High Variability of Facial Muscle Innervation by Facial Nerve Branches: A Prospective Electrostimulation Study

Dissertation
zur Erlangung des akademischen Grades
doctor medicinae (Dr. med.)

vorgelegt dem Rat der Medizinischen Fakultät
der Friedrich-Schiller-Universität Jena, 2019

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Tag der öffentlichen Verteidigung: 29.06.2021
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List of abbreviations

| Abbreviation | Description                  |
|--------------|------------------------------|
| CN           | Cranial Nerve                |
| EMG          | Electromyography              |
| FN           | Facial Nerve                  |
| FNM          | Facial Nerve Monitoring       |
| HD           | High Definition               |
| HNO          | Hals-, Nasen- und Ohren       |
| Hz           | Hertz                         |
| M.           | Musculus                      |
| mA           | Milliampere                   |
| N.           | Nervus                        |
Zusammenfassung:
Anatomisch ist der makroskopische und mikroskopische Verlauf des peripheren Nervus facialis zu den mimischen Muskeln vielfach beschrieben, aber es gibt keine detaillierten Berichte über die funktionelle Verbindung der Äste des N. facialis zu den einzelnen Gesichtsmuskeln und deren funktionellen Einheiten (Berkovitz 2005, Yang et al. 2013). Bei der Präparation des Nervus facialis während einer Parotidektomie kann eine eindeutige Zuordnung des Nervus facialis zu den Hauptästen (Rami temporales, zygomatici, buccales, marginalis mandibulae und colli) aufgrund der Variabilität seiner Verzweigung schwierig sein. Zudem erfolgt die Überwachung des Gesichtsnervs durch das Nervenmonitoring weiterhin hauptsächlich mit Zweikanalsystemen. Zumeist wird nur der M. orbicularis oris und der M. orbicularis oculi überwacht. Dies bedeutet, dass nur die Gesichtsnervenäste überwacht werden, die funktionell mit diesen beiden Muskeln verbunden sind. Dies ist von Bedeutung für den HNO-Chirurgen, da er aufgrund des individuellen Verzweigungsmusters des N. facialis und den Signalen des Monitorings über die weitere Präparation entscheiden und eine Nervenschädigung vermeiden muss (Sood et al. 2015). Darüber hinaus ist es für zukünftige bionische Anwendungen zur Wiederherstellung der Funktionen des Gesichtsnervs individuell erforderlich zu wissen, welche Nervenäste welche Gesichtsbewegung und welche Variabilität aufweisen (Frigerio et al. 2015).

Daher wurde diese prospektive klinische Studie durchgeführt, um die Lücke zwischen Anatomie und Neurophysiologie durch systematische intraoperative elektrische Stimulation der peripheren Gesichtsnervenäste zu schließen und die Zuordnung der Nervenäste für funktionell wichtige Gesichtsbewegungen zu bewerten.

Bei einer standardisierten lateralen Parotidektomie wurden bei 7 Patienten (vier Männer, drei Frauen, Durchschnittsalter 62 Jahre) der Gesichtsnerv und seine Hauptäste unter einem Operationsmikroskop freigelegt. Dann wurde systematisch der Hauptstamm und alle Aufzweigungen zweiter und dritter Ordnung mit einer Stimulationselektrode mit einer Frequenz von 3 Hz und 30 Hz und von 0,1 bis 2 mA stimuliert. Der Verlauf und die Verteilung aller Äste wurden auf Video aufgezeichnet. Gleichzeitig wurde die Reaktion
der Gesichtsmuskelkontraktion über eine zweite Kamera aufgezeichnet, die über dem Gesicht des Patienten angeordnet war. Offline wurden die beiden synchronen HD-Videosequenzen von zwei Untersuchern qualitativ analysiert. Die Stimulation der temporofazialen Division führte bei 4 Patienten zu einer Kontraktion des M. orbicularis oculi und des M. zygomaticus, bei 3 Patienten des M. nasalis und des M. orbicularis oculi und des M. frontalis, während der obere Ast bei 4 Patienten eine Kontraktion des M. orbicularis oculi proximal bewirkte. Distal wurde eine selektive Kontraktion des oberen Augenlides bei 5 Patienten und des M. frontalis bei 2 Patienten erzeugt. Der untere Ast verursachte bei 4 Patienten eine Kontraktion des unteren Augenlides und bei 3 Patienten eine Kontraktion des M. orbicularis oculi. Die Stimulation der zervikofazialen Division führte bei 3 Patienten zu einer Kontraktion des M. orbicularis oris und des M. depressor anguli oris, bei 2 Patienten zu einer Stimulation des M. orbicularis oris durch den kranialen Ast bei 7 Patienten zu einer Kontraktion des M. orbicularis oris und bei 3 Patienten des M. depressor anguli oris. Bei 6 Patienten stimulierte der kaudale Ast den M. depressor anguli oris. Die Stimulation des gesamten Spektrums von Ästen der temporofazialen Division konnte zum Augenverschluss führen. Die Stimulation des Spektrums der Nervenäste der zervikofazialen Division konnte zu Stimulationen im Mittelgesicht (nasale und zygomatische Muskeln) sowie im Mundbereich (M. orbicularis oris und M. depressor anguli oris) führen. Frontal- und Augenregion wurden ausschließlich von der temporofazialen Division versorgt. Die Mund- und Halsregion wurde ausschließlich von der zervikofazialen Division versorgt. Es gab eine funktionale Überlappung im Mittelgesicht.

