Defining the Optimal Segment for Neurotization—Axonal Mapping of Masseter Nerve for Facial Reanimation

Terence Goh, LH, MB, BS, FAMS, Chuan Han Ang, MB, BS, Jolie Hwee, MB, BS, MRCS, and Bien-Keem Tan, FRCS

Background: Recently, there has been renewed interest in using the motor nerve to the masseter for facial reanimation. This article aims to identify the ideal segment of the masseter nerve for facial reanimation by mapping its anatomy and studying the axonal count in its branches.

Methods: Fifteen fresh cadaveric heads with 30 masseter nerves were dissected under the microscope. The masseter muscle was exposed with a preauricular incision, the course of the nerve followed and measurements of the nerve and its branches were taken to identify the topography of the nerve. The nerve was then harvested en bloc, fixed, and axon counts of cross-sections of the nerve recorded with ImageJ (an image analysing software). The data were analyzed using Microsoft Excel.

Results: The masseter consists of 3 discrete muscle layers, and the nerve to the masseter that entered the muscle between the middle and deep layers in all specimens was dissected. The average length of the masseter nerve from the mandibular notch to the last branch was 49.1 ± 10.5 mm. At origin, the nerve diameter was 0.80 ± 0.2 mm and had 1395 ± 447 axons. After the first major branch at a distance of 19.3 to 29.9 mm from the origin, the axon count of the main trunk ranged from 655 to 1025.

Conclusions: The segment of the masseter nerve which has an axon count of 600 to 800 is located after the first branch of the masseter nerve at a distance of 29.9 ± 7.2 mm from the start of its intramuscular course. Given that an axon count of 600 to 800 approximates that of the zygomatic branch of the facial nerve it is postulated that nerve coaptation at this level is able to produce a clinically satisfactory smile.

Key Words: masseter nerve, axon, facial nerve, reanimation

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The motor nerve to the masseter is increasingly favored as a donor motor nerve for facial reanimation[1–10] and emerging as the cranial nerve of choice to power the facial muscle to create a smile.[11] It is the standard source of innervation for free muscle flaps in cases of bilateral Mobius syndrome.[1,7] Its role has extended to include direct nerve transfer (masseter-to-facial nerve transfer) in some cases due to unavailability of the contralateral facial nerve. However, some surgeons are still wary of using this nerve due to the absence of a truly spontaneous smile and the potential to cause an overpowering smile.

A review of the literature revealed that there have been an increasing number of papers on the anatomy of the masseter nerve. Numerous authors have reported on the consistency of the origin of the masseter nerve with reference to the zygomatic arch and the tragus as well as techniques to identify the origin of the nerve.[6,12–14] Several Korean authors have reported on the course of the masseter nerve within the muscle and its relevance to botulinum toxin injection.[15,16] Proponents of masseter nerve transfer have reported that the masseter function can be preserved by sparing the proximal branches of the nerve.[6] Although this is borne out by clinical evidence, no study exists that details the axon count of the masseter nerve or its branches at different distances from the start of its intramuscular course. Such information will be extremely useful in achieving optimal results for neurotization of free muscle transfers.

The objective of this study was to investigate the intramuscular course of the motor nerve to the masseter under the microscope, so as to provide a detailed topographic roadmap for safe and expedient exposure of the nerve for neurotization. Counting axons using a computed assisted software as the nerve branches provided axonal counts at specific branch points of the nerve. This information provides the clinician with greater confidence to select the most ideal part of the nerve for neurotization while preserving innervation to the masseter.

METHODS

Cadaver Dissection

Fifteen fresh adult Caucasian cadavers (8 men and 7 women) were obtained from Science Care Inc., Arizona, USA with funding from a grant (SHF/FG409S/2009). The specimens used were fresh cadaver heads that have not been formalinized. They were stored at −20°C and thawed at −4°C for 24 hours 1 day before dissection. The heads were then thawed at room temperature 4 hours before dissection.

