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Anatomic Analysis of Masseteric-to-zygomatic Nerve Transfer in Rat and Pig Models

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Background: Nerve transfer from the masseteric branch of the trigeminal nerve is a widely performed procedure for facial reanimation. Despite achieving powerful muscle force, clinical and aesthetic results leave room for improvement. Preclinical animal models are invaluable to establishing new therapeutic approaches. This anatomical study aimed to establish a masseteric-to-zygomatic nerve transfer model in rats and pigs.

Methods: The masseteric branch of the trigeminal nerve and the zygomatic branch of the facial nerve were dissected in 30 swine and 40 rat hemifaces. Both nerves were mobilized and approximated to achieve an overlap between the nerve ends. Over the course of dissecting both nerves, their anatomy, length, and branching pattern were documented. At the coaptation point, diameters of both nerves were measured, and samples were taken for neuromorphometric analysis.

Results: Anatomic details and landmarks were described. Tension-free coaptation was possible in all rat and pig dissections. In rats, the masseteric branch had an average diameter of 0.36 mm (±0.06), and the zygomatic branch average diameter was 0.46 mm (±0.13). In pigs, the masseteric branch measured 0.52 (±0.16) mm and the zygomatic branch, 0.59 (±0.16) mm. No significant differences were found between the diameters and axon counts of both nerves in pigs. In rats, however, their diameters, axon counts, and fascicular areas were significantly different.

Conclusion: Our study demonstrated the feasibility of direct masseteric-to-zygomatic nerve transfer in rats and pigs and provided general anatomic knowledge of both nerves. *(Plast Reconstr Surg Glob Open 2023; 11:e5344; doi: 10.1097/GOX.0000000000005344; Published online 18 October 2023.)*

INTRODUCTION

Peripheral facial paralysis causes severe facial disfigurement and can result in significantly reduced social interactions and quality of life.[1] Numerous reconstruction techniques have been developed to restore facial symmetry.[2–5] Dynamic reconstruction aims to re-innervate muscles of facial expression to restore facial symmetry in a moving face. These procedures typically involve nerve repair via neurorrhaphy, nerve grafting, or nerve transfer.[6] Nerve transfer is a common reconstructive approach when the proximal nerve stump is not available.[7,8] Donor nerve axons regenerate along the distal facial nerve branches to re-innervate the mimetic muscles.[9,10] Several variables should be taken into account when selecting a donor nerve to maximize functional outcomes. Donor site morbidity needs to be carefully weighed. The donor nerve should approximately match the diameter and axon count of the recipient nerve. The donor nerve should also be close enough, or long enough and mobile enough to reach the recipient nerve to allow tension-free nerve coaptation without the need for an interposition graft.[11,12] Ideally and if available and dispensable, the donor nerve should drive a synergistic movement to facilitate posttransfer functional training. Widely used donor nerves for facial paralysis include the masseteric branch of the trigeminal nerve and the hypoglossal nerve.[7,8,13,14] The masseteric nerve gained popularity due to its proximity to the facial nerve as well as accessibility for free

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muscle transfers. Furthermore, it provides reliable, powerful muscle force due to its high number of axons. Great consistency is observed in its anatomic course, and utilization of the nerve leads to little to no co-morbidity.[8,15] Nevertheless, despite the advances in facial nerve reconstruction, facial reanimation outcomes are often unpredictable, subpar, and largely variable between patients.[16–18] Preclinical animal models are invaluable in refining surgical approaches. This study aimed to establish a small and a large animal model of masseteric-to-zygomatic nerve transfer, including anatomical knowledge of the zygomatic branch of the facial nerve and the masseteric branch of the trigeminal nerve and demonstrating feasibility of the nerve transfer.

Takeaways

Question: Is rat or pig a suitable model animal for masseteric-to-zygomatic nerve transfer procedures?

Findings: Dissection of the masseteric branch of the trigeminal nerve and the zygomatic branch of the facial nerve was conducted in swine and rat hemifaces to document their anatomic courses, branching patterns, length, diameter, and mobility for nerve transfer. Histology of both nerves was done to determine the match in their fascicular area and axon count. Our results showed that direct masseteric-to-zygomatic nerve transfer is achievable in both rats and pigs.

Meaning: Rat and pig masseteric-to-zygomatic nerve transfer are suitable models for facial reanimation studies.