Daraus lässt sich schlussfolgern, dass die Topographie eines bei der Parotidektomie präparierten Astes des Gesichtsnervs keine verlässlichen Rückschlüsse auf die damit verbundene Zielmuskel- und Gesichtsbewegung zulässt. Darüber hinaus zeigte die vorliegende Studie, dass es mehrere Konstellationen gab, insbesondere für intraparotidale Nervenäste jenseits der ersten Verzweigung, bei denen eine zweikanalige oder sogar vierkanalige Überwachung möglicherweise nicht zuverlässig ist. Funktionell wichtige Gesichtsbewegungen, wie Augenschluß oder
Bewegungen zum Öffnen des Mundes, wurden variabel über mehrere periphere Nervenäste vermittelt. Eine solche redundante Innervation könnte erklären, warum eine Läsion eines peripheren Nervenastes in der Gl. parotis nicht zwangsläufig zu einer Funktionsstörung des Gesichtsnervs führt. Zukünftige bionische Geräte benötigen eine patientenspezifische Untersuchung, um die korrekten peripheren Nervenäste zu stimulieren und selektiv gewollte Muskelkontraktionen auszulösen.
1. Introduction:
The facial nerve (FN) is the seventh cranial nerve (CN). It is a mixed nerve with motor supply to the facial muscles being most crucial. It exhibits diversity in its course, dimensions and anatomic relations especially in the extratemporal part (Moore et al. 2010). As it exits through the stylomastoid foramen in the base of skull, the extracranial portion of the facial nerve immediately gives branches off the main trunk to the auricular muscles, the posterior belly of the digastric and the stylohyoid muscles (Kwak et al. 2004).

The nerve then courses ventrally and at the posterior edge of the parotid gland it splits into the upper and lower divisions (Rodriguez et al. 2009). Within the parotid gland, there is further branching with multiple individual variations (Davis et al.1956). The upper (temporofacial) division of the FN gives off the temporal, zygomatic and buccal branches whereas the lower (cervicofacial) division gives off the marginal mandibular and cervical branches (Solares et al. 2003).

It is the most frequently injured of all the CNs, causing paralysis of the muscles of facial expression. Although it is a mixed nerve, the motor component is the most important due to the significance of facial palsy (Pather et al. 2006).

1.1 Branching patterns
Numerous micro- dissection studies have demonstrated that branching patterns and anastomoses between branches both within the parotid and on the face exhibit considerable individual variation (Katz and Catalano 1987, Kapuz et al. 1994, Solares et al. 2003, Kwak et al. 2004, Kalaycioğlu et al. 2014).

In parotid surgery, these nerve anastomoses are important and presumably explain why accidental or deliberate division of a small branch often fails to result in the expected FN weakness (Standring et al. 2008). McCormack et al. studied 100 FNs from cadavers and described the surgical anatomy with special reference to the parotid gland (McCormack et al. 1945). They described a complex classification of 8 patterns of the FN branching and anastomosis (Fig.1).
Dargent and Duroux presented 5 main types of FN distribution. The authors dissected 68 FNs from within the substance of the parotid gland. They noted two major classes and five "types" of FN branching from 59 of the 68 dissections. Class 1 (35 cases): FN without anastomoses between branches after their initial branching from the trunk. Class 2 (24 cases): FN with anastomoses between the cervicotemporal branches which form intraglandular plexuses (Dargent and Duroux 1946).

Davis et al. dissected 350 cadaveric facial halves and categorized the branching pattern of the FN into 6 distinct types (Fig. 2) (Davis et al. 1956).
Baker and Conley reviewed the extratemporal FN anatomy in about 2000 parotidectomy cases. Their findings suggested that the FN branching pattern was more variable than that noted in the Davis cadaveric studies including the presence of a FN trunk trifurcation with a direct buccal branch in a few instances (Baker and Conley 1979).

Katz and Catalano found in a study of 100 patients during parotid surgery significant variations in the FN branching (Fig. 3) (Katz and Catalano in 1987).

Kopuz et al. in a cadaveric study found also intraparotideal configuration of the FN and classified as Katz and Catalano did in 1987. Twenty four per cent of the FNs had no anastomoses (Type I); 12% had a ring-like shape anastomosis between the buccal and the zygomatic branches (Type II); 14% of the anastomoses were between the buccal and the other branches in a ring-like shape (Type III); 38% of the FN had multiple complex anastomoses and were named as multiple loops (Type IV); 12% had two main trunks (Type V) (Kopuz et al. 1994).
Fig 3: Patterns of extratemporal facial nerve branching (Katz and Catalano 1987).
(T: temporal, Z: zygomatic, B: buccal, M: marginal, C: cervical)

Kwak et al. 2004 classified the branching patterns of the FN according to the origin of the buccal branch into four types (Fig.4) (Kwak et al. 2004).