The heads were dissected using an operating microscope. Using a preauricular facelift incision, a sub-SMAS (superficial musculoaponeurotic system) dissection was performed to expose the masseter muscle and the zygomatic arch. The key landmarks, the tragus, and the zygomatic arch were marked, and the masseter was divided from the zygomatic arch sharply. The aponeurotic layer of each muscle layer of the masseter was divided until the masseter nerve was exposed (Fig. 1). Upon identification of the masseter nerve at the mandibular notch, the nerve was traced intramuscularly, documenting the branching pattern, caliber, and number of branches of the nerve. The origin of the nerve was defined to be the point of exit from the mandibular notch, and the end of the nerve was defined to be at the final branch. The nerve travelled anteromedially in an oblique fashion from the deep to superficial plane giving off multiple branches into the different muscle layers.

Measurements were taken from the tragus and the zygomatic arch to the origin of the masseter nerve, and of the length of the nerve and the distances of the branches from the origin (Fig. 1). Meticulous care was taken during the dissection to follow the nerve branches as they pierced the muscle layers. The muscle layers were cut so as to preserve the nerve. Once the nerve was dissected free, it was explanted and fixed on blotting paper before the nerve was preserved in 5% formaldehyde. The nerve was then sent for processing followed by sectioning for histological analysis.

Histological Preparation

The harvested nerve was sectioned at its origin and before every branching point (Fig. 2). Every branch was also sectioned. The

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From the Department of Plastic Surgery, Singapore General Hospital, Outram, Singapore.

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Reprints: Terence Goh LH, MB, BS, MMed, 20 College Road, Academia. Level 4 No. 376, 169856, Singapore. E-mail: terence.goh@sgh.com.sg.

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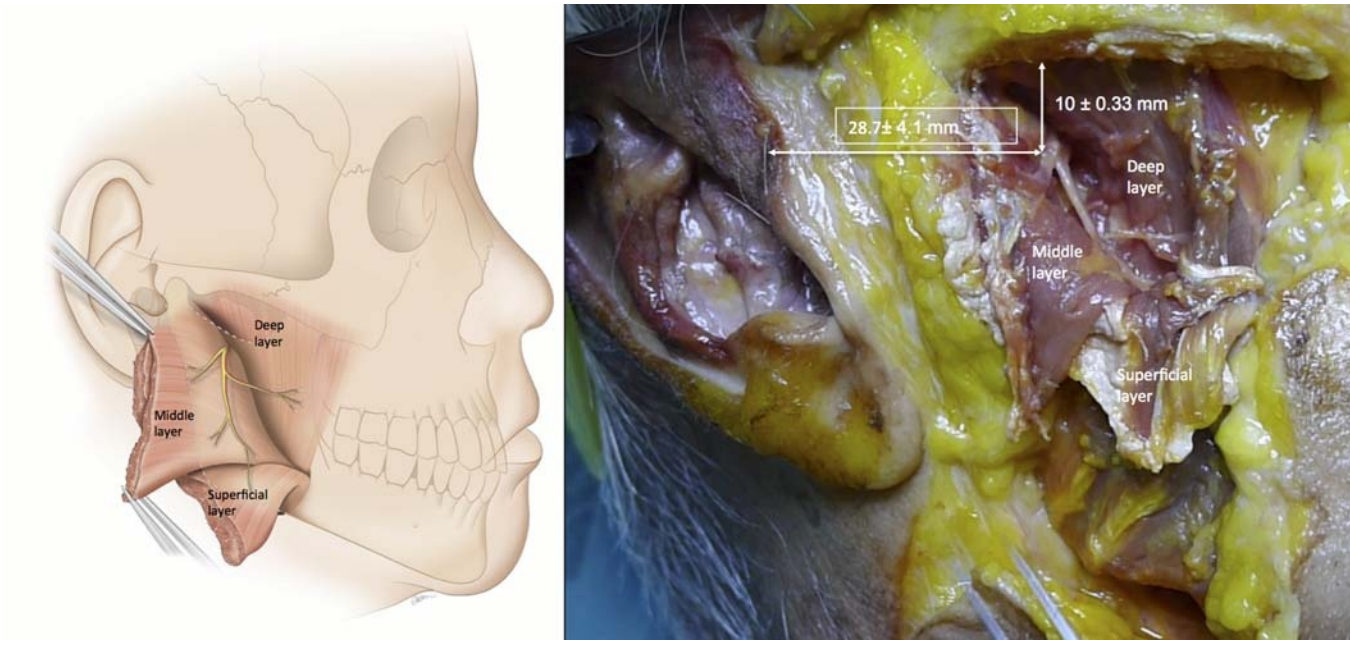


