


# Intraoperative Neurophysiological Monitoring: What the Anesthesiologist Should Know. Narrative Review

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## Abstract

Intraoperative neurophysiological monitoring (IONM) allows monitoring and evaluating the integrity of the motor and sensory systems in procedures that put the nervous system (NS) at risk and prevents complications. For an appropriate IONM, it is fundamental to comprehend current techniques, how they work, as well as the influence of anesthetics and other variables on the records.

**Objective:** Review peer-reviewed publications; identify and describe the main IONM techniques and the impact of anesthetic and perioperative management on them.

**Methods:** A systematic and narrative search and review was carried out in English and Spanish in the Medline, Scopus and PubMed databases, using the MeSH terms: "Intraoperative Neurophysiological Monitoring"; "Anesthesia"; "Neuroanesthesia"; "Perioperative Management"; "Neurological surgery", "Complications", "Safety".

**Results:** Current national and international clinical guidelines for intracranial and spine surgery recommend multimodal IONM to evaluate the functional integrity of the NS and reduce complications. Total intravenous anesthesia (TIVA) with propofol is recommended as the technique of choice for a better recording of motor evoked potentials (MEP) and somatosensory evoked potentials (SSEP).

**Conclusions:** It is essential to understand the clinical bases of the different IONM techniques and to interpret alerts and alarm criteria in a timely manner to obtain optimal surgical results and prevent neurological injuries. The neuroanesthesiologist must ensure an adequate state of anesthetic depth, physiological, hemodynamic and cerebrospinal perfusion pressure stability, avoiding modifications that could alter the recordings.

**Keywords:** Anesthesia; Neuroanesthesia; Intraoperative Neurophysiological Monitoring; Neurological surgery; Complications; Safety.

## What do we know about this problem and what is the contribution of this study?

Despite the wide circulation and recommendation of the use of IONM by international guidelines, there is little diffusion and understanding among anesthesiologists about techniques, objectives, importance, and impact of anesthetic and perioperative management.

This study identifies and describes the main IONM techniques, as well as the impact of drugs, hemodynamic variables, anesthetic and perioperative management on IONM — all essential knowledge for every anesthesiologist.



## Introduction

Intraoperative neurophysiological monitoring (IONM) allows the identification of nerve structures, as well as real-time monitoring and evaluation of the functional integrity of the cerebral cortex, brainstem, spinal cord (SC), nerve roots, and cranial and peripheral nerves, avoiding motor and/or sensory postoperative deficits — transient or permanent — in procedures that put the nervous system (NS) at risk, through various neurophysiological techniques.<sup>1-6</sup> To ensure adequate monitoring, it is recommended to record baseline or reference and control waveforms.<sup>1</sup>

Due to the influence of most anesthetics on the inhibitory pathways,<sup>3,7</sup> the knowledge and understanding of the main IONM techniques and the impact of anesthetic management and various physiological variables on the recordings is crucial for optimal results.

## Methodology

A systematic and narrative search and review was undertaken in the Medline, Scopus and PubMed databases — in English and Spanish — using the MeSH terms: "Intraoperative Neurophysiological Monitoring"; "Anesthesia"; "Neuroanesthesia"; "Perioperative Management"; "Surgery neurological"; "Complications"; "Safety". Narrative statistics and recommendations based on the results are presented.

## Results

IONM is a useful technique for evaluating the neurological status of patients under general anesthesia.<sup>8</sup> For over 30 years, it has been used to detect and prevent injuries in various intracranial and spinal surgeries.<sup>3,9,10</sup> It is effective to identify a possible peripheral nerve injury, predict risk of paraparesis, paraplegia and quadriplegia (sensitivity 100%; specificity 91%); predict improvement of the facial nerve in facial hemispasm surgery and guide the resection of epileptogenic areas in epilepsy surgery.<sup>5</sup> Likewise, it is used in endonasal surgery and resection of skull base tumors, for identifying and reducing the risk of cranial nerve injury or critical neurovascular structures;<sup>11-12</sup> prevention of ischemic complications and neurological deficits in endovascular<sup>13</sup> or surgical<sup>14-15</sup> management of intracranial aneurysms; prevention and prediction of perioperative neurological deficits in posterior fossa surgery,<sup>16</sup> aortic<sup>1,17</sup> or thoracic spinal fusion surgery.<sup>9</sup>

Its use has been more widespread in spinal surgery, in which, despite favorable results, nerve elements can be damaged

by direct (compression, traction, section laceration, avulsion) or indirect mechanisms (ischemic phenomena due to vascular elongation or compression of the spinal cord), and generate complications secondary to surgical treatment.<sup>18-20</sup>

The recording of evoked potentials (EP) represents the electrophysiological technique with the greatest capacity to provide quantitative, objective and opportune measurements for monitoring the functional integrity of the SC, nerve roots, along with adequacy of the vascular supply to these elements,<sup>4,9,21</sup> since it allows rapid and real-time recognition of functional changes in motor, sensory and nerve structures pathways that comprise them (anterior tracts and posterior cords).<sup>5, 21-22</sup>

Solid Class I evidence ratifies its use —including recording of somatosensory evoked potentials (SSEP) and transcranial motor evoked potentials (TcMEP)—, as a reliable diagnostic adjunct, as well as a valid method to assess SC integrity in the perioperative setting of column surgery.<sup>23</sup>

Multimodal IONM is the combination of different neurophysiological techniques and is the gold standard for preventing and reducing the incidence of postoperative neurological complications<sup>24-26</sup> (Figure 1); the combination of SSEP (Figure 2) and MEP (Figure 3) through sensory and motor cortex stimulation evaluates transmission pathways through the recording of the evoked response (Figure 4), which can suggest modifications during the procedure, detecting, preventing and reducing the incidence of postoperative neurological deficit up to 60% in spinal surgery<sup>22,26</sup> with proven benefit in scoliosis correction;<sup>9,27-29</sup> and as a routine component in surgeries for deformities or intramedullary tumors, reducing complications and maximizing adequate resection.<sup>23</sup>

Despite the evident advantage of IONM to reduce expenses derived from possible complications,<sup>5</sup> the cost-benefit in anterior cervical fusion surgery is considered controversial,<sup>8,30</sup> whereas in the rest of the spine procedures, in duly justified cases, it can be a useful, valid and sensitive tool to detect neurological damage in high-risk conditions or with pre-existing myelopathy, especially with the use of multimodal IONM,<sup>4</sup> provided that it is used as a diagnostic tool and not a therapeutic one.<sup>23</sup>

Current international clinical guidelines recommend multimodal IONM to assess the functional integrity of the NS, SC and nerve roots.<sup