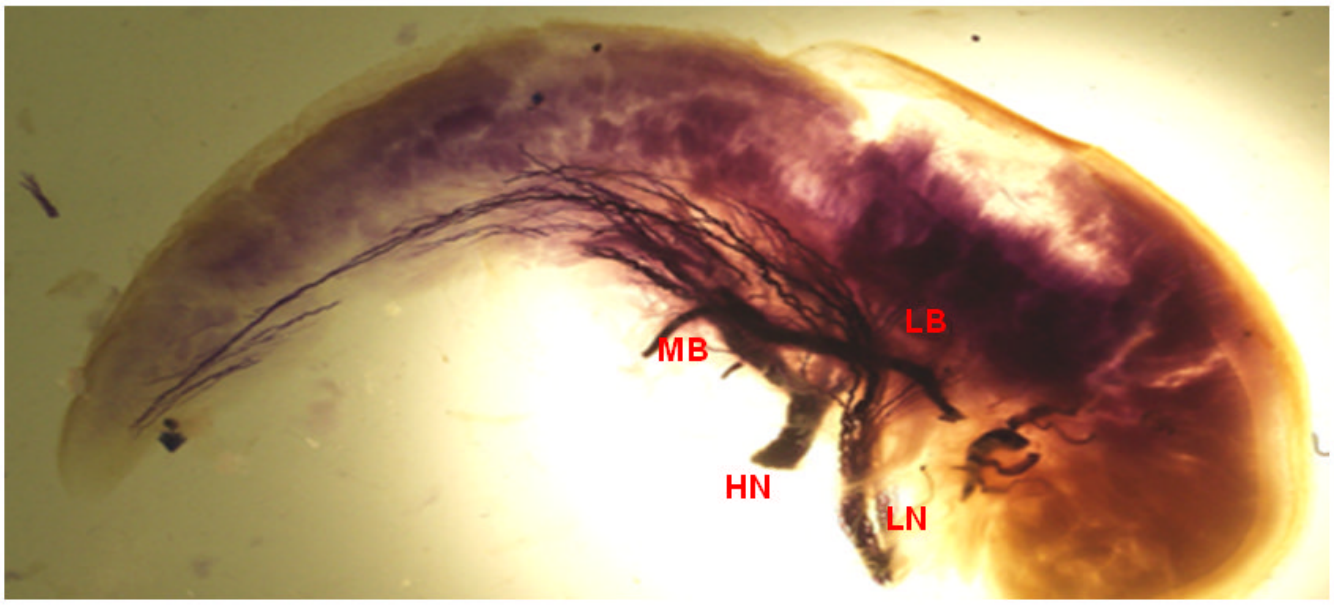

Figure 5.
Sihler's stained whole tongue specimen. Reduction tongue, group B. **HN**: hypoglossal nerve; **LN**: lingual nerve; **MB**: medial branch of the HN; **LB**: lateral branch of HN.