FIGURE 1. Topography of the masseter nerve. The masseter consistently has 3 muscle layers. The nerve to the masseter travels between the middle and deep layers of the masseter. The masseter nerve crosses the mandibular notch 3 cm anterior to the tragus and 1 cm inferior to the zygomatic arch. The nerve travels inferomedially at an angle of 53.9 degrees from the zygomatic arch. The average length of the masseter nerve measures 49.1 mm.

processing of the nerve sections involved overnight staining with Luxol fast Blue solution at 57°C followed by washing with 95% alcohol and distilled water. Counterstaining was done with Nuclear Fast Red before the specimens were dehydrated and mounted for microscopy to count the axons.

Computer-Assisted Nerve Counting

Slides were scanned using an Ariol slide-scanning microscope (Leica Microsystems). Analysis was performed using the Ariol image analysis software. For each section, the nerve was manually outlined. Axons were counted using ImageJ (Rasband, W.S., ImageJ; US National Institutes of Health, Bethesda, MD, http://imagej.nih.gov/ij/; 1997–2011) and a custom-written macro for manual counting. The data were computed and analyzed with Microsoft Excel software. A major branch was defined as one with more than 100 axons, and a minor branch as one with less than 100 axons.

RESULTS

Gross Anatomy

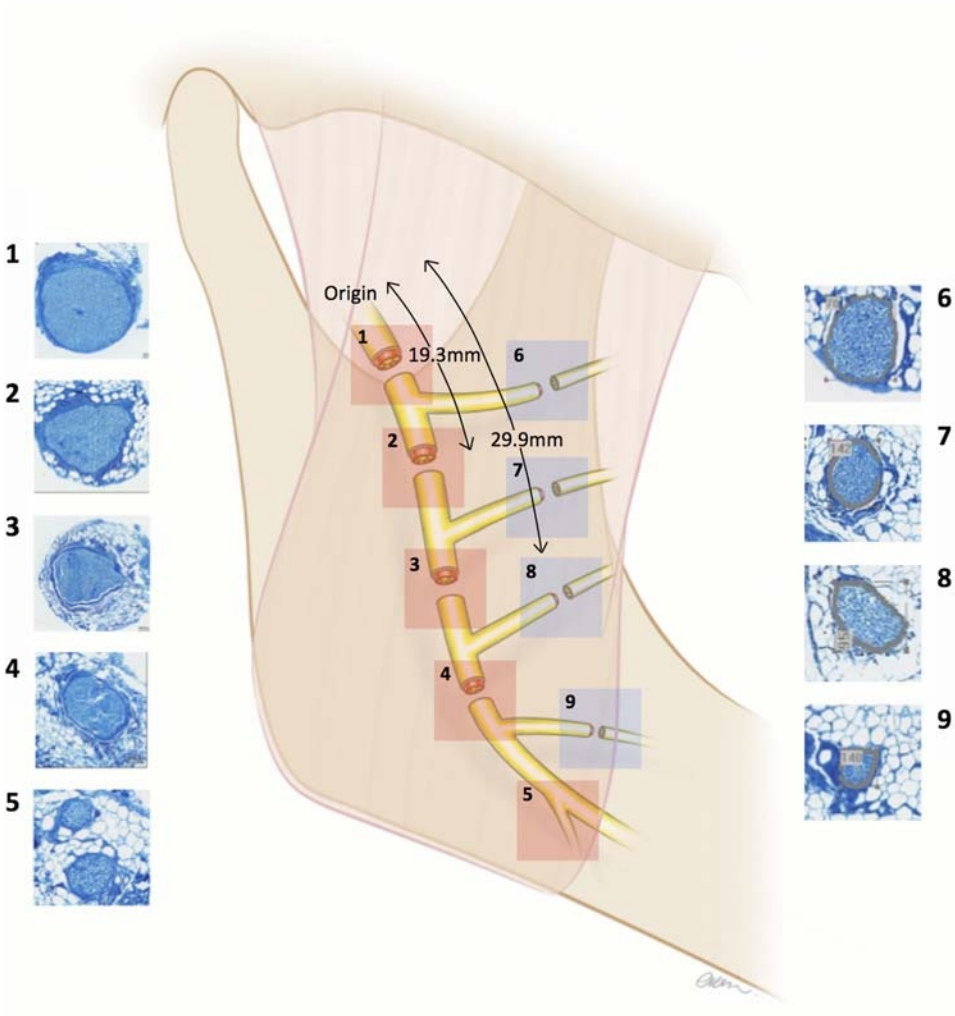
The masseter muscle comprised 3 muscle layers in all the 30 specimens and the masseter nerve crossed the mandibular notch and entered the muscle between the middle and deep layers consistently, travelling obliquely in an inferomedial direction from deep to superficial as it sent branches into the different layers of the masseter (Video 1, http://links.lww.com/SAP/A175, masseter anatomy; Fig. 1). The average length of the masseter nerve (from the mandibular notch to the last branch) measured 49.1 ± 10.5 mm, and it travelled at an angle of 53.9 (47–60) degrees from the zygomatic arch.