Fig. 4 Patterns of extratemporal facial nerve (Kwak et al 2004).
Several authors have reported the possibilities of trifurcation, quadrifurcation, or even a plexiform branching pattern of the FN trunk (Table. 1)

| Author                  | Bifurcation % | Trifurcation % |
|-------------------------|---------------|----------------|
| Davis et al., 1956      | 100           | 0              |
| Park and Lee., 1976     | 95.6          | 4.4            |
| Katz and Catalano., 1987| 100           | 0              |
| Kopuz et al., 1994      | 82            | 18             |
| Ekincli., 1999          | 81.4          | 18.6           |
| Salame et al., 2002     | 97.8          | 2.2            |
| Tsai and Hsu., 2002     | 100           | 0              |
| Kwak et al., 2004       | 86.7          | 13.3           |
| Kalaycioğlu et al., 2013| 81.3         | 18.8           |
| Myint et al., 1991      | 96.2          | 3.8            |

Table 1. Bifurcation and trifurcation of the FN trunk according to various authors.

1.2 Minor trunks

While many articles have carefully described the length of the FN trunk and branching patterns, the minor trunk of the FN is rarely reported. Katz and Catalano reported three cases as presenting with two main trunks known as the major and minor trunks with the latter joining the larger temporofacial division, the origin of the main buccal branch (Katz and Catalano 1987). Similar to this description, Kopuz et al. reported 18% of cases with a minor trunk (Kopuz et al. 1994).

Park and Lee reported 4.4% in their series. Thus, a surgeon should bear in mind that even after finding two main facial nerve trunks, a third minor trunk could still be present and could be exposed to injury (Park and Lee 1977).

1.3 Electrophysiological facial nerve monitoring during parotidectomy

Intraoperative facial nerve monitoring (FNM) by direct visualization of facial muscle movement was first performed in 1898 (Delgado et al. 1979). Since these early reports, its application has been significantly refined, starting with the introduction of electromyography in the 1970s (Minahan and Mandir 2011).

The goals of facial nerve monitoring during parotidectomy are the same as those during otologic and neurotologic surgery and include early facial nerve identification, warning to the surgeon of unexpected facial nerve stimulation, mapping of the course of the nerve, reduction of mechanical trauma to the
nerve, and evaluation and prognosis of nerve function at the conclusion of
the procedure (Silverstein and Rosenberg 1991)
Electrophysiological monitoring is generally the preferred method of facial
nerve monitoring because it is more sensitive and specific than visual
monitoring of facial movements. The degree of facial muscle activity
detected is also quantifiable. An adjustable pulsed stimulator allows for
electrically evoked EMGs. With facial nerve stimulation, either related to
surgical manipulation or from electrical stimulation of the nerve, an
immediate EMG response is visible, with a characteristic waveform and
amplitude. In addition, the response is immediately audible and its volume
correlates directly with its EMG amplitude (Eisele et al. 2010).
Electrophysiological facial nerve monitoring is safe. Because electrically
evoked facial nerve responses during electrophysiologic facial nerve
monitoring are obtained using a pulsed nerve stimulator, facial nerve injury
arising from overstimulation that theoretically may occur from prolonged
stimulation with a battery-powered direct current nerve stimulator is unlikely
(Love and Marchbanks 1978)
In primary cases of parotidectomy, intraoperative FNM decreases the risk
of immediate postoperative facial nerve dysfunction but does not appear to
influence final outcome of facial nerve weakness (Sood et al. 2015).
2. Aim of the work:
Facial expression is an important part of non-verbal human communication that any paralysis or even deficiency of facial movements tends to be not only immediately noticeable, but provide a serious social stigma for the patient. Furthermore Facial paralysis is an unsatisfactory pathology to treat, and the results of neural reconstruction are still unsatisfactory. The anatomic pathways followed by the FN and its relations are very important and carry great significance for both surgeons and clinicians in order to make accurate diagnosis and effective surgical intervention regarding facial nerve disorders. An intimate knowledge of facial nerve anatomy is also critical to avoid its inadvertent injury during rhytidectomy, parotidectomy, maxillofacial trauma surgery and ideally in any surgery of the head and neck region. A standard assignment of the main facial nerve branches does not exist due to the variability of the nerve branching network, this is of importance because only such an assignment allows the surgeon to decide if facial nerve monitoring recording of only a limited number of muscles will track nerve stimulation and alert him before damaging the nerve. Facial nerve monitoring using two-channel systems with recording from the orbicularis oris and orbicularis oculi muscle allows only the facial nerve branches which are functionally connected to these two muscles to be tested. Furthermore, identification of such anatomical and functional connection between facial nerve branches and their corresponding target mimic muscles is necessary for future bionic applications to restore facial nerve functions. Hence, the present prospective clinical study was performed to establish these anatomical variations of the extratemporal part of the facial nerve as well as to bridge the gap in knowledge between anatomy and physiology through systematic stimulation of the exposed facial nerve and its branches during parotidectomy to evaluate the alignment of the branches to functionally important facial movement.
High Variability of Facial Muscle Innervation by Facial Nerve Branches: A Prospective Electrostimulation Study

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Objectives/Hypothesis: To examine by intraoperative electric stimulation which peripheral facial nerve (FN) branches are functionally connected to which facial muscle functions.

Study Design: Single-center prospective clinical study.

Methods: Seven patients whose peripheral FN branching was exposed during parotidectomy under FN monitoring received a systematic electrostimulation of each branch starting with 0.1 mA and stepwise increase to 2 mA with a frequency of 3 Hz. The electrostimulation and the facial and neck movements were video recorded simultaneously and evaluated independently by two investigators.

