

# Facial Nerve Monitoring During Parotidectomy: A Case Series

J of Neurophysiological Monitoring 2023; 1(1): 1-12.

ISSN 2995-4886

Wasiq Nadeem <sup>1,2</sup>  
Wadana H. Khattak <sup>1,3</sup>  
Rafia H. Jahangiri <sup>1,3</sup>  
Hooria Asad <sup>1,3</sup>  
Laila Farooq <sup>1,3</sup>  
James Blaylock <sup>4</sup>  
Leah Crounce-Solano <sup>4</sup>  
Rudy R. Garza <sup>4</sup>  
William R. Fell <sup>5</sup>  
Irfan S. Basha <sup>6</sup>  
Faisal R. Jahangiri <sup>1,7,8</sup>

<sup>1</sup>Global Innervation LLC, Dallas, Texas, USA.

<sup>2</sup>UT Health, San Antonio, San Antonio, Texas, USA.

<sup>3</sup>Khyber Medical College, Khyber Medical University, Peshawar, KPK, Pakistan

<sup>4</sup>Axis Neuromonitoring LLC, Richardson, Texas, USA.

<sup>5</sup>Covenant Medical Center, Lubbock, Texas, USA.

<sup>6</sup>Division of ENT, Department of Surgery, Sheikh Khalifa Medical City, Abu Dhabi, UAE

<sup>7</sup>Department of Neuroscience, School of Behavioral & Brain Sciences, The University of Texas at Dallas, Richardson, Texas, USA.

<sup>8</sup>Laboure College of Healthcare, Milton, Massachusetts, USA.

**KEYWORDS:** Facial nerve, Parotidectomy, ionm, electromyography, emg, corticobulbar mep, comep.

**CITE AS:** Nadeem W, Khattak WH, Jahangiri RH, Asad H, Farooq L, Blaylock J, Crounce-Solano L, Garza RR, Fell WR, Basha IS, Jahangiri FR. Facial Nerve Monitoring During Parotidectomy: A Case Series. J of Neurophysiological Monitoring 2023; 1(1): 8-20. doi: 10.5281/zenodo.8206378.

## ABSTRACT

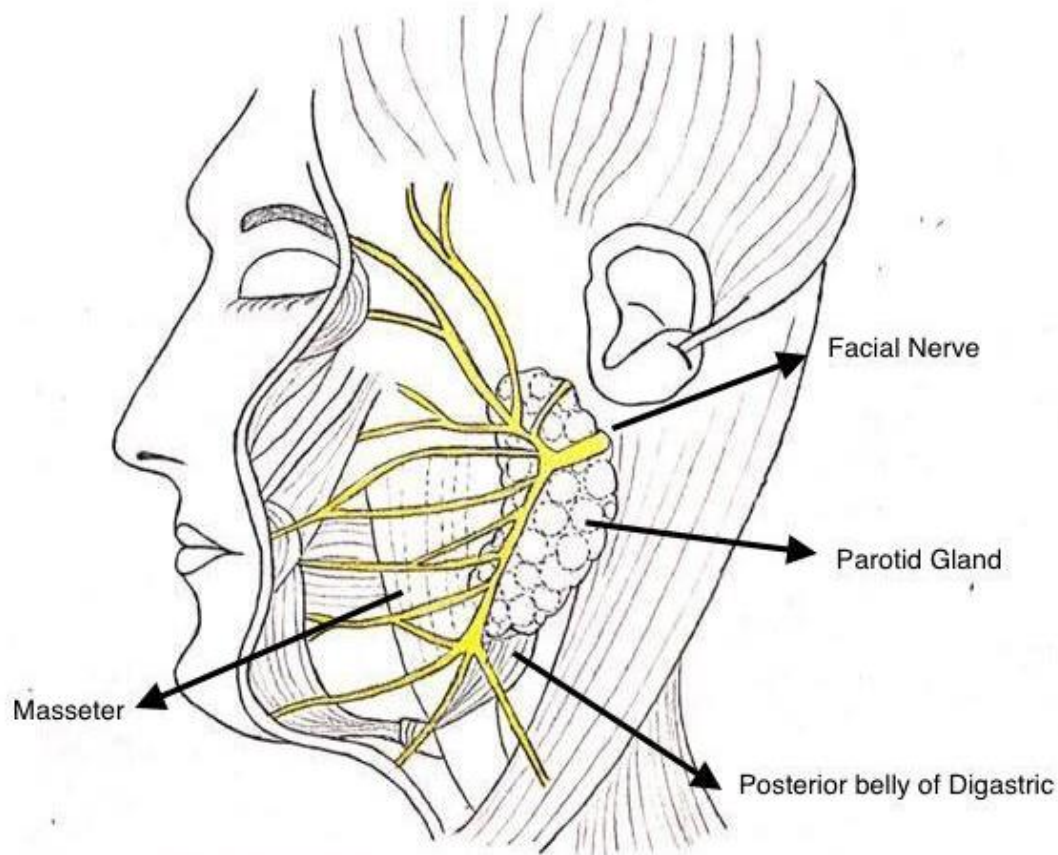
The parotid gland constitutes the largest of the salivary glands. It is an irregular lobulated mass located anterior to the ear and is an important anatomical landmark as the facial nerve courses and divides through the parotid gland. These facial nerve branches are at risk during parotidectomy, especially when a parotid mass is involved. In addition to surgeon experience and anatomical knowledge, intra-operative facial nerve monitoring (IONM) is an extremely useful tool in identifying and preserving the facial nerve during parotidectomy and reducing post-operative complications. A retrospective analysis was conducted. Twenty-three patients met inclusion criteria and underwent parotidectomies from 11/20/2012 to 1/13/2023. 14 male and nine female patients with a mean patient age of 63 years were included. All patients underwent intraoperative facial nerve monitoring and had successful procedures without incidence of facial nerve injury or other post-operative complications. Intraoperative Facial nerve monitoring is an incredibly important tool during parotidectomy and can help reduce post-operative complications such as transient and long-term facial nerve weakness. It should be employed during all parotidectomy cases, by surgeon experience, especially when the deeper lobe of the parotid gland is involved or in the case of malignancy. IONM helps reduce post-operative facial nerve weakness post-parotidectomy and should be employed during parotidectomy., quantitative, stress, non-REM sleep.