The following findings were obtained from studying the masseter nerve under the microscope (×10 magnification).

(1) Branching pattern of the masseter nerve (Fig. 3). In all the specimens, the masseter nerve travelled with the vascular pedicle, and the masseter artery was noted to cross the nerve in 60% of specimens; in 40% of specimens, the artery travelled parallel to the nerve. This was verified by sending the specimens for microscopy (Fig. 4). There were 3 main branching patterns (Figs. 3A–C). In all the patterns, the masseter nerve consisted of a single dominant descending trunk. As the nerve travelled down the length of the muscle, it gave off numerous nerve branches to supply the different layers of the masseter. *Type I*—2 specimens (6.7%) consisted of a single dominant descending trunk with no major branches but only minor nerve branches (<100 axons). *Type II*—single major branch, 5 specimens (16.7%) had 1 major branch (>100 axons). *Type III*—21 specimens (76.6%) had 2 or more major branches.

(2) Microscopy and axon count. Nineteen specimens were monofascicular, 9 specimens were bi-fascicular and 2 specimens were trifascicular. In the specimens with more than 1 fascicle, there was consistently 1 large fascicle and the second or third fascicles were noticeably smaller (Fig. 5).
The axon count at origin of the intramuscular portion was 1395 ± 447 axons, and the diameter of the nerve was 0.8 ± 0.2 mm. As the nerve branched distally, the axon count at the main trunk dropped (Fig. 2). The axon count after the first major branch, at a distance of 19.3 ± 6.7 mm from the origin averaged 1025 ± 423 axons with a diameter of 0.65 ± 0.2 mm. The axon count after the second major branch, at a distance of 29.9 ± 7.2 mm from the origin averaged 655 ± 204 axons with a diameter of 0.52 ± 0.1 mm. The axon counts after the third and fourth major branches were 426 ± 152 and 301 ± 186, respectively.

(3) Application of axon count. The ideal axon count for powering a natural smile has been approximated to that of the zygomatic branch, which is about 600–800 axons. At a distance of 19.3 to 29.9 mm from the origin at the mandibular notch (after the first major branch), the axon count of the masseter nerve main trunk was between 625 and 1025. This makes for an ideal point for nerve coaptation of the masseter nerve while preserving the first major branch (Fig. 1)—possible in 28 of 30 (93.3%) specimens.



Nerve Section	Diameter (mm)	Axon Count
1	0.8±0.20	1395±447
2	0.65±0.20	1025±423
3	0.52±0.10	655±204
4	0.4±0.08	426±152
5	0.29±0.08	301±186
6	0.34±0.17	404±411
7	0.26±0.12	222±135
8	0.25±0.08	233±120
9	0.25±0.08	216±78

FIGURE 2. Diagram of a cross-section of the masseter nerve at the main trunk and branches. Cross-sections of the trunks (prior to every major branch) and branches were sent for histological mapping to ascertain the axonal counts. The distances of the length of the nerve and the distances of the branch points from the origin were also recorded. At a distance of 19.3 to 29.9 mm from the origin, the average axonal count is 1025 (range, 655–1025).