METHODS

Fifteen domestic market hog cadavers and 20 Sprague Dawley rat cadavers were used following euthanasia under Institutional Animal Care and Use Committee–approved studies at our institution. This anatomic study was exempt from Institutional Animal Care and Use Committee approval. Previous studies did not alter nerve physiology or head and neck anatomy of these animals.

Surgical Approach

The masseteric branch of the trigeminal nerve and the zygomatic branch of the facial nerve were dissected in 30 swine hemifaces and 40 rat hemifaces. The anatomical dissection took place under 2.5× magnification with surgical loupes either immediately after euthanasia or after freeze-thawing. In pigs, a preauricular incision was made, and the skin was separated from the underlying superficial musculoaponeurotic system (SMAS). The parotid gland was retracted to fully expose the facial nerve root, as the gland was located superficially to the nerve. After identifying the zygomatic branch coming off the facial nerve root, it was traced distally toward its branches to the orbicularis oculi muscle (Fig. 1). In rats, the skin superior to the edge of the mandibular border was lifted, and an incision was made into the skin, thereby preventing injury to deeper structures. The SMAS was identified as a thin musculoaponeurotic layer, which was tightly attached to the skin. By further separating the skin from the underlying musculature, the facial nerve was easily exposed, running on top of the masseter and temporalis muscle. The parotid gland was removed to allow better access to the facial nerve root. The zygomatic branch was then identified and traced distally (Fig. 2). In both animal models, the masseteric branch was exposed by separating the masseter muscle fibers at its insertion at the inferior edge of the zygomatic bone. Over the course of dissecting both nerves, their anatomy and branching pattern was documented. The zygomatic branch was mobilized from the facial nerve root to the intramuscular branches at the orbicularis oculi muscle and was cut proximally where it came off the facial nerve. The masseteric nerve was mobilized from below the zygomatic bone to its intramuscular branches and cut distally where it gave off branches. By rotating the distal end of the masseteric branch superiorly toward the zygomatic bone and the proximal end of the zygomatic branch toward the snout, overlap of both nerve ends was achieved. The diameter of both nerves at the coaptation point was measured with a digital caliper. The coaptation point was the point on the re-routed zygomatic branch where the distal end of the masseteric branch reached, where nerve coaptation would take place. The excess length of the zygomatic nerve where it overlapped with the masseteric branch was also measured. The regenerative distance from the coaptation site to the intramuscular branches of the zygomatic nerve was measured before taking histology samples of each nerve.

Histology

Five left hemifaces and five right hemifaces were randomly selected in pig and rat cadavers that were immediately dissected after euthanasia to collect nerve samples for histomorphometric analysis. An approximately 3-mm-long segment of the masseteric nerve and zygomatic nerve was harvested at the overlap and placed in Trump's fixative.[19] This made for 10 masseteric nerve samples and 10 zygomatic nerve samples each in rats and pigs. After fixation, nerve samples were epoxy-embedded, cut into 1-µm-thick cross-sections, and stained with 1% toluidine blue O.[19] These nerve cross-sections were scanned at 20× using MoticEasyScan Pro 6 (MOTICEUROPE, S.L.U., Barcelona, Spain). These images were then analyzed with Image J to measure total fascicular area and with Ilastik-1.4.0b15-OSX cell density counting[20] to acquire axon count.

Statistical Analysis

All quantitative measurements were presented as mean (±SD). To compare data between the donor and recipient nerves, a Mann Whitney *U* test was used. Calculations were performed using GraphPad Prism Version 9.2.0. A *P* value of less than 0.05 was considered significant.

RESULTS

Pig Anatomy

After branching off the facial nerve root, the zygomatic branch continued its course toward the orbicularis