Copyright: ©2023 Nadeem, W et al. This open-access article is distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

## INTRODUCTION

The parotid gland constitutes the largest pair of salivary glands. It secretes saliva into the mouth via the parotid, or Stensen's, duct. It is an irregular lobulated mass located in front of the ear and bound by the external acoustic meatus and zygomatic arch above, the mandible below, anteriorly by the masseter, and posteriorly by the sternocleidomastoid muscle [1]. This gland is an important anatomical landmark as the facial nerve divides into branches. The facial nerve takes a tortuous path from the brainstem through the skull base before it exits the skull via the stylomastoid foramen and enters the parotid gland. Here it divides,

first into two major branches at the pes anserinus, a bifurcation of the facial nerve into the temporofacial (upper) and cervicofacial (lower) branches. It then divides into its five major branches: temporal, zygomatic, buccal, marginal mandibular, and cervical (**Figure 1**). These nerves innervate the muscles of facial expression but do not provide innervation to the parotid gland, which is innervated by the auriculotemporal nerve [2].



**Figure 1:** Anatomy of course of the facial nerve as it passes and divides into its branches at the parotid gland (illustration by Laila Farooq).

Many afflictions can affect the parotid gland – these include but are not limited to infections, tumors, and mechanical obstruction. Concerning infection, the parotid gland is most infected by disease-causing agents through the bloodstream, most commonly with viral agents such as mumps. However, bacterial infections, dental issues, and mechanical obstructions through stones or tumors can also cause parotitis. Tumors of the Parotid gland are usually benign. However, most (80%) salivary gland tumors begin in the parotid glands, so removing them is usually preferred before the tumor can spread [3]. The most common parotid gland tumor is a benign pleomorphic adenoma, which increases in malignant potential the longer it remains, from 1.5% in the first five years to 9.5% after 15 years. Parotidectomy, both superficial or total, is a standardized surgical procedure for benign and malignant parotid gland conditions and the treatment of choice for parotid gland tumors. One of the major potential risks of parotidectomy is injury to facial nerve branches as they course through the gland [4]. The nerve damage may be due to mechanical injury such as stretching, ligation, or compression of the nerves, nerve ischemia, or a thermal or electrical injury [4]. Nerve

injury can also be a necessary byproduct of surgery, especially in a tumor that may have encased or invaded certain branches, necessitating the sacrifice of those branches.

Upon literature review, temporary facial nerve paralysis, affecting some or all the facial nerve branches, has occurred in 9.3% to 64.6% of parotid gland surgeries. Permanent facial paralysis has been reported to occur at a lower rate below 8% [5]. The marginal mandibular branch of the facial nerve is particularly susceptible to damage during parotidectomy. Older patients seem more vulnerable to facial nerve damage [5]. Given the potential for damage to such an important nerve with potential debilitating consequences for patients, including facial asymmetry, incomplete eye closure, smile function, and psychosocial disturbances, amongst other effects, it is vitally important that the integrity of the facial nerve is monitored both through surgeon experience as well as intra-operative neurophysiological monitoring (IONM) to ensure it is preserved. IONM has become increasingly used during such procedures and has been shown to decrease the risk of immediate postoperative facial nerve weakness (Sood et al., 2015).

IONM helps monitor the cranial nerves' structural and functional integrity in head and neck surgeries [6]. During parotidectomy procedures, a multimodality IONM approach is employed by utilizing Corticobulbar Motor Evoked Potentials (CoMEP), continuous and triggered electromyography (sEMG/tEMG), and Train of Four (TOF) [7]. This aids in minimizing any nerve damage caused by a surgical procedure and provides the surgeon with real-time feedback. IONM helps minimize complications that would otherwise have been detected post-operatively using a physical exam.

## MATERIALS AND METHODS

### *Patient selection:*

Patient with a parotid gland mass suitable for treatment with parotidectomy.