In the remaining 2 specimens, this will not be possible. This distance also allows the nerve to be turned up for coaptation to the donor nerve.

Clinical Case (Fig. 6)

This patient was a 34-year-old female who had previously undergone gamma-knife ablation of an acoustic neuroma on the right side. She had developed complete paralysis of the right hemi-face. She was referred for facial reanimation 23 months after loss of function. Due to the time that had elapsed, the decision was made to perform a free gracilis muscle transfer with the ipsilateral masseter nerve as a donor nerve for neurotization.

The masseter muscle was exposed using a pre-auricular incision with an extension behind the ear. Adequate exposure of the zygomatic arch (up to the malar prominence) and masseter was obtained using this approach. The posterior part of the first two leaves of the masseter muscle was sharply divided from the zygomatic arch, and flipped anteriorly to expose the masseter nerve, which was located on the deep layer of the masseter. A proximal branch of sufficient caliber was noted and spared. The nerve was dissected further for up to 2 cm and the distal segment was reflected up to be coapted to the obturator nerve. Due to the size mismatch, the largest fascicle within the obturator nerve was identified and coapted to the masseter nerve. Additional perineural sutures were used to bolster the repair. The nerve repair was reinforced with fibrin glue. The patient recovered uneventfully post-operatively. She regained movement of the gracilis muscle at 120 days after surgery and was able to obtain a full smile at 180 days (Video 2, http://links.lww.com/SAP/A176).

DISCUSSION

The masseter muscle originates from the zygomatic arch and inserts into the angle of the mandible. In all our specimens, the masseter consisted of 3 muscle layers, and the masseter nerve crossed the mandibular notch to enter the muscle in between the middle and deep layers consistently. There are 2 reported approaches to expose the masseter nerve. The first method uses the intersection of a point 1 cm inferior to the zygomatic arch and 3 cm anterior to the tragus as a surgical landmark to locate the masseter nerve. Blunt dissection is then performed, and the nerve is located at a depth of 1 to 2 cm from the surface of