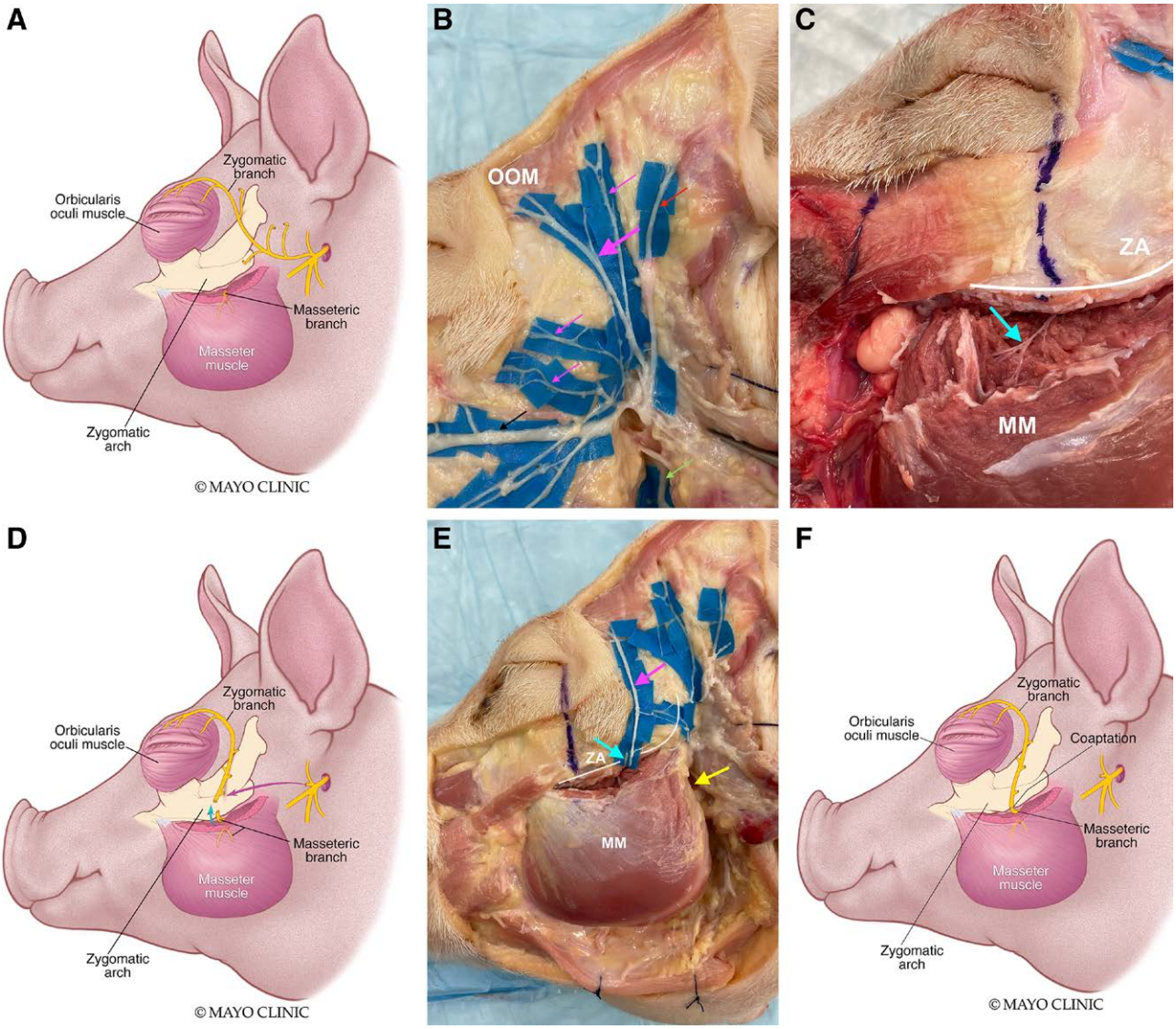

Fig. 1. Pig masseteric-to-zygomatic nerve transfer model. A, Schematic drawing of the anatomy of the zygomatic branch and the masseteric branch. The zygomatic branch is shown to be one of the five motor branches after the facial nerve has emerged from the stylomastoid foramen. It courses toward and innervates orbicularis oculi muscle (OOM) while giving off multiple branches along the way. The masseteric branch of the trigeminal nerve runs deeper to the masseter muscle after emerging below the zygomatic arch (ZA). B, Hemiface dissection photograph showing the zygomatic branch (pink arrows) that passes across the ZA toward the OOM while traveling from deep to superficial. The other peripheral motor branches of the facial nerve, temporal (red arrow), buccal (black arrow), and cervical branch (green arrow) are also shown. C, Exposure of the masseteric branch (arrow) after detaching the masseter muscle (MM) from the zygomatic arch (ZA). D, Schematic drawing of re-routing the zygomatic branch (pink arrow) and the masseteric branch (blue arrow) for the nerve transfer. The zygomatic branch was cut proximally and rotated anteriorly after several secondary branches were cut to mobilize it. The masseteric branch was cut distally and rotated superiorly to meet the zygomatic branch. E, The zygomatic branch (pink arrow) and the masseteric branch (blue arrow) were aligned next to each other to measure the overlapping distance, starting from the distal end of the masseteric branch to the proximal end of the zygomatic branch. The yellow arrow points toward the level of the facial nerve root. F, Schematic drawing of the completed masseteric-to-zygomatic nerve transfer. Drawings A, D, and F used with permission from Mayo Foundation for Medical Education and Research. All rights reserved.