Results: A uniform functional allocation of specific peripheral FN branches to a specific mimic movement was not possible. Stimulation of the whole spectrum of branches of the temporofacial division could lead to eye closure (orbicularis oculi muscle function). Stimulation of the spectrum of nerve branches of the cervicofacial division could lead to reactions in the midface (nasal and zygomatic muscles) as well as around the mouth (orbicularis oris and depressor anguli oris muscle function). Frontal and eye region were exclusively supplied by the temporofacial division. The region of the mouth and the neck was exclusively supplied by the cervicofacial division. Nose and zygomatic region were mainly supplied by the temporofacial division, but some patients had also nerve branches of the cervicofacial division functionally supplying the nasal and zygomatic region.

Conclusions: FN branches distal to temporofacial and cervicofacial division are not necessarily covered by common facial nerve monitoring. Future bionic devices will need a patient-specific evaluation to stimulate the correct peripheral nerve branches to trigger distinct muscle functions.

Key Words: Electrostimulation, facial nerve branches, mimetic muscles, facial movements, nerve innervation, nerve course, communication, monitoring.

Level of Evidence: 4

INTRODUCTION

There have been many anatomical reports regarding the macroscopic and microscopic course of the peripheral facial nerve to the mimetic muscles. Typically, coming out of the stylomastoid foramen, the main trunk of the facial nerve is divided into a temporofacial division and a cervicofacial division. The temporofacial division should have temporal, zygomatic and buccal branches. The cervicofacial division most frequently has a mandibular branch and a cervical branch. The allocation of these five main branches to the mimetic muscles from anatomical textbooks is shown in Supporting Table 1 in the online version of this article. Actually, a clear anatomical allocation of main peripheral branches to individual mimetic muscles during facial nerve dissection, for instance during parotidectomy, is often not possible because the peripheral branching and the intercommunication of the branches are highly variable. The temporofacial division most often has a plexiform arrangement formed by dichotic and anastomotic divisions. The cervicofacial trunk resembles merely a simple large loop, but up to about 50% of its fibers show anastomoses with the temporofacial division. In nearly half of the cases buccal branches arising from the two main divisions are interconnected with the zygomatic branch. Even when preparing the facial nerve in an antegrade direction beginning at the main trunk and beyond the bifurcation into temporofacial and cervicofacial division, as it is mainly performed during parotidectomy, a clear assignment of the just-prepared nerve branch to the main branches (temporal, zygomatic, buccal, mandibular, cervical) might be difficult due to the variability of the nerve
branching network. However, this is of importance because only such an assignment allows the surgeon to decide if facial nerve monitoring recording of only a limited number of muscles will track nerve stimulation and alert him before damaging the nerve. Facial nerve monitoring is still performed mostly with two-channel systems with recording from the orbicularis oris and orbicularis oculi muscle. This means that only the facial nerve branches are monitored, which are functionally connected to these two muscles. Due to the above-mentioned reasons this can be very variable. Therefore, when always using the same standard recording positions, the quality of the monitoring is variable. Furthermore, for future bionic applications to restore facial nerve functions, it is necessary to know which nerve branches reveal which facial movement and with which variability.

Hence, the present prospective clinical study was performed to systematically stimulate the exposed facial nerve and its branches during parotidectomy to evaluate the alignment of the branches to functionally important facial movements.

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was performed from June 2014 to December 2015 at the Department of Otoblocksurgery, Jena University Hospital, Jena, Germany. Approval for the study was obtained through the local institutional review board, and informed consent was obtained from all study participants. Seven patients were investigated (four male, three female; median age 62 years). Standard lateral parotidectomy (three right side, four left side) was performed because of a benign tumor (four patients), malignan tumor (two patients), and chronic sialadenitis (one patient). Standard facial monitoring (CLEO Nerve Monitor; inomed, Emmenndingen, Germany) with recording electrodes (SDN electrodes RT/SW and Trigron GN; inomed) in the frontal, orbicularis oculi, orbicularis oris, and depressor labii inferioris muscles was performed. Facial nerve function was normal before and after surgery in all cases.

Electrostimulation, Facial Monitoring, and Recording of Facial Movements

During parotidectomy, the main trunk of the facial nerve and all main branches were exposed under an operating microscope until the anterior border of the parotid gland. The peripheral facial nerve and its branches were systematically stimulated first with a monopolar electrode with ball tip (straight, length = 13 cm, no. 552529; inomed) and then with a bipolar concentric stimulation electrode (B8 probe, length = 13 cm, no. 522106; inomed) beginning with the main trunk, all branches of first order, and all branches of second order. The monopolar stimulation electrode has a larger stimulation field allowing a faster screening of the tissue for nerve branches. The bipolar electrode has a small stimulation field allowing a meticulous scanning of a once-discovered facial nerve branch. Each branch was stimulated from proximal to distal. The stimulation at each stimulation point started with 0.1 mA and was increased stepwise to 2 mA with a frequency of 3 Hz. Switching between the frequency of 3 Hz to 30 Hz was useful to verify the direction of the facial movements. The stimulation with 3 Hz evoked only short contractions of the subdermal muscles visible only as a shiver of the skin. When stimulated with 30 Hz, a tetanic contraction of the whole stimulated muscle was visible, and the direction of the muscle move. Each was shown to see. Each stimulation was repeated at least once time. The exposed facial nerve and its branches and the whole electrostimulation procedure were video recorded via a microscope camera (Image 1 HD HS3-M; Karl Storz, Tuttingen, Germany). Simultaneously, the facial contractions were video recorded by an endoscope camera (Image 1 HD HS3-Z, Karl Storz) with a supporting arm over the face. Both camera signals were recorded simultaneously together with one audio channel for audio comments into the same video documentation system (Advanced Image and Data Acquisition NEO; Karl Storz).