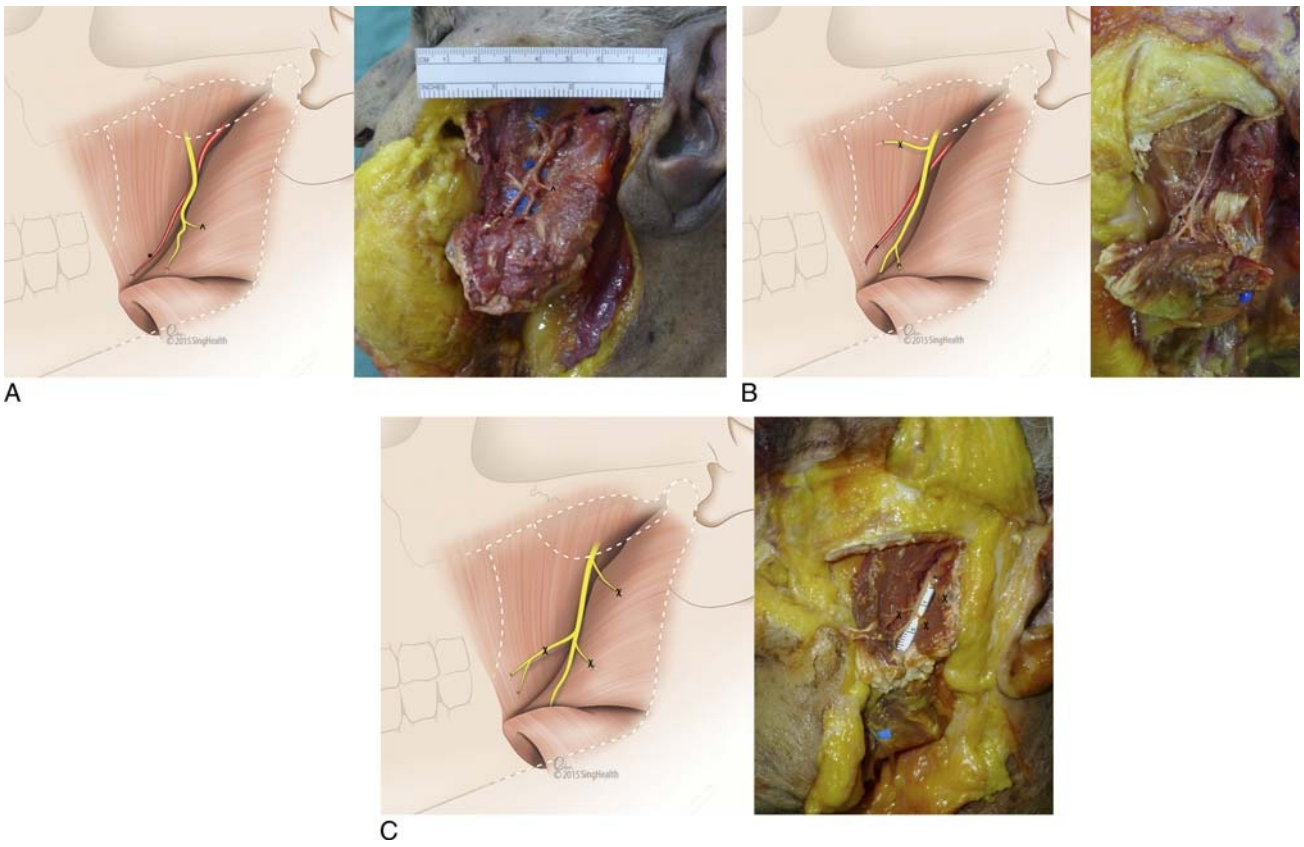


FIGURE 3. Pattern of nerve branching of the masseter nerve. The masseter nerve shows three distinct branching patterns. In all patterns, the masseter nerve consisted of a single dominant descending trunk. *Type I* (A) 6.7% of the specimens consisted of a single dominant descending trunk with no major branches but only minor nerve branches (<100 axons). * The masseter artery. The branch(^) of the masseter nerve shown here was a minor branches. *Type II* (B)—16.7% of the specimens had only one major branch (>100 axons). (*) denotes masseter artery. The major branch is marked (χ). The distal branch(^) of the masseter nerve shown here was a minor branch. *Type III* (C)—76.6% of the specimens had 2 or more major branches, shown here as χ. The masseter artery is not seen in this specimen.

the masseter.[13] The disadvantage of blunt dissection through the muscle layers of the masseter is that there is a higher incidence of causing bleeding or injury to the nerve. In some patients, the masseter muscle is bulky and blunt dissection may expose the distal branches of the masseter nerve "end-on," making it difficult to differentiate it from the main descending trunk of the nerve. The second method involves detaching the superficial and middle layers of the masseter from the zygomatic arch sharply. After the superficial and middle layers have been detached from the arch as a composite layer, the nerve is visualized clearly between the middle and deepest layers of the muscle. This method allows visualization of the intramuscular part of the nerve in its entirety. In our dissections, we have found that the masseter nerve consistently ran close to the masseter artery; in 60% of specimens, the artery crossed the nerve, and in 40%, the artery ran parallel to the nerve. Detaching the masseter muscle sharply is an expeditious method that avoids random splitting of the muscle. In our approach, we ligate the masseter artery under magnification if it crosses the nerve. Ligation of this vessel is important to facilitate dissection of the nerve safely.

In our earlier dissections using loupe magnification, we found that some of the branches of the masseter artery were mistaken for nerve branches and vice versa. The dissection of the masseter nerve requires fresh cadavers and adequate magnification for differentiating between the nerve branches and the vascular pedicle. To ensure accuracy, we decided to proceed with dissection and confirm the presence of nerve fibre in the cut ends under the microscope.