oculi muscle. At first, the nerve ran through a thick fatty tissue compartment, rostral to the parotid gland. While traveling from deep to superficial, it gave off multiple delicate branches to the lower eye lid and midface. On average, the zygomatic nerve gave off its first branch 15.6 (±7.6) mm distal to the facial nerve root. The second was found after 24.7 (±9.2) mm and the third after 33.1 (±13.9) mm. The fourth, fifth, and sixth secondary branch were an average of approximately 10 mm apart from each other. The seventh branch measured 67.9 (±14.4) mm, the eighth branch 71.8 (±14.8) mm, and the ninth branch 79.2 (±13.8) mm away from the stylomastoid foramen. One pig demonstrated 12 secondary branches, the last of which was a distance of 90 mm from

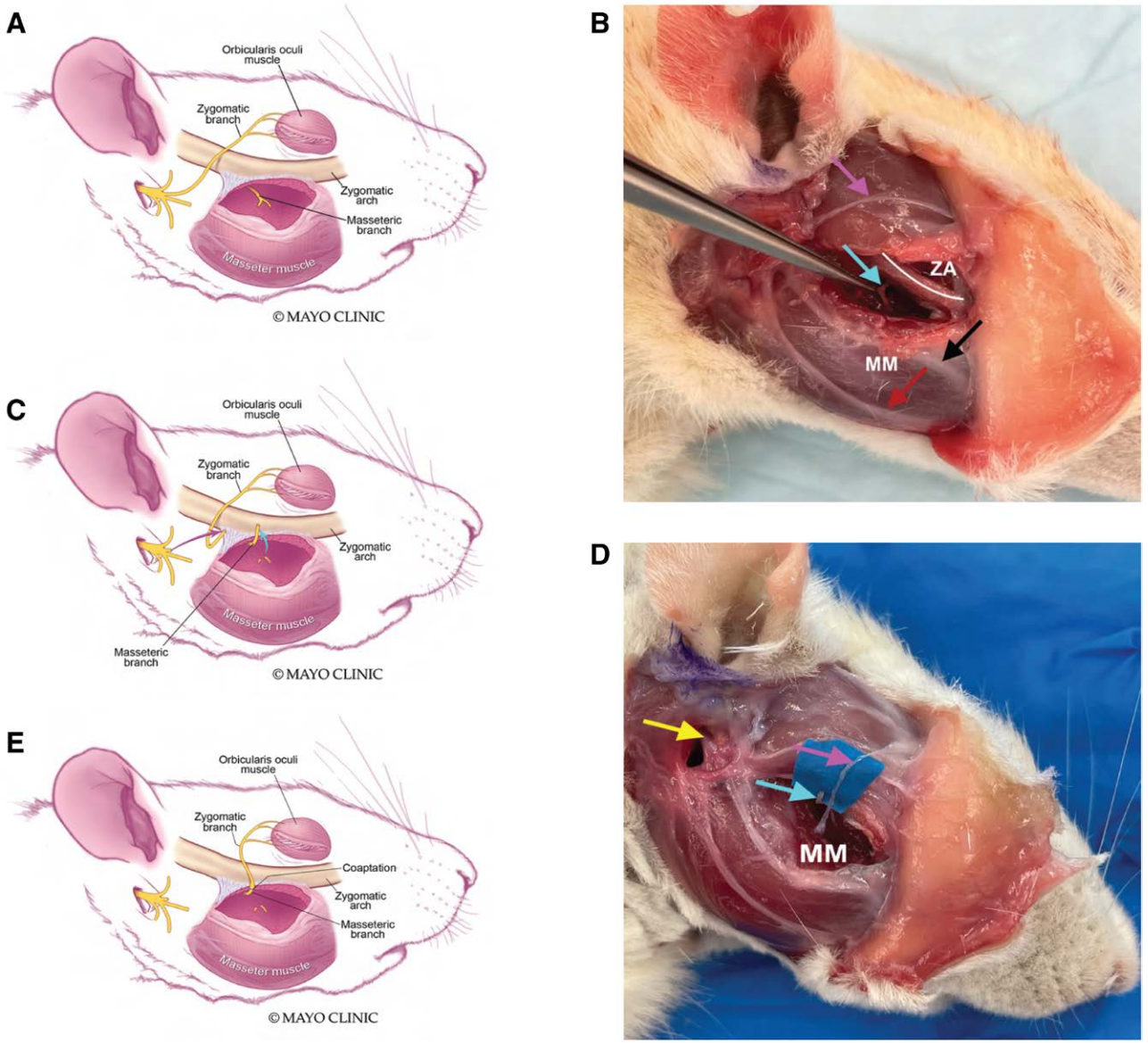

Fig. 2. Rat masseteric-to-zygomatic nerve transfer model. A, Schematic drawing of the anatomy of the zygomatic branch of the facial nerve and the masseteric branch of the trigeminal nerve. The zygomatic branch also appears to be one of the five motor branches coming off the facial nerve root. It, however, has much fewer secondary branches compared with the pig model. B, The facial nerve branches were readily visible after removing the skin flap. The zygomatic branch (pink arrow) was identified as passing across the zygomatic arch (ZA) toward the orbicularis oculi muscle. The buccal branch (black arrow) and the marginal mandibular branch (red arrow) were seen coursing on top of the masseter muscle (MM). The masseteric branch (blue arrow) was exposed via an incision detaching the muscle fibers of the MM from the zygomatic arch. C, Schematic drawing of re-routing the zygomatic branch (pink arrow) and the masseteric branch (blue arrow) for the nerve transfer. The zygomatic branch was cut proximally and rotated anteriorly, whereas the masseteric branch was cut distally and rotated superiorly. D, The zygomatic branch (pink