Evaluation of the Facial Movements

The evaluation was performed offline independently by two investigators. All facial movements were documented on a standardized electronic form. Depending on the individual facial nerve branching, the stimulation of seven to 12 branches was recorded and analyzed. To be able to compare the highly variable nerve branches and summarize the results of all patients, the branches of the facial nerve were numbered in a standardized manner (see Supporting Figure 1 in the online version of this article). The main trunk of the facial nerve was number 0. After bifurcation, the first cranial main branch (temporofacial division) was number 1, the first caudal main branch number (sensitofacial division) was number 2. A trifurcation was not seen. Following this nomenclature, we continued to number the branches of the second order starting from cranial with number 11, 12 for sub-branches of the first cranial main branch number 1. In the same manner, the numbers 21, 22, and so on from cranial to caudal were used for the sub-branches of the second cranial main branch number 2. Branches of third order were numbered 211, 212, and so on in the same order. If the pattern of facial muscle movements changed during the proximal to-distal stimulation of a branch, this was also classified adding a letter (a, b) to the number of the branch. For instance, the second branch of the temporofacial division (number 12) showed a different stimulation pattern during proximal stimulation (number 12a) than during distal stimulation (number 12b). The videos were evaluated according to the following facial movements: 1) frowning (frontal muscle), 2) eye closure (orbicularis oculi muscle) separately for B1 upper lid and B2 lower lid movement, 3) nose wrinkle (nasal muscles), 4) showing teeth (smiling; zygomaticus muscles), 5) pursing lips (orbicularis oris muscle), 6) depressor lips (depressor anguli oris muscle), and 7) neck movement (platysma). The allocation of the visible movement to muscles was controlled by prior electric recording from the underlying mimics muscles, but continuous electric monitoring during surgery was performed only from the muscles A, B1, E, and F (see above). Each investigator documented independently the facial movements for each facial nerve branch in all cases. Finally, the results were compared, and all cases with divergent evaluation were reviewed together until a consensus assessment was obtained. A disagreement in the first evaluation between the two reviewers occurred in three out of the seven patients for 16 out of 612 movement analyses without discernable pattern; overall 68 facial nerve branches were stimulated and nine different movements evaluated.

RESULTS

Peripheral Branching of the Facial Nerve

During parotidectomy, all peripheral facial nerve branches were dissected systematically in an anterograde direction beginning from the main trunk. All branches were pursued until the anterior border of the

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Fig. 1. Parotidectomy on the left side. Detailed view of facial nerve stimulation [different stimulation points have different numbers] and response of different facial muscles. Stimulation parameter: 1 mA and 3 Hz. Activated muscles are color coded. Overlapping activation by different facial nerve branches is color coded in overlapping colors. [Color figure can be viewed in the online issue, which is available at www.laryngoscope.com.]

Pareotid gland. Three examples of the peripheral facial nerve branching are shown in Figures 1 to 3. As branching of first order, the main trunk (0) always divided into a temporofacial division (branch 1) and a cervicofacial division (2). A trifurcation was not seen. In one case a nerve loop was seen between the main trunk and the temporofacial division (Fig. 2). This loop was clearly and reliably excitable by electric stimulation. As branching of second order, the temporofacial division divided either into two branches, an upper branch (11) and a lower
branch (12; in three patients) or into three branches, an upper (11), middle (12), and lower branch (13; in four patients). The cervicofacial division divided either into two branches, an upper (21) and a lower branch (22) in six patients or into three branches, an upper (21), middle (22), and lower branch (23) in only one patient. The upper branch of the temporofacial division (11) divided in three patients into an upper branch (111) and lower branch (112) in one case, and into an upper (111), middle (112), and lower branch (113) in two cases. The
other four cases did not show a further subdivision until the anterior border of the parotid gland. The lower branch of the temporofacial division (12 and/or 13) never showed a further subdivision. The upper branch of the cervicofacial division (21) also showed no further subdivision. The lower branch of the cervicofacial division (22) divided into an upper (221) and lower branch (222) in two cases. The other five cases presented no further branching until the anterior border of the parotid gland.

In summary, all patients showed within the parotid gland a bifurcation of the main trunk into a temporofacial and a cervicofacial division. Furthermore, all patients showed a branching of the temporofacial division into at least two branches (second order branching). All but one patient also showed a branching of the cervicofacial division into at least two branches within the plane of the parotid gland. Branching of the third order was very rare and maximally seen in three patients within the plane of the parotid gland.