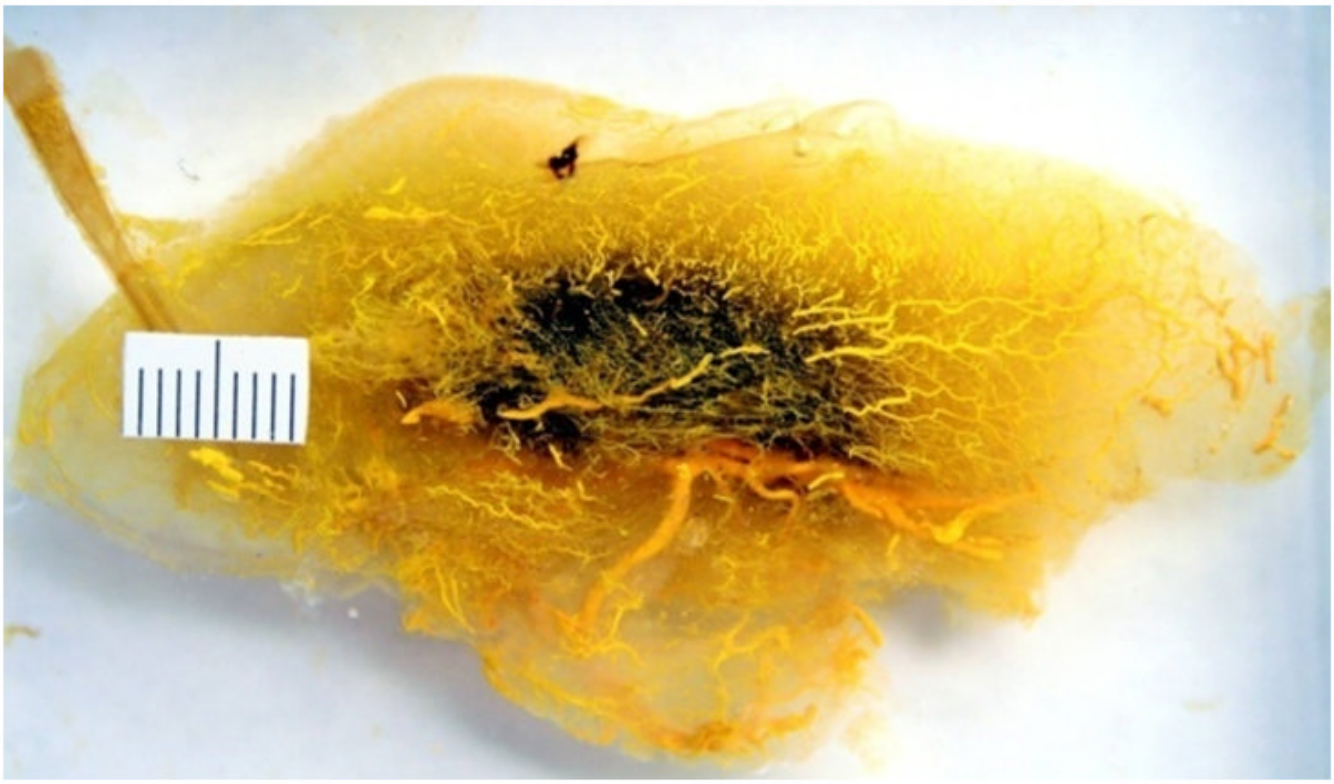

Figure 6.
A Microfil casting of vasculature of a reduction tongue specimen, group B.