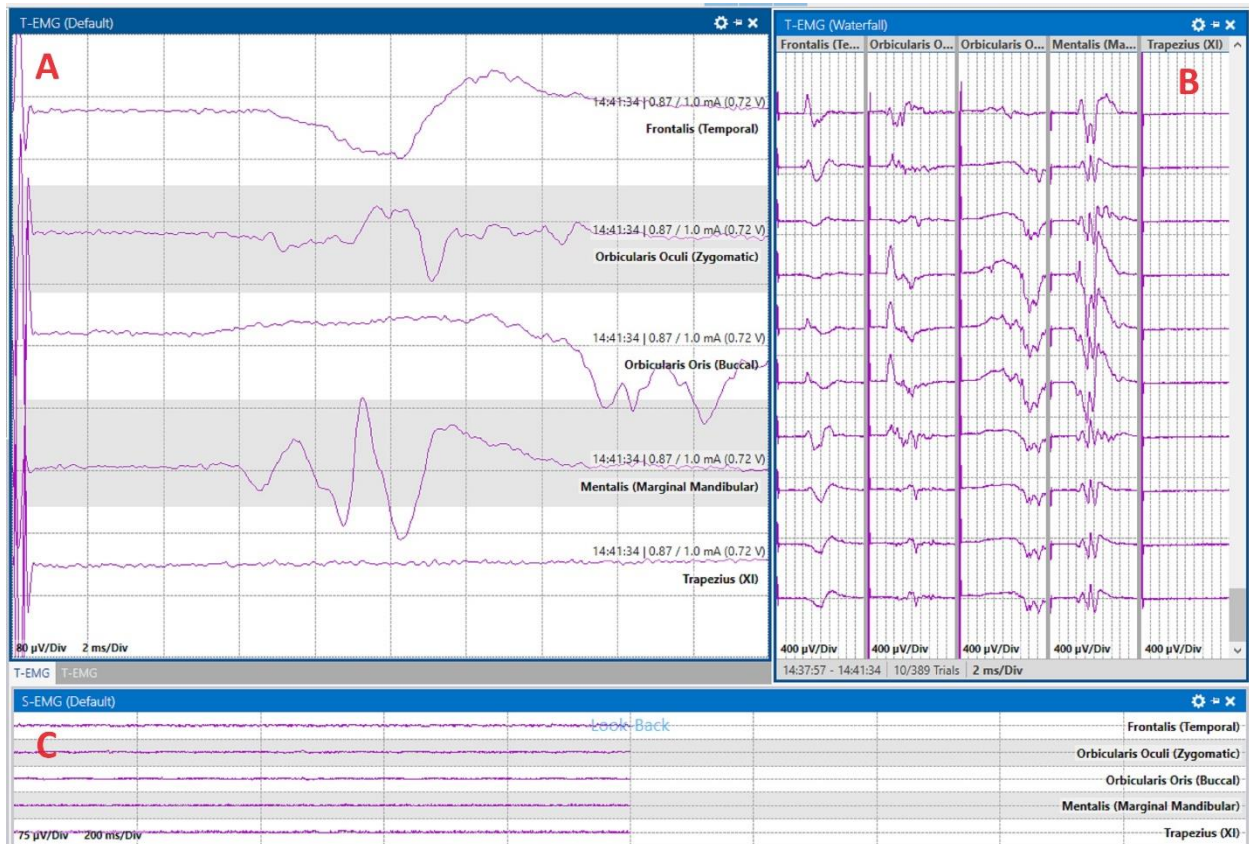
### *Anesthesia:*

General anesthesia with total intravenous anesthesia (TIVA) was given with no muscle relaxant. A train of four (TOF) was used during this surgery by stimulating the right median nerve and recording from APB muscles.