**Electrostimulation of the Peripheral Facial Nerve**

The results of the stimulation of the branches of the peripheral facial nerve are summarized in Table I. Stimulation of the main trunk of the facial nerve led to a reliable and visible stimulation of the whole hemiface in all but one patient. Neck movements were reliably visible only in four cases. Interestingly, the stimulation of the temporofacial division led to reliable eye closure but at the same time also to movements in the midface related to the function of the zygomatic muscles (in six out of seven patients). Stimulation of the cervicofacial division was followed by activation of the midface in only one patient but always of the mouth and mental region related to the orbicularis oris muscle and the depressor anguli oris muscle. Neither any branch of the temporofacial division nor of the cervicofacial division (of a second or third order) led to a movement related to single mimic muscle function.

From the perspective of the different mimic muscles, an allocation of specific peripheral nerve branches to a specific movement or muscle movement was not possible (compare Table I and Figs. 1–3). Stimulation of the whole spectrum of branches of the temporofacial division could lead to eye closure (orbicularis oculi muscle function). Stimulation of the spectrum of nerve branches of the cervicofacial division could lead to reactions in the midface (nasal and zygomatic muscles) as well as around the mouth (orbicularis oris and depressor anguli oris muscle function). A separate stimulation of upper versus lower eye lid was possible only in some patients. From a functional point of view, the frontal and eye region (frontalis muscle and orbicularis oris muscle) was exclusively supplied by the temporofacial division. The region of the mouth (orbicularis oris, the depressor anguli oris muscle area) and the neck were exclusively supplied by the cervicofacial division. There was a functional overlap in the midface. The nose and zygomatic region were mainly supplied by the temporofacial division, but two patients had also nerve branches of the cervicofacial division functionally supplying the nasal and zygomatic region.

**DISCUSSION**

The presented analysis showed that the topography of a facial nerve branch dissected during parotidectomy does not allow reliable conclusions on the related target muscle and facial movement. The innervation pattern of the peripheral branches, especially distal to the temporofacial and cervicofacial division was highly variable. Furthermore, within the parotid gland, an allocation of a nerve branch to an individual muscle function was not possible. Most stimulations led to synchronous movements of different mimic functions. Looking from the muscle side, what is important for the reliability of standard facial nerve monitoring, monitoring of an individual mimic muscle does not automatically mean that all peripheral facial nerve branches of the same region are monitored.

The methodology used had limitations. Only seven patients were investigated with a time-consuming electrostimulation mapping of the peripheral facial nerve. Nevertheless, the high variability of the anatomical facial nerve branching and the highly variable functional connection of the electrostimulated nerve branch and facial target region suggest that this high variability is a common phenomenon of peripheral facial nerve function. Only the facial nerve branches within the parotid region were analyzed. Therefore, we cannot exclude that more distal facial nerve branch stimulation might lead to selective stimulation of individual mimic muscles and facial movements. It seems to be unlikely that unattended antidiromic stimulation along the facial nerve trunk caused some of the stimulation effects via an F wave. Much higher supramaximal stimulation is needed to trigger a facial nerve F wave.11

A comparison to other studies is not possible, as comparable studies were not performed for the facial nerve. In contrast, the innervation of laryngeal muscles has been investigated in detail in the recent years by functional electrostimulation providing a better understanding of laryngeal neuroanatomy.12–14 The two former studies were performed intraoperatively in two patients, respectively. This should be taken into account when considering that the present study with high intraoperative effort of time was performed in only seven patients.

From many anatomical studies it is well known that the five main extratemporal branches of the facial nerve (frontal, zygomatic, buccal, marginal, and cervical) are anatomically connected not to a single target muscle but with some variation to several mimic muscles, and that these five peripheral branches exhibit, again with a high degree of variability, a variety of nerve fiber interconnections and sometimes also nerve loops within the parotid gland.4,5,7,15 It has to be emphasized that even the terminal branches outside the parotid gland (distal to the facial plexus within the parotid gland) often show temporal-zygomatic, zygomatic-buccal, buccal-mandibular, and mandibular-cervical nerve anastomoses.16 We can now confirm that this anatomical condition is mirrored by the physiological functional situation.

For facial nerve monitoring during parotid surgery, usually two or four channels are used for the recording from the facial muscles.17 For the mostly used two-
### TABLE 1
Overview About the Results of the Electrostimulation of the Different Peripheral Facial Nerve Branches and Activated Mimic Muscles in Seven Patients.