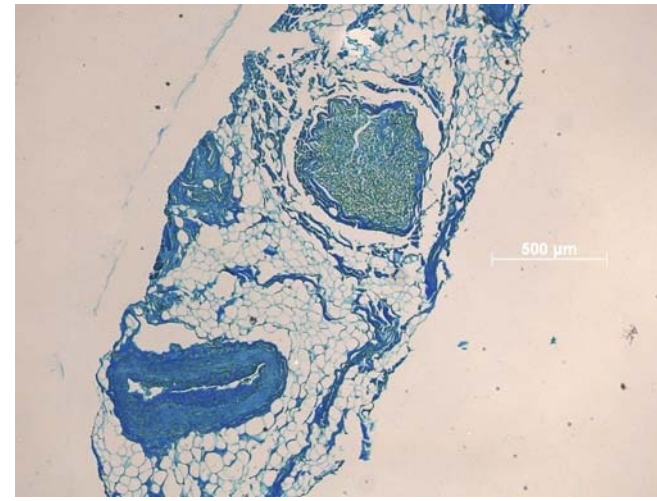


FIGURE 4. Histology showing the masseter nerve and artery. Histological examination confirms that the masseter nerve travels in close proximity to the maxillary artery. The artery is often about the same size or larger in diameter than the main trunk of the masseter nerve. The diameter of the artery in this slide measures 0.7 mm whilst the masseter nerve measures 0.5 mm.

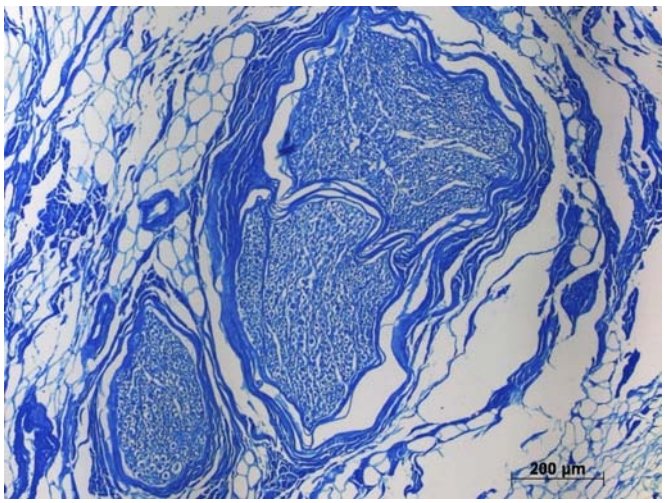


FIGURE 5. Histology showing fascicles of the masseter nerve. Majority of the specimens showed that the masseter nerve is monofascicular. In the specimens where there is more than 1 fascicle, the second was noticeably smaller.

The masseter nerve consisted of a single descending trunk that was dominant, being of larger caliber and having a higher axonal density than its branches. The main trunk was predominantly monofascicular, and even if there were more than 1 fascicle, the other fascicles were significantly smaller and obviated the nerve as a recipient. These findings were similar to that of Hontanilla and Qiu[17] and Cotrufo et al.[13] The branching pattern of the masseter nerve arborized like a tree—because the main trunk traversed obliquely through the muscle toward the ipsilateral oral commissure, sending numerous branches through the different layers of the muscle.

The following observations were consistent in 93.3% (n=28) of specimens: (1) Presence of major branches (defined as one with more than 100 axons) and (2) presence of a major branch within 2 cm of the origin. This allows the preservation of the muscle function when a side branch of the masseter is used for neurotization. Preserving neurotization to the remnant masseter is important as it is believed that a functioning masseter provides a resting tone by an intact feedback loop (personal communication - Klebuc).

Although the ideal axon count of a recipient nerve for facial reanimation has not been ascertained, most experts concur that 800 to 1000 axons are adequate.[18] Studies have shown that the distal end of a cross-facial nerve graft has an axon count of about 200.[6,18] This may explain the weak muscle contraction and consequent poor excursion of cross facial nerve-neurotized transfers compared with those innervated by a masseter nerve. On the other hand, the nerve to masseter has a count of 1400 to 2000 axons,[6,14] and several authors have indicated this to be overpowering. We postulate an ideal axon density to be 600 to 800 axons which is the axon count of the buccal nerve used for facial reanimation. Upon compiling the cross-sectional axon counts, we were able to estimate the "axonal drop" along the course of the masseter nerve. It is estimated that the region between the first and second branches of the masseter nerve should have an axon density of 1025 ± 423 to 655 ± 204. This segment of the nerve lies approximately 2 to 3 cm from the origin of the nerve and has a diameter of 0.52 to 0.66 mm. This is the ideal site for coaptation for facial reanimation.