### *Electromyography (EMG)*

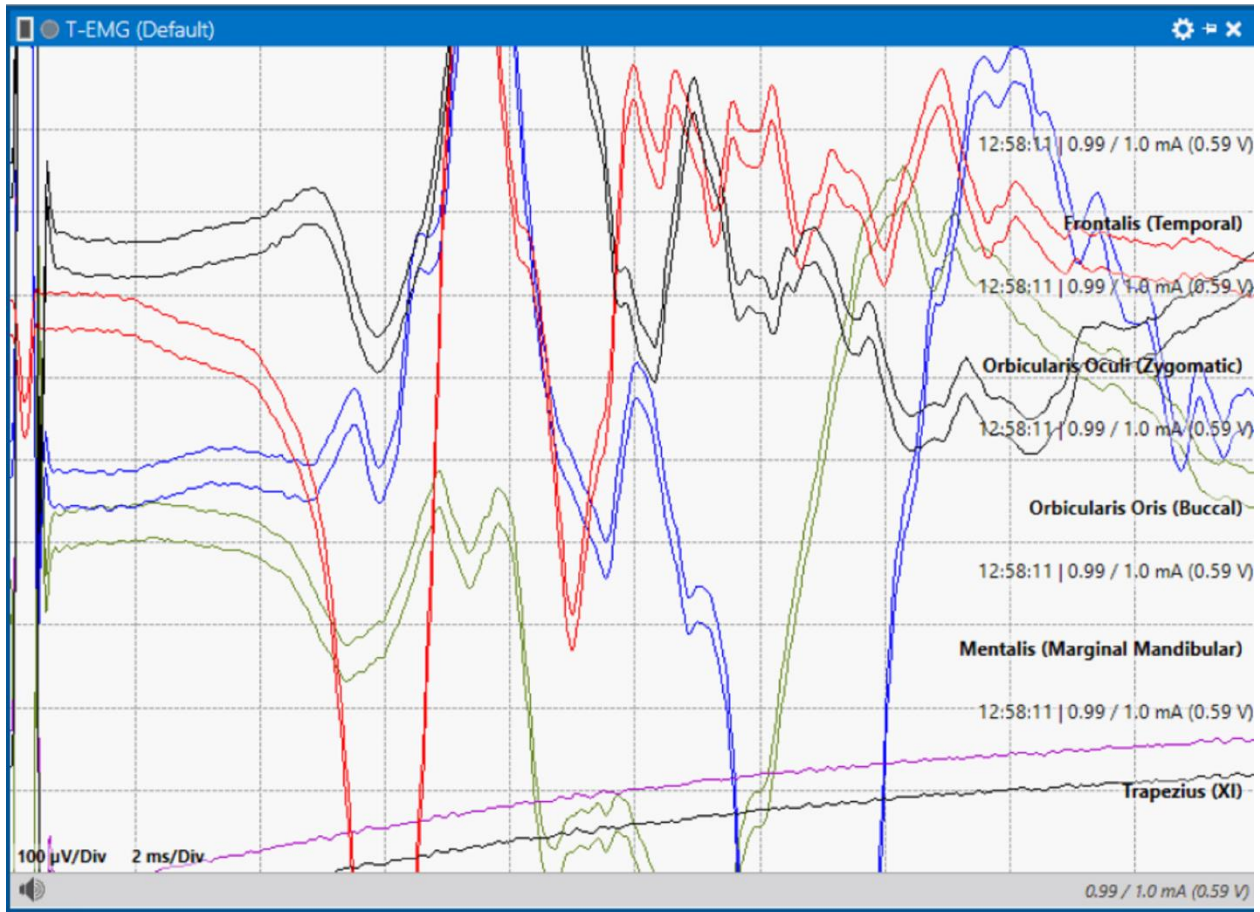
Electromyography (EMG) is used to monitor the functional integrity of the nerve supplying a muscle. The five branches of the facial nerve, temporal, zygomatic, buccal, marginal mandibular, and cervical, are monitored by the EMG of the frontalis, orbicularis oculi, buccinator, mentalis, and platysma muscles, respectively. Subdermal needle electrodes are placed in these muscles. Two types of EMG are used: spontaneous (s-EMG) and triggered (t-EMG). T-EMG is done by stimulating a nerve and monitoring its response to the corresponding muscles (**Figures 2-5**). In s-EMG, subdermal needles are placed in muscles corresponding to nerves at risk and are then monitored passively without any nerve root stimulation.

For the intraoperative monitoring of facial nerves in parotid gland surgeries, a sensitivity of 50-200 uV/div is used. The low-frequency filter is kept at 10 Hz, and the high-frequency filter is kept at 5000 Hz. The sweep for s-EMG is kept at 300ms/div. For t-EMG, the sweep is kept at 1.0-1.5 ms/div, and the stimulation intensity is set to 0.05-4.0 mA. The stimulation duration should be 200 microseconds, and the stimulation rate should be set at a repetition of 2.66-3.79/sec.

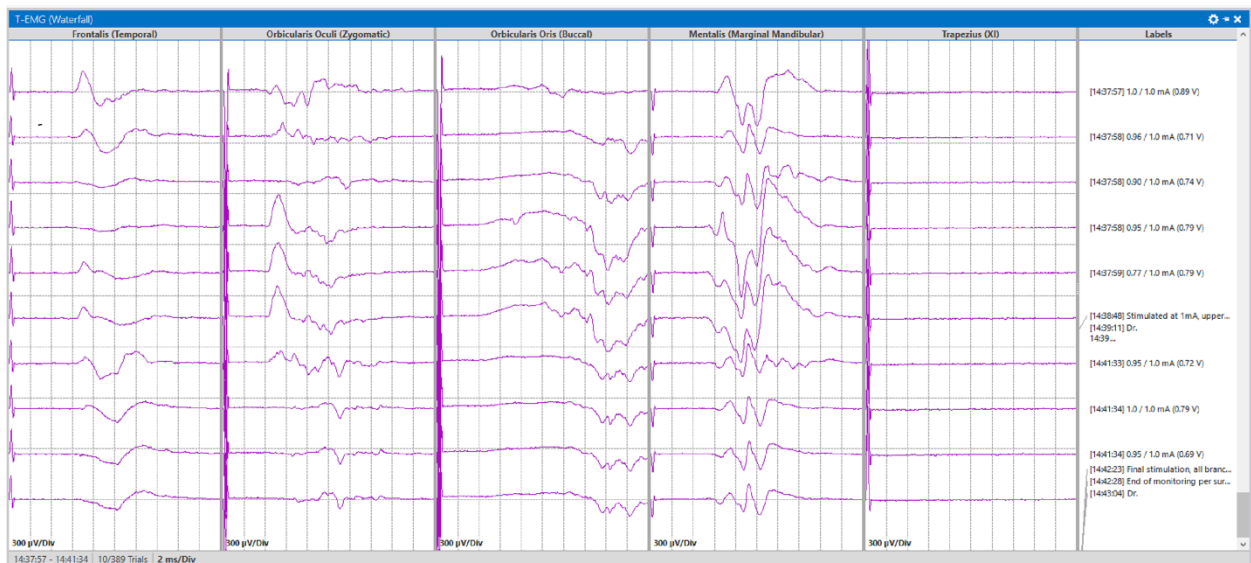


**Figure 2:** Facial nerve (CN VII) EMG responses. (A) Triggered EMG (t-EMG) responses with a monopolar hand-held probe at 1.0 mA. (B) Stack view of t-EMG responses from facial muscles with trapezius muscle as a control. (C) Spontaneous EMG (s-EMG) responses from the muscles of the facial expression.