| Branch | No. of Patients With This Branch | Stimulation | Frowning | Eye Closure | Upper Eye Lid Movement | Lower Eye Lid Movement | Nose Wrinkling | Smiling Teeth | Pouting Lips | Depressing Lips | Neck Movement |
|--------|---------------------------------|-------------|-----------|-------------|------------------------|------------------------|----------------|---------------|--------------|----------------|----------------|
| 9      | 7                               | Main trunk  | 6         | 6           | 6                      | 6                      | 7              | 7             | 6            | 6              | 4              |
| 1      | 7                               | TF division | 2         | 6           | 6                      | 6                      | 7              | 7             | 6            | 6              | 4              |
| 11a    | 7                               | First branch of TF division, proximal | 3          | 4           | 3                      |                        | 1              |               |              |                |                |
| 11b    | 1                               | First branch of TF division, distal   | 1          |              |                        |                        | 1              |               |              |                |                |
| 111    | 3                               | First subdivision of first branch of TF division | 1          |              |                        |                        | 2              |               |              |                |                |
| 112    | 3                               | Second subdivision of first branch of TF division | 1          |              |                        |                        | 3              |               |              |                |                |
| 113    | 2                               | Third subdivision of first branch of TF division | 1          |              |                        |                        | 1              |               |              |                |                |
| 12a    | 7                               | Second branch of TF division, proximal | 4          |              |                        |                        | 3              |               |              |                |                |
| 12b    | 3                               | Second branch of TF division, distal   | 1          |              |                        |                        | 1              |               |              |                |                |
| 13a    | 3                               | Third branch of TF division, proximal  | 2          |              |                        |                        | 1              |               |              |                |                |
| 13b    | 1                               | Third branch of TF division, distal    | 1          |              |                        |                        |                 |               |              |                |                |
| 21     | 6                               | CF division | 1          | 1           | 7                      | 7                      | 2              |               |              |                |                |
| 21a    | 6                               | Second branch of CF division, proximal | 2          | 1           | 5                      |                        | 2              |               |              |                |                |
| 22a    | 1                               | Second branch of CF division, distal   | 1          |              |                        |                        | 1              |               |              |                |                |
| 221    | 1                               | First subdivision of second branch of CF division | 1          |              |                        |                        | 1              |               |              |                |                |
| 222    | 1                               | Second subdivision of second branch of CF division | 1          |              |                        |                        | 1              |               |              |                |                |
| 23     | 1                               | Third branch of CF division            | 1          |              |                        |                        |                 |               |              |                |                |

For each facial muscle, the number of patients with muscle movements during stimulation of the different nerve branches is shown.

CF = cervicofacial; TF = temporalis.
channel monitoring, the needle electrodes are most frequently inserted in the superior portion of the orbicularis oris and into orbicularis oculi muscles. These recording sites are sufficient to monitor the main trunk of the facial nerve, but will lead to false-negative results if peripheral facial nerve branches that innervate other facial muscles are stimulated or injured. The present study showed that there are several constellations,
especially for intraparotid nerve branches beyond the first branching, where two-channel or even four-channel monitoring might not be reliable.

Facial nerve reanimation surgery and muscle transfers are the standard procedures for reconstruction of facial function. Nevertheless, functional results are limited, and surgery is often not possible in cases of chronic denervation. Therefore, bionic devices with artificial electrical stimulation of extramussular facial nerve branches, intramuscular nerve branches, or direct stimulation of facial muscles have been discussed for some time. Recent studies have shown the feasibility of facial pacing in animal models and in humans. Successful rehabilitation via facial nerve branch pacing will depend on the selectivity of the electrical stimulation. For instance, for selective pacing of eye closure, it will be necessary to stimulate the orbicularis oculi muscle itself, using an electrode array with contact to large parts of the muscle. Alternatively, one could stimulate a facial nerve branch, selectively innervating the orbicularis oculi muscle. The present results suggest that the way of extramussular facial pacing will be successful when targeting peripheral facial nerve branches, sometimes even beyond the parotid gland.

CONCLUSION

This prospective intraoperative clinical study of seven patients demonstrated that a uniform functional allocation of specific peripheral facial nerve branches to a specific mimic movement was not possible. Functionally important facial movements, such as eye closure or movement for the opening of the mouth, were related to several peripheral nerve branches. Such a redundant innervation might explain why a lesion of a peripheral nerve branch within the parotid gland does not automatically lead to facial nerve dysfunction. It was frequently not possible to activate a specific isolated muscle function by intraparotid peripheral facial nerve stimulation, which has implications on planning the site of nerve stimulation of future bionic devices to restore facial nerve function. Furthermore, the present study showed that there are several constellations, especially for intraparotid nerve branches beyond the first branching, where two-channel or even four-channel monitoring might not be reliable.

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4. Discussion

In an agreement to anatomical studies (Katz and Catalano 1987, Kapuz et al. 1994, Solares et al. 2003, Kwak et al. 2004, Kalaycioğlu et al. 2014) confirmed this study that the five main extratemporal branches of the facial nerve (frontal, zygomatic, buccal, marginal, and cervical) are anatomically connected not to a single target muscle but with some variation to several mimic muscles, and that these five peripheral branches exhibit, again with a high degree of variability, a variety of nerve fiber interconnections and sometimes also nerve loops within the parotid gland.

Not only anatomically but also on the basis of neurophysiology, the presented analysis showed that the topography of a facial nerve branch dissected during parotidectomy does not allow reliable conclusions on the related target muscle and facial movement. The innervation pattern of the peripheral branches, especially distal to the temporofacial and cervicofacial division was highly variable.

Furthermore, within the parotid gland, an allocation of a nerve branch to an individual muscle function was not possible. Most stimulations led to synchronous movements of different mimic functions. Looking from the muscle side, what is important for the reliability of standard facial nerve monitoring, monitoring of an individual mimic muscle does not automatically mean that all peripheral facial nerve branches of the same region are monitored.

When facial nerve monitoring is planned for a given case, the surgeon can explain its use and benefits to the patient. This information reinforces the fact that the surgeon will use all means available to preserve and protect the facial nerve, this can be greatly reassuring to a patient (Eisele et al. 2010).