arrow) and the masseteric branch (blue arrow) were aligned next to each other to measure the overlapping distance from the distal end of the masseteric branch to the proximal end of the zygomatic branch. The yellow arrow points toward the facial nerve root. E, Schematic drawing of the completed masseteric-to-zygomatic nerve transfer. Drawings A, C, and E used with permission from Mayo Foundation for Medical Education and Research. All rights reserved.

the nerve root. After the nerve passed over the zygomatic arch, it continued to traverse from deep to superficial toward the facial muscles. In total, the zygomatic nerve had an average of 11 branches (±4) (Table 1). In all dissections, at least one zygomatic branch innervated the orbicularis oculi muscle. This branch was chosen as the recipient nerve for the masseteric-to-zygomatic nerve transfer. The zygomatic nerve measured 65.9 (±11.9) mm long from where it came off the facial nerve root to where it entered orbicularis oculi muscle. The masseter muscle fibers were detached from superficial to deep off of the zygomatic bone, and the masseteric nerve was found 7.18 (±1.94) mm deep to the zygomatic bone. The masseter muscle measured 72.9 (±2.9) mm in

Table 1. Comparison of the Zygomatic and Masseteric Branch in the Rat and Pig Models

	Rat	Pig
Zygomatic nerve diameter (mm)	0.46 ± 0.11	0.59 ± 0.16
Masseteric nerve diameter (mm)	0.36 ± 0.06	0.52 ± 0.16
Zygomatic nerve length (mm)	17.7 ± 3.19	65.95 ± 11.89
Masseteric nerve length (mm)	4.25 ± 1.59	11.68 ± 2.60
Zygomatic nerve axon count	282.36 ± 103.92	861.91 ± 928.86
Masseteric nerve axon count	526.2 ± 144.71	818.81 ± 223.44
Zygomatic nerve fascicular area (mm^2)	0.020 ± 0.08	0.150 ± 0.13
Masseteric nerve fascicular area (mm^2)	0.111 ± 0.05	0.160 ± 0.03
Zygomatic nerve fascicle quantity	1.54 ± 0.68	6.40 ± 3.20
Masseteric nerve fascicle quantity	1.20 ± 0.42	5.20 ± 2.25
Regenerative distance (mm)	9.17 ± 2.53	57.92 ± 10.6