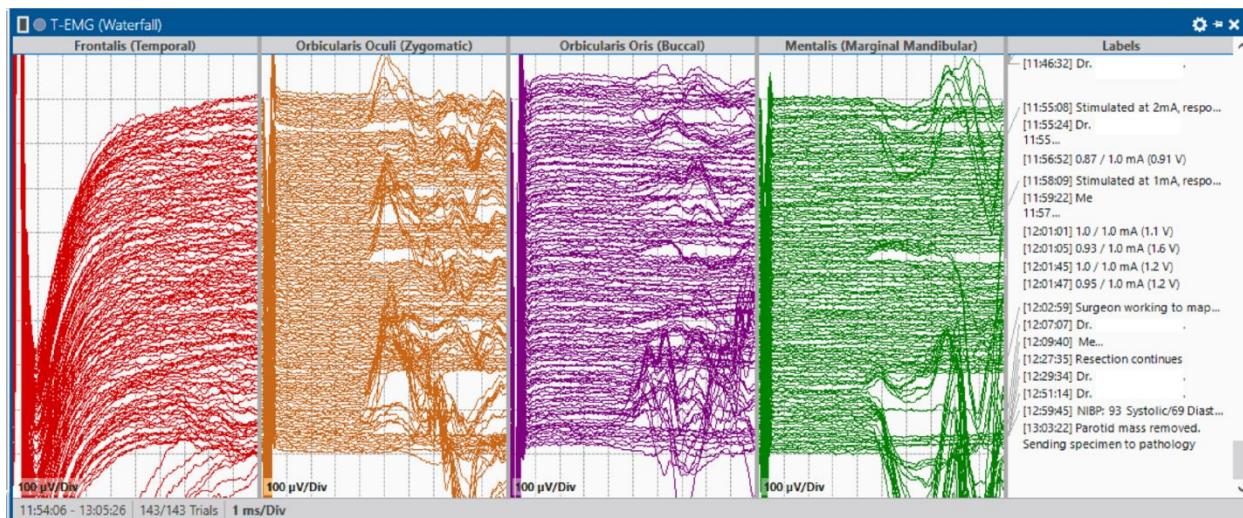




**Figure 3:** Facial nerve (CN VII) triggered EMG (t-EMG) responses with a monopolar hand-held probe at 1.0 mA. Responses can be seen in the frontalis, orbicularis oculi, orbicularis oris, and mentalis muscles.



**Figure 4:** Facial nerve (CN VII) triggered EMG (t-EMG) responses with a monopolar hand-held probe at 1.0 mA. Stack view of t-EMG responses from facial muscles with trapezius muscle as a control.



**Figure 5:** Facial nerve (CN VII) triggered EMG (t-EMG) responses with a monopolar hand-held probe at 1.0 mA. Stack view of t-EMG responses from facial muscles with trapezius muscle as a control.

### ***Corticobulbar MEP (CoMEP)***

Facial nerve corticobulbar motor evoked potentials (FN-CbMEPs) are a neurophysiological test used to assess the integrity and function of the corticobulbar pathways that control facial muscle movements. These pathways involve the communication between the motor cortex of the brain and the facial nerve nuclei in the brainstem, which ultimately control voluntary movements of the facial muscles.

The test involves the use of transcranial motor stimulation (TCMEP), which is a technique to stimulate specific regions of the brain, such as the motor cortex. The goal of FN-CbMEPs is to measure the time it takes for the stimulation to elicit a response in the facial muscles. Electrodes are placed on the face to record the muscle responses, known as evoked potentials. By analyzing the timing and amplitude of these evoked potentials, clinicians can assess the functional connectivity and conduction along the corticobulbar pathways that innervate the facial muscles.

FN-CbMEPs are particularly useful in various clinical settings, including:

**Neurosurgery:** Before performing surgical procedures near the facial nerve, such as tumor removal, surgeons may use FN-CbMEPs to map the motor cortex and assess the risk of damaging the facial nerve during the surgery.

**Diagnosis and Monitoring:** FN-CbMEPs can help diagnose and monitor conditions that affect the corticobulbar pathways, such as facial nerve disorders, motor neuron diseases, and multiple sclerosis.

**Research and Rehabilitation:** Researchers use FN-CbMEPs to study brain function, motor control, and plasticity. In rehabilitation, the test can help assess the effectiveness of interventions for facial muscle weakness or paralysis.

It's important to note that FN-CbMEPs should be performed by trained professionals and interpreted in the context of other clinical and diagnostic information. The test provides valuable insights into the neural

pathways controlling facial muscles and contributes to a comprehensive understanding of neurological function.

In intraoperative neurophysiological monitoring (IONM), facial muscle corticobulbar motor evoked potentials (FMCoMEP) are used to measure facial nerve motor function (FaNMF) during the surgical procedure (**Figure 6**). The hookwire electrodes or subdermal needles are placed on the same muscles employed for EMG monitoring, including the frontalis, orbicularis oculi, nasalis, orbicularis oris, mentalis, and platysma muscles. Corkscrew electrodes were placed on the scalp for stimulation at C3/Cz for left cortical stimulation and C4/Cz for right cortical stimulation. The stimulation duration was 50 or 75, with an interstimulus interval (ISI) of 2.0 ms and a frequency of 500 Hz. A double train stimulation was used with an Intertrain interval (ITI) of 90ms with three pulses followed by one pulse. A total sweep of 120ms was used. Recording filters with locut of 10 Hz hicut 5000 Hz and a sensitivity of 200uV per division were used. A drop in amplitude (AMP) to 50% is the warning criterion that is most frequently employed [3–10].



**Figure 6:** Corticobulbar MEP (CoMEP) from Facial nerve (FN-CbMEPs) Responses in stack view from mentalis muscles. Red arrow: Single pulse peripheral response followed by centrally conducted train CoMEP responses. Green arrow: absence of single pulse peripheral response with persistent centrally conducted CoMEP responses.

### ***Train of Four (TOF):***

The train of four (TOF) is crucial to examine surgical relaxation, intubation, and complete flaccidity during surgical procedures. During parotidectomy, TOF is used throughout the surgery to monitor the integrity of facial expression muscles. The stimulation rate for the TOF is optimized at 2Hz for a duration of 0.2ms with a sweep of 20ms/div, a sensitivity of 200 $\mu$ V, and a current ranging from 0-50mA.

### **Parotidectomy Steps and Considerations:**



Parotidectomies are performed for a multitude of reasons, including but not limited to neoplasms, chronic parotitis, or as a part of resection for other malignancies such as cutaneous melanomas or squamous cell carcinomas as part of deep margins of resection. Specific complications of parotidectomy include salivary fistulas, permanent facial nerve injury, Frey's syndrome, and First bite syndrome. Regarding potential facial nerve injury, rates of paresis and paralysis increase with the level of parotid tissue removed, lowest at partial parotidectomies, followed by superficial, and highest at total parotidectomies (Iowa Head and Neck protocols).

Once the patient is per-operatively prepped and draped, with appropriate anesthesia induction and positioning, operative steps begin with initial incision and flap elevation. A preauricular incision is made above the level of the zygomatic arch and extended posteriorly to the mastoid, which allows for the mobilization of the tail of the parotid. The flap is continually elevated above the level of the peri parotid fascia, with a thicker skin flap allowing a decreased risk of Frey's syndrome. In most cases, the posterior branches of the great auricular nerve can also be preserved. The parotid gland is then mobilized further to allow for facial nerve dissection. This mobilization and exposure occur by initially elevating the parotid off the sternocleidomastoid (SCM) muscle and the tragal cartilage. Once done, the facial nerve can be identified, with the five most common methods of identification including the tympanomastoid suture, the tragal pointer, the digastric muscle, within the fat deep to the parotid-mastoid fascia or using the facial nerve stimulator. Once located, the nerve is dissected further, with the direction of dissection dependent on tumor location and pathology. Parotid tissue is then removed, with the amount being dependent on the tumor size and type and sent for frozen section analysis.

Of note, during this last section of the case, the dissection of the facial nerve and resection of parotid tissue increases the chance of facial nerve injury, given the proximity and close dissection. Facial nerve sacrifice is dependent upon frozen section results and is generally sacrificed in the case of malignancy, with malignancy also indicating a need for further exploration, whether that is the removal of all parotid tissue or possibly even neck dissection. It is generally preferred to preserve facial nerve function, even in the case of positive microscopic tumor margins. Efforts should be maintained to preserve facial nerve function as much as possible, especially if the function was present before the procedure. It is here where intra-operative facial nerve monitoring can greatly benefit through the meticulous dissection of the facial nerve away from the parotid gland and removing parotid tissue.

## CASE INFORMATION

Twenty-three patients met inclusion criteria with parotidectomies due to a parotid mass performed between 11/20/2012 and 1/13/2023. Fourteen patients were male, and nine were female (**Table 1**). Thirteen patients underwent right-sided parotidectomies, and ten patients underwent left-sided parotidectomies (**Table 2**). The patients' ages ranged between 28 and 94 years, with a mean age of 63 years (**Table 3**). All patients underwent their procedures with appropriate intraoperative facial nerve monitoring.

Electromyography (EMG) is an important tool now being applied to assess the functional integrity of the nerve innervating the muscle. The EMG of the frontalis, orbicularis oculi, buccinator, orbicularis oris, mentalis, and platysma muscles, respectively, is used to monitor the five branches of the facial nerve: the temporal, zygomatic, buccal, marginal mandibular, and cervical. This aids in the real-time identification and functional evaluation of nerves involved in surgery. The motor branch of the facial nerve, superior laryngeal, recurrent and inferior laryngeal are usually monitored in head and neck surgeries.