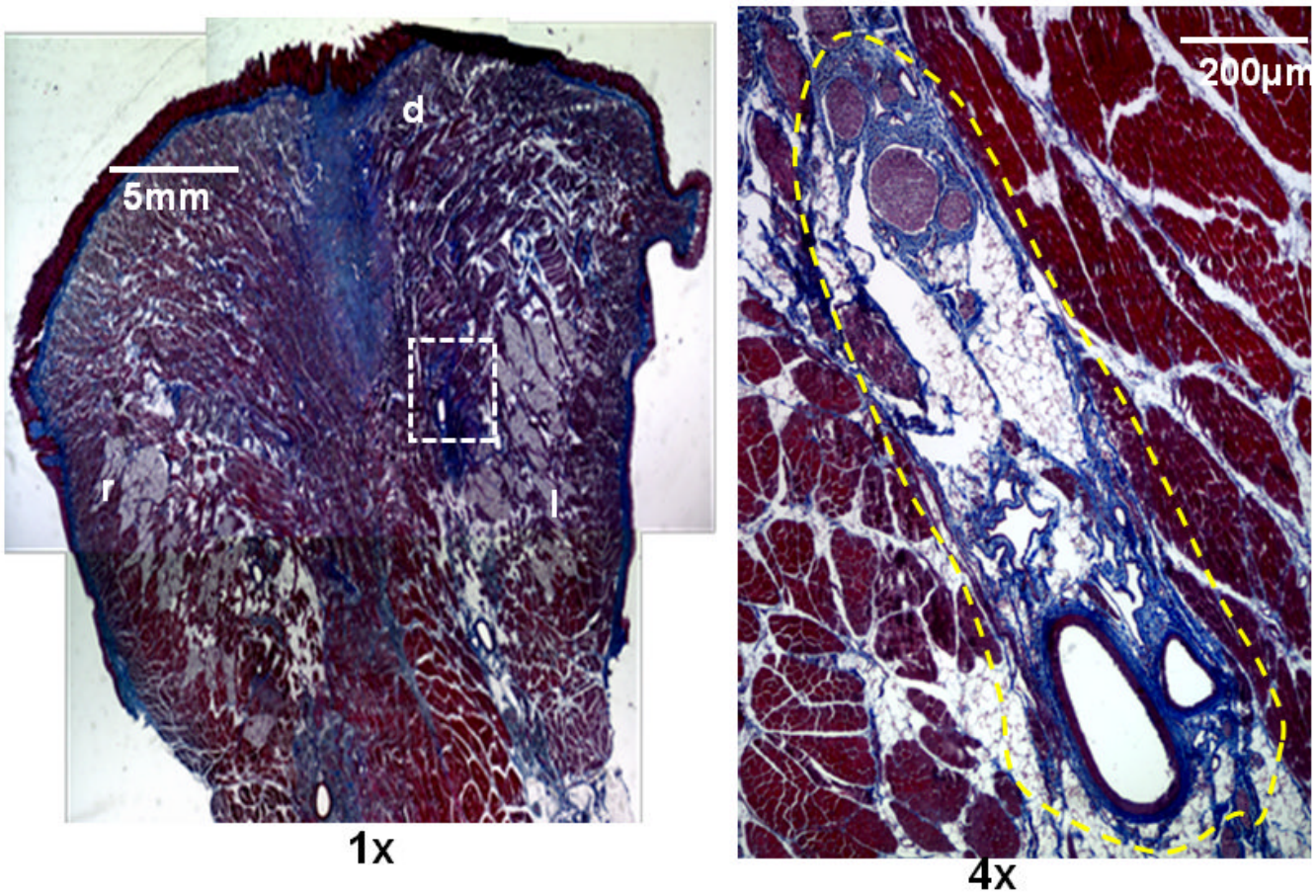

Figure 7.
Coronal sections of the tongue body (trichrome, 1x and 4x objectives) from the block12C (refer to Fig. 2). White boxes in A and C images indicate the sampling locations. Yellow dashed lines circumscribe the area of neurovascular bundles of the tongue. Lower-case letters indicate the orientation of the sections. **d**; dorsal, **r**: right side; **l**: left side.