width horizontally, and the motor nerve was 34.2 (±6.6) mm away from the posterior border of the muscle. As the nerve emerged below the zygomatic bone, it usually consisted of one to two branches giving off two (±1.3) intramuscular branches inferiorly. In total, it had a mean of three branches (±1.4). The first intramuscular branch was found 7.1 (±4.4) mm after the masseteric branch emerged below the zygomatic bone, followed by the second branch at 9.9 (±3.7) mm. The total length of the masseteric nerve measured 11.7 (±2.6) mm, starting from the edge of the zygomatic bone to where it gave off the first intramuscular branches.

Nerve Transfer in the Pig Model

Tension-free masseteric-to-zygomatic nerve coaptation was possible with a minimum of 0.5 mm overlap between the donor and recipient nerves by cutting four (±2.2) secondary branches of the zygomatic nerve to mobilize it. No overlap was possible without cutting at least one branch of the zygomatic nerve. In 60% of the dissections, the nerve transfer was possible without cutting any of the masseteric branches, in 23.3% one branch had to be cut, in 6.6% two branches had to be cut and in 3.3% three branches had to be cut. At the distal end of the masseteric nerve, a diameter of 0.52 (±0.2) mm was measured, whereas the diameter of the zygomatic branch was 0.59 (±0.2) mm at the overlapping point (Table 1). Histologically, the masseteric branch had a fascicular area of 0.16 (±0.03) mm^2 and an axon count of 818.81 (±223.4). Similar results were observed in the zygomatic branch, with a fascicular area of 0.15 (±0.1) mm^2 and an axon count of 861.91 (±928.9). No significant difference was detected in nerve diameter ($P = 0.067$) and axon count ($P = 0.25$) between the two nerves. However, significant differences were detected comparing the total number of branches ($P < 0.0001$) and the fascicular area ($P = 0.035$) of the nerves. Both nerves were multifascicular; the masseteric nerve had 5.2 (±2.3) fascicles and the zygomatic branch 6.4 (±3.2) fascicles (Fig. 3). No significant difference was found in fascicular quantity between the nerves ($P = 0.3775$). The regenerative distance measured 57.92 (±10.6) mm from the coaptation site to the intramuscular branches to the orbicularis oculi muscle.

Rat Anatomy

As little subcutaneous fat tissue existed in the rat face, the facial nerve branches were readily identified, passing across the zygomatic arch toward the orbicularis oculi muscle. Only one zygomatic branch was seen coming off the facial nerve root in all dissections. This gave rise to one (±0.8) secondary branch an average of 14.0 (±5.9) mm distal to the facial nerve root. After resection of the

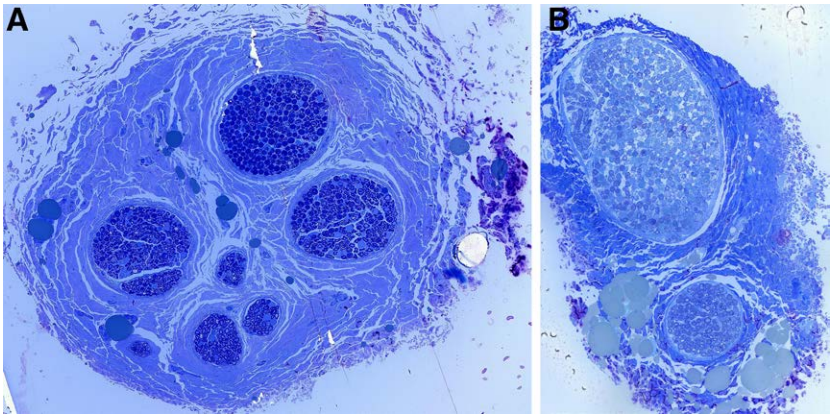

Fig. 3. Cross-sections of pig zygomatic branch (A) and masseteric branch (B) stained with 1% toluidine blue. Both nerves were multifascicular. The zygomatic branch had 6.4 (±3.2) fascicles, whereas the masseteric branch had 5.2 (±2.3) fascicles.