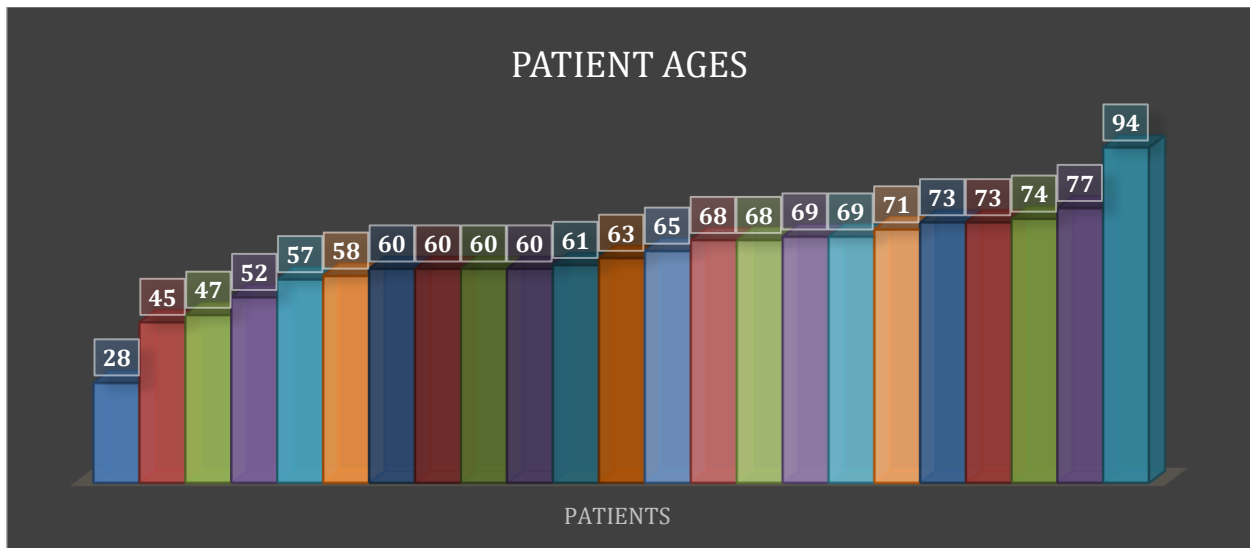


<b>Gender Distribution of the Parotid Gland Tumor</b>		
<b>Gender</b>	<b>Frequency</b>	<b>Percentage</b>
<b>Male</b>	14	60.9
<b>Female</b>	9	39.1
<b>Total</b>	23	100

**Table 1.** Gender distribution of the parotid gland tumor.

<b>Surgical Side of the Parotid Gland Tumor</b>		
<b>Side</b>	<b>Frequency</b>	<b>Percentage</b>
<b>Right</b>	13	56.5
<b>Left</b>	10	43.5
<b>Total</b>	23	100

**Table 2.** Frequency and percentage of the parotid gland tumor on right and left sides.



**Table 3.** Ages of the twenty-three patients who underwent the parotid gland tumor resection with facial nerve monitoring.

## DISCUSSION

Head and neck muscles, such as orbicularis oris (CN VII), Orbicularis oculi (CN VII), and false vocal cords (CN X), are used to monitor these nerves. EMG can be monitored intra-operatively as spontaneous (s-EMG) or triggered (t-EMG). Any injury to nerves in the form of pulling, stretching, heating, or compression is detected by s-EMG as an abnormal activity such as bursts, spikes, trains, and neurotonic discharge. Spontaneous EMG provides immediate feedback to the surgeon, minimizing nerve root damage, but it can only be used without muscle relaxants, minimizing its use. T-EMG is used for mapping the nerve pathways, identifying nerve roots, and confirming the functional continuity of the nerve roots.

The corticobulbar tract (CBT) contains seven different tracts innervating the different cranial nerve motor nuclei. The facial corticobulbar tract is commonly monitored during surgical procedures; therefore, its functional integrity can be continuously monitored. A double train transcranial electrical stimulation (dt-TES) for motor evoked potentials (MEP) consisting of initially three pulses facilitating a train of stimuli was followed by another pulse as a testing train. A control pulse, either 20 msec before or 40 or 90 msec after the pulse train, is added to differentiate corticobulbar stimulation from peripheral stimulation. Parameters were chosen so that only the TES pulse train elicited a response for the facial nerve target muscles, and the control pulse did not. A fixed pattern of stimulus intensity at 30 mA was used to determine the baseline FMcoMEP stimulation threshold before the skin incision. This was then increased by 5-mA increments until one of the target muscles responded to the stimulation.

Regarding potential facial nerve injury in partial, superficial, or total parotidectomies, total parotidectomy has a substantially greater chance of facial nerve paralysis than partial or superficial parotidectomies [9]. Though facial nerve weakness is primarily temporary post-operatively, rates of paresis are lowest in partial parotidectomies at 1.9%, to 22% in superficial, and up to 46% in total parotidectomies. Rates of facial nerve paralysis range from 0.7% in partial to 2% in superficial and up to 10% in total parotidectomies (Iowa Head

and Neck Protocols). In both cases of paralysis and paresis of the facial nerve, total parotidectomies have the worst rates of injury.

After anatomical preservation following parotid surgery, several explanations have been proposed for facial nerve dysfunction. Mechanical trauma, such as crushing and compression during surgery or ischemia injury during nerve dissection, could be the cause. According to Dulguerov et al., nerve stretching is the most probable cause of facial nerve dysfunction following anatomical preservation. [10] For transient facial weakness, facial dysfunction ranges from 14 to 66%, and persistent facial weakness ranges from 0 to 9%. Drooling, asymmetry of the face, trouble chewing, and corneal ulcers are all symptoms of facial nerve injury [11]. Additionally, there can be damage and injury to branches of the great auricular nerve following parotidectomy during initial dissection, resulting in hypoesthesia. The numbness normally disappears a year after the surgery, but a small skin patch could be permanently anesthetized [9].

Given this array of potential complications, intra-operative nerve monitoring must be utilized whenever possible to monitor and preserve the facial nerve. Given the benefit of intra-operative nerve monitoring and the reduction reported in post-operative transient facial nerve weakness and palsy grade (Savvas et al. 2016), it should be utilized whenever available. This decrease in weakness is likely a result of the accurate identification of nerve branches and, as a result, decreased operative mechanical stress on the nerve itself through decreased stretching or manipulation when unnecessary. The use of IONM should be considered even more so in cases of parotid gland malignancy, where there is deep lobe involvement or a necessity for total parotidectomy with further exploration. As rates of facial nerve injury only increase as the grade of parotid tissue is removed, IONM should be considered highly similar as further tissue is removed. This requires even further attention to the facial nerve. Further studies can be done to delineate the facial nerve injury outcomes using IONM in partial, superficial, and total parotidectomies.

## CONCLUSION

The parotid gland is the most affected gland when it comes to salivary gland neoplasms. As a result, it is a subject of surgery to remove these neoplasms, and given the anatomic course of the facial nerve through this gland, it is of utmost importance to find ways to increase facial nerve preservation through these procedures. Intra-operative nerve monitoring provides a safe and reliable way to monitor the facial nerve, especially in deep lobe involvement or total parotidectomies. It should be utilized to decrease potential facial nerve injury and palsy post-operatively.

## ORCID

Faisal R. Jahangiri	<a href="https://orcid.org/0000-0002-1342-1977">https://orcid.org/0000-0002-1342-1977</a>
Wasiq Nadeem	<a href="https://orcid.org/0000-0002-2194-8488">https://orcid.org/0000-0002-2194-8488</a>
Wadana H. Khattak	<a href="https://orcid.org/0009-0001-9138-5924">https://orcid.org/0009-0001-9138-5924</a>
Rafia H. Jahangiri	<a href="https://orcid.org/0009-0009-3210-6612">https://orcid.org/0009-0009-3210-6612</a>
Laila Farooq	<a href="https://orcid.org/0009-0004-9166-7608">https://orcid.org/0009-0004-9166-7608</a>
Hooria Asad	<a href="https://orcid.org/0009-0005-0610-1958">https://orcid.org/0009-0005-0610-1958</a>



## REFERENCES

1. Drake, R., Vogl, A. W., & Mitchell, A. W. (2009). *Gray's anatomy for students E-book*. Elsevier Health Sciences.
2. Kochhar, A., Larian, B., & Azizzadeh, B. (2016). Facial nerve and parotid gland anatomy. *Otolaryngologic Clinics of North America*, 49(2), 273-284.
3. Stryjewska-Makuch, G., Kolebacz, B., Janik, M. A., & Wolnik, A. (2017). Increase in the incidence of parotid gland tumors in the years 2005–2014. *Polish Journal of Otolaryngology*, 71(2), 29-34.
4. Chiesa-Estomba, C. M., Saga-Gutiérrez, C., González-García, J. Á., Calvo-Henríquez, C., Larruscain, E., Sistiaga-Suárez, J. A., ... & Quer, M. (2021). Intraoperative monitoring of the facial nerve during parotid gland surgery in Otolaryngology services—Head and Neck Surgery. *Acta Otorrinolaringologica (English Edition)*, 72(3), 158-163.
5. Wiertel-Krawczuk, A., & Huber, J. (2020). Iatrogenic injury and regeneration of the facial nerve after parotid gland tumour surgery: a pilot study with clinical and neurophysiological assessment. *Journal of Medical Science*, 89(1), e385-e385.
6. Marchese-Ragona, R., De Filippis, C., Marioni, G., & Staffieri, A. (2005). Treatment of complications of parotid gland surgery. *Acta Otorhinolaryngologica Italica*, 25(3), 174.
7. Kennelly, K. D. (2019). Clinical neurophysiology of cranial nerve disorders. *Handbook of clinical neurology*, 161, 327-342.
8. López, J. R., & Legatt, A. D. (2022). Monitoring surgery around the cranial nerves. In *Handbook of Clinical Neurology* (Vol. 186, pp. 319-351). Elsevier.
9. Thahim, K., Udaipurwala, I. H., & Kaleem, M. (2013). Clinical manifestations, treatment outcome and post-operative complications of parotid gland tumours-an experience of 20 cases. *J Pak Med Assoc*, 63(12), 1472-1475.
10. Siddiqui, A. H., Shakil, S., ur Rahim, D., & Shaikh, I. A. (2020). Post parotidectomy facial nerve palsy: A retrospective analysis. *Pakistan Journal of Medical Sciences*, 36(2), 126.
11. Chiang, F. Y., Wang, C. C., Wu, C. W., Lu, I. C., Chang, P. Y., Lin, Y. C., Lien, C. F., Wang, C. C., Huang, T. Y., & Hwang, T. Z. (2021). Correlation between Electrophysiological Change and Facial Function in Parotid Surgery Patients. *Journal of clinical medicine*, 10(24), 5730. <https://doi.org/10.3390/jcm10245730>