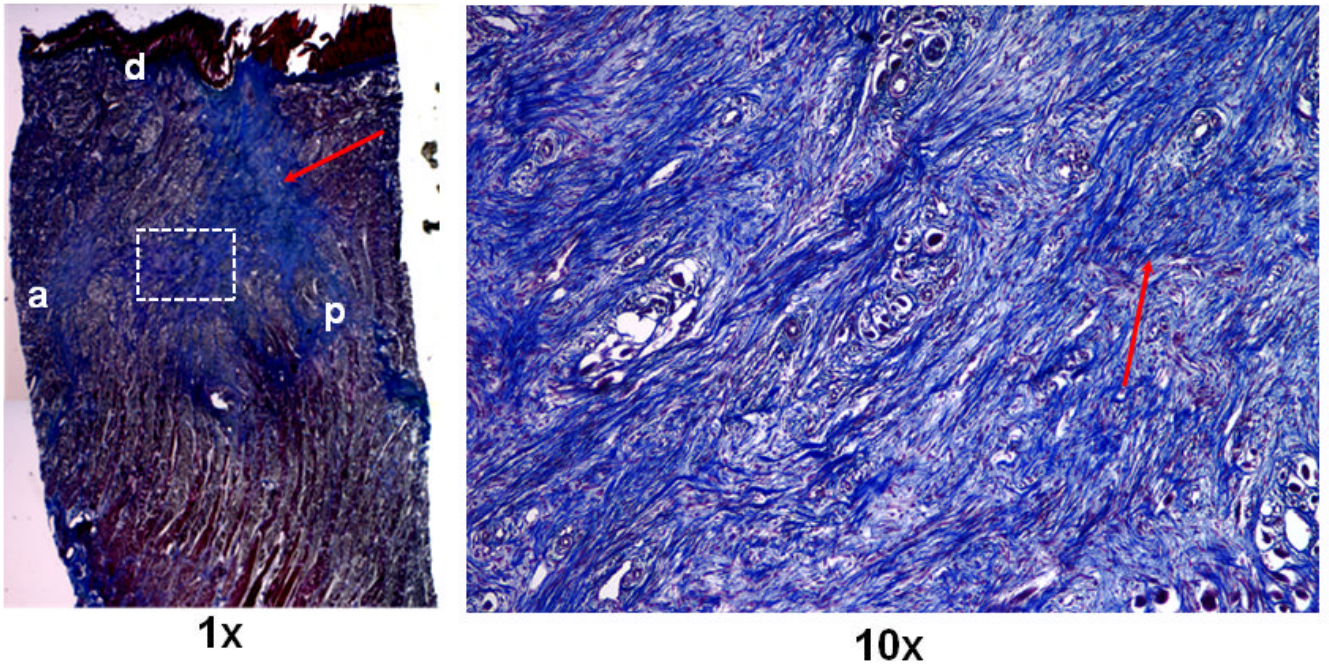

Figure 8.

Mid-sagittal sections of the tongue body (trichrome, 1x, 4x and 40x objectives) from the block 5S (refer to Fig.2). White boxes indicate the sampling locations. Red arrows indicate collagen-rich scar tissue, and yellow arrows indicate myofibers. Lower-case letters indicate the orientation of the sections. **d**; dorsal, **a**: anterior; **p**: posterior.

Table 1

Measurements of Postmortem Tongues

	N	Length (mm)			
Group		Blade	Body	Base	Total
A	6	39.2±1.1	65.9±4.5	33.8±3.3	138.8±5.9
B	6	20.9±2.6***	52.5±3.1***	34.2±2.1	107.5±6.1***
C	5	40.6±2.8	56.8±3.2	35.3±1.5	134.5±5.0
D	5	17.1±2.7***	52.9±2.8*	34.8±1.6	104.7±2.3***
	N	Width (mm)			
Group		Blade	Body	Base	
A	6	33.9±3.5	33.7±2.2	23.5±1.0	
B	6	15.0±1.7***	25.1±1.9***	23.9±1.1	
C	5	37.3±2.5	35.7±2.4	29.4±1.0	
D	5	23.0±0.9***	30.2±1.6**	27.5±0.9*	
	N	Thickness (mm)			
Group		Blade	Body	Base	Thickest
A	6	8.4±0.4	29.4±2.0	28.4±3.6	33.5±3.7
B	6	6.4±1.3**	28.8±3.5	26.3±3.7	32.1±2.7
C	5	9.9±0.9	37.1±1.7	29.0±1.5	37.7±1.9
D	5	9.0±0.5	34.5±2.9	26.8±1.3*	35.1±1.3*
	N	Weight (mm)			
Group		Weight	Loss%		
A	6	75.7±5.5	n/a		
B	6	59.2±1.4***	17.2±1.2%		
C	5	71.4±1.5	n/a		
D	5	59.4±1.3***	15.2±0.8%		
	N	Volume (mm^3)			
Group		Volume	Loss%		
A	6	75.5±4.8	n/a		
B	6	59.5±1.2***	17.2±1.0%		
C	5	72.0±2.2	n/a		
D	5	60.6±0.9***	15.0±0.7%		

Asteriks indicate significant differences between groups A and B and between groups C and D (non-paired t tests).

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$.

Table 2

Measurements of Tongue Casts

Groups		Length		Width		Thickness
		Blade	Body	Blade	Body	Blade
C	before	40.2±2.5	63.2±2.9	34.3±1.5	25.9±1.8	7.9±0.4
	4W after	45.6±2.9*	67.5±3.3	36.2±2.3	31.5±2.3**	9.2±0.6**
D	before	41.7±3.2	62.8±1.6	33.2±2.8	24.7±1.2	8.0±0.3
	4W after	16.5±2.6***	66.4±3.6	23.2±2.6***	27.5±0.6**	10.5±0.3***
Non-paired *t*-test	Before	-	-	-	-	-
	4W after	C > D###	-	C > D##	C > D#	C > D#

* Comparisons before and after the surgery (paired *t* tests)

Comparisons between the two groups (non-paired *t* tests).

* $p < 0.05$

** $p < 0.01$

*** $p < 0.001$

\# $p < 0.05$

\#\# $p < 0.01$

\#\#\# $p < 0.001$