The stimulation probe is useful in assisting the surgeon to identify the main trunk of the facial nerve when nerve localization is difficult due to tumor or scar from previous surgery, to identify a peripheral nerve branch during retrograde facial nerve dissection, and to distinguish facial nerve from sensory nerve or non-nerve tissue during parotid dissection. After surgical dissection of the facial nerve, electrical stimulation of the nerve with the probe is recommended to allow assessment of the functional integrity of the
nerve and to aid in prediction of postoperative facial nerve function (Bhattacharyya et al. 2004). Electrical confirmation of the identity of the facial nerve can reassure the surgeon and improve surgical confidence (O’Regan et al. 2007).

For facial nerve monitoring during parotid surgery, usually two or four channels are used for the recording from the facial muscles (Eisele et al. 2010). For the mostly used two-channel monitoring, the needle electrodes are most frequently inserted in the superior portion of the orbicularis oris and into orbicularis oculi muscles. These recording sites are sufficient to monitor the main trunk of the facial nerve, but will lead to false-negative results if peripheral facial nerve branches that innervate other facial muscles are stimulated or injured. The present study showed that there are several constellations, especially for intraparotid nerve branches beyond the first branching, where two-channel or even four-channel monitoring might not be reliable.

Facial nerve monitoring equipment of the future will likely be more refined. A recent report describes optical nerve stimulation using pulsed infrared optical radiation (Teudt et al. 2007). This new method may contribute to improved spatial selectivity of stimulation.

Only the facial nerve branches within the parotid region were analyzed. Therefore, we cannot exclude that more distal facial nerve branch stimulation might lead to selective stimulation of individual mimic muscles and facial movements. Also a comparison to other studies is not possible, as comparable studies were not performed for the facial nerve.

Facial nerve reanimation surgery and muscle transfers are the standard procedures for reconstruction of facial function (Volk et al. 2011). Nevertheless, functional results are limited, and surgery is often not possible in cases of chronic denervation. Therefore, bionic devices with artificial electrical stimulation of extramuscular facial nerve branches, intramuscular nerve branches, or direct stimulation of facial muscles have been discussed for some time. Recent studies have shown the feasibility of facial pacing in animal models and in humans (Sood et al. 2015, Sachs et al. 2007, Kurita et al. 2010). Successful rehabilitation via facial nerve branch pacing will depend on the selectivity of the electrical stimulation. For instance, for
selective pacing of eye closure, it will be necessary to stimulate the orbicularis oculi muscle itself, using an electrode array with contact to large parts of the muscle (McDonnell et al. 2013) Alternatively, one could stimulate a facial nerve branch, selectively innervating the orbicularis oculi muscle. The present results suggest that the way of extramuscular facial pacing will be successful when targeting peripheral facial nerve branches, sometimes even beyond the parotid gland.
5. Conclusions:

This prospective intraoperative clinical study of seven patients demonstrated the high variability of the anatomical facial nerve branching and mirrored this functionally with the highly variable functional connection of the electrostimulated nerve branch and facial target region which confirm that this high variability is a common phenomenon of peripheral facial nerve function.

This phenomenon might explain why a lesion of a peripheral nerve branch within the parotid gland may not lead to facial nerve dysfunction and may pass unnoticed.

Furthermore, the present study showed that there are several constellations, especially for intraparotid nerve branches beyond the first branching, where two-channel or even four-channel monitoring might not be reliable.

Functionally important mimic movements, such as blinking or smiling were related to several peripheral nerve branches. It was frequently not possible to activate a specific isolated muscle function by intraparotid peripheral facial nerve stimulation, which has implications on planning the site of nerve stimulation of future bionic devices to restore facial nerve function.
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7. Anhang
7.1 Ehrenwörtliche Erklärung
Hiermit erkläre ich, dass mir die Promotionsordnung der Medizinischen Fakultät der Friedrich-Schiller-Universität bekannt ist, ich die Dissertation selbst angefertigt habe und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben sind, mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts unterstützt haben: Univ.-Prof. Dr. med. Orlando Guntinas-Lichius und PD Dr. med. habil. Gerd Fabian Volk. 

die Hilfe eines Promotionsberaters nicht in Anspruch genommen wurde und dass Dritte wederunmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, dass ich die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht habe und dass ich die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung nicht bei einer anderen Hochschule als Dissertation eingereicht habe.

Ort, Datum,
Suhl, 01.08.2021

Unterschrift des Verfasser
7.2 Danksagung

Danken möchte ich meinen Eltern und meine Ehefrau. Danken für das Privileg eine gute Ausbildung genossen zu haben. Für das Privileg in allen Lebensbereichen gefördert, unterstützt und geliebt zu sein. Ohne Euch wäre diese Dissertationsschrift nicht entstanden.

Mein Dank gilt des Weiteren insbesondere meinem Doktorvater Univ.-Prof. Dr. med. Orlando Guntinas-Lichius. Sie waren buchstäblich rund um die Uhr und von überall auf der Welt für meine Fragen erreichbar und haben mich in jeder Hinsicht unterstützt und ermutigt. Die gemeinsame Arbeit mit Ihnen war mir stets eine große Freude.

Danken möchte ich Herrn PD Dr. med. habil. Gerd Fabian Volk für die großartige Unterstützung.