parotid gland, the facial nerve root was fully exposed. In 33% of the dissections, one facial nerve branch (buccal) was observed piercing through the glandular tissue. Starting from the nerve root to the orbicularis oculi muscle, the zygomatic nerve measured 17.7 (±3.2) mm in length with two (±0.8) branches in total (Table 1). The masseter muscle measured 19.9 (±1.4) mm from posterior to anterior, in which the motor nerve was found 8.5 (±2.2) mm anteriorly to the posterior edge of the muscle and 4.1 (±0.7) mm deep in the muscle tissue. It gave off intramuscular branches 3.15 (±1.4) mm inferior to the zygomatic arch. Two intramuscular branches (±1.1) were counted that coursed inferiorly in a majority of the dissections. The masseteric nerve measured 4.3 (±1.6) mm from where it emerges below the zygomatic arch to its intramuscular branches.

Nerve Transfer in the Rat Model

Tension-free masseteric-zygomatic nerve coaptation was achieved in all dissections, without cutting any tethering branches of either nerve in most dissections. On average, the overlapping length measured 5.9 mm (±2.6). At the overlapping point, the masseteric nerve was 0.36 (±0.1) mm in diameter and the zygomatic branch measured 0.46 (±0.1) mm in diameter (Table 1). Histologic analysis revealed an axon count of 526.2 (±144.7) in the masseteric branch with a fascicular area of 0.11 (±0.1) mm^2. The zygomatic branch had an average of 228.36 (±103.92) axons and a fascicular area of 0.020 (±0.01) mm^2. Significant differences were found regarding axon count ($P = 0.001$), fascicular area ($P < 0.001$), and diameter ($P < 0.001$) between the two nerves at the overlap. The masseteric branch was mostly monofasicular with 1.2 (±0.4) fascicles, whereas the zygomatic branch had approximately 1.5 fascicles (±0.7) (Fig. 4). No significant difference was determined when comparing fascicular quantity ($P = 0.3104$) between the two nerves. The regenerative distance was 9.17 (±2.5) mm.

DISCUSSION

Animal models are crucial for biomedical research. It is important to consider the advantages and limitations of various models in selecting small or large animal models. Small animals have low acquisition and maintenance cost, as well as uncomplicated housing requirements. Consequently, they are widely used for studies that require high sample sizes to assess efficacy and side effects of a novel drug or technique.[21–23] For nerve regeneration research, the most commonly used small animal model is rat.[24] Rats possess an exceptional regenerative capacity. This might seem beneficial; however, it subsequently increases the risk of false interpretation and concurrently reduces the results' applicability to human practice.[25] Brenner et al[25] had demonstrated that selecting the optimal time window to interpret results of nerve regeneration in rodents is crucial to unmasking potential key differences. Despite the higher cost and husbandry requirements, research with large animal models is frequently performed before moving a novel treatment approach to clinical application, as large animals such as sheep and pigs resemble human anatomy and physiology more closely.[26–28] Great similarities have been observed when comparing pig and human nerves. Particularly, pig nerve regeneration occurs at the same rate as in humans, thereby increasing clinical translation.[29]

Masseteric nerve transfer has played a prominent role in facial paralysis treatment.[30] To explore novel techniques (eg, electric stimulation devices) in enhancing functional outcomes of facial reanimation, we developed a masseteric-to-zygomatic nerve transfer model in both rats and pigs. In the rat model, anatomic dissections were less challenging, as both nerves were readily accessible for quick exposure and mobilization. Nerve coaptation, on the other hand, was more technically demanding, as the diameters of both nerves were quite small. In the pig model, anatomic dissection was more challenging and more time-consuming. Attention must be paid when dissecting the