The masseter nerve is the ideal recipient cranial nerve donor for facial reanimation for bilateral Mobius syndrome and is the preferred nerve for ipsilateral neurotization of the facial nerve stump or free muscle transfer for selected cases. The advantages for the masseter nerve are: (1) location within the same operative field and proximity to facial nerve for free muscle transfer, obviating the need for nerve grafting and hence reducing the time for neurotization; (2) high axon density giving strong muscle recovery; (3) reliable and constant anatomy with branching patterns that allow preservation of masseter function; (4) potential for cerebral adaptation due to cross-talk between facial nerve and trigeminal nerve nuclei; and (5) synergistic actions of biting and smiling (in contrast to shoulder shrugging [accessory nerve] or tongue movement (hypoglossal nerve). However, some authors still prefer the accessory nerve as a donor as they feel that the shoulder shrug is more synergistic. The limitation of using the masseter nerve is that the patients find it difficult to smile with their mouths open, and sykinesis can lead to unwanted muscle spasm during biting.

CONCLUSIONS

This study yields findings that allow better understanding of the axon distribution as the masseter nerve branches within the muscle. The axonal count of 600 to 800, which will provide a clinically satisfactory smile is located after the first branching of the masseter nerve at a distance of 29.9 ± 7.2 mm from origin. This information provides

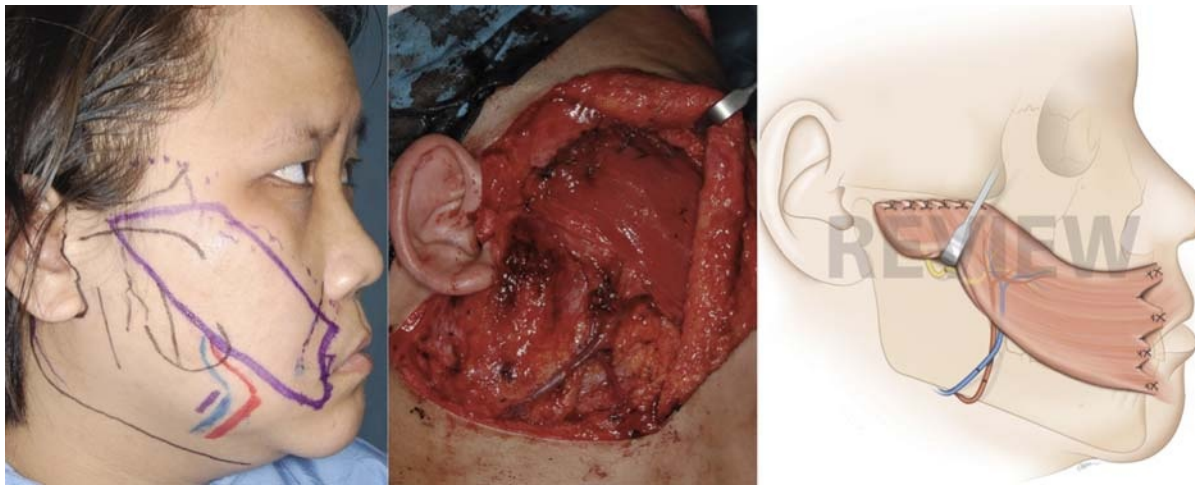


FIGURE 6. Single-stage facial reanimation using gracilis free tissue transfer and neurotization by the ipsilateral masseter nerve. (Left) Preoperative marking for planning of the anastomosis and nerve coaptation. (Middle) Gracilis inset with anastomosis to the facial artery and vein. (Right) Nerve coaptation of the obturator nerve to the masseter nerve.

the clinician with a road map to confidently select the ideal segment for facial reanimation.

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Informed consent was received for publication of the figures in this article.

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