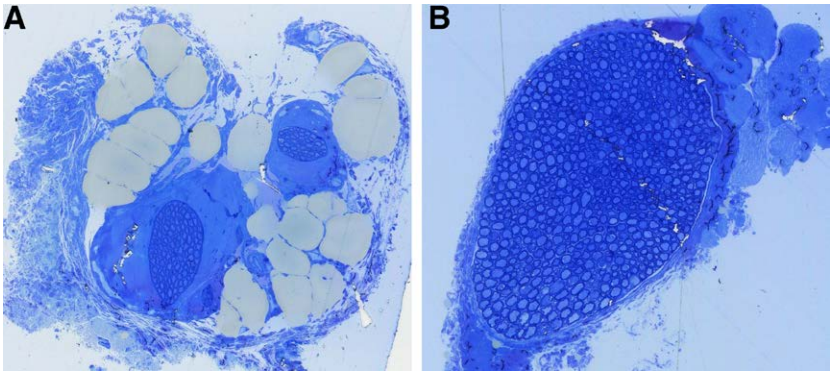

Fig. 4. Cross-sections of rat zygomatic branch (A) and masseteric branch (B) stained with 1% toluidine blue. The zygomatic branch had substantial amount of loose connective tissue around the nerve fascicles, which attributed to its larger gross diameter measurement despite the relatively smaller microscopic fascicular area measurement. The zygomatic branch had 1.5 (±0.7) fascicles, whereas the masseteric branch had 1.2 (±0.4) fascicles.

zygomatic branch inferior to the zygomatic arch, where it emerges from a deep fatty compartment, confounded by multiple secondary branches that arise from the zygomatic branch.[31] An average of four zygomatic secondary branches had to be cut to mobilize the zygomatic nerve enough so tension-free direct coaptation with the masseteric nerve could be achieved. No significant difference was detected in either axon count nor diameter of the pig masseteric and zygomatic branch at the overlapping point, highlighting their compatibility for a nerve transfer. Schreiber et al[11] showed that a donor-to-recipient axon count ratio greater than 0.7:1 positively correlated with successful outcomes. In our study, the calculated axon count ratio was 0.95:1 in pigs and 1.8:1 in rats. Due to the higher donor-to-recipient axon count ratio, it would be possible to spare one of the intramuscular branches of the masseteric nerve to better preserve donor site function while using the rat model.

Masseteric-to-zygomatic nerve transfer has gained great popularity in facial reanimation in the clinic due to its successful reinnervation and powerful muscle force.[7,8,32–34] When comparing this technique in humans with our rat and pig models, both parallels and differences were observed. Gross anatomy of the facial nerve and the masseteric branch seem identical to human anatomy, except for the relationship of the parotid gland to the facial nerve. In humans, the facial nerve divides into the upper and lower divisions in between the parotid gland tissue before branching off the five distinct peripheral motor branches.[35] This was not observed in either rats or pigs. In the rat model, only a buccal branch was found to pierce the parotid gland in 33% of the dissections. In the pig model, no facial nerve branch was seen to run through the parotid gland, as the gland was located superficially to the nerve root. This anatomical difference eases the surgical approach in the rat and pig models in that there is no need to dissect through the glandular tissue to expose the nerve. Borschel et al[15] investigated the anatomic location of the masseteric nerve in relation to standard surgical landmarks in a human cadaver study, and successfully located the nerve 3 cm anterior to the tragus and 1 cm inferior to the zygomatic arch. In our pig model, the masseteric nerve was found 3.4 cm anterior to the posterior edge of the masseter muscle with the tragus being even further away. Furthermore, human masseteric branch was previously described to be consistently monofascicular,[15] whereas an average of five fascicles was observed in the pig model. This presents an opportunity for selective fascicular transfer where the most indispensable fascicles could be spared to reduce donor site morbidity.[36] Regarding axon count, great comparability was recognized between the zygomatic branch in humans and pigs, as both had an average of around 845 nerve fibers. The masseteric nerve had fewer fibers in pigs than in humans.[7] No similarities were observed when comparing the axon counts in the rat model to human anatomy.

One limitation of this study stems from it being an anatomic study with euthanized animals. A cadaver study cannot mimic natural elasticity of the nerve tissue. This might potentially impact nerve mobility. Nevertheless, our dissections have shown that there was more than enough overlap between the donor and recipient nerves to ensure a tension-free direct nerve transfer. We have used the pig masseteric-to-zygomatic nerve transfer model in five animals where we investigated the effect of electrical pacing on blink function. Tension-free direct nerve transfer was achieved in all animals with signs of successful nerve regeneration, attesting to the usefulness of this model.

CONCLUSIONS

This study provided the anatomic knowledge and neuromorphometric data of the zygomatic branch of the facial nerve and the masseteric branch of the trigeminal nerve in pigs and rats. A direct masseteric to zygomatic nerve transfer was feasible in both model animals. These models can be used in future studies to develop novel therapeutic approaches for facial reanimation.

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DISCLOSURE

The authors have no financial interest to declare in relation to the content of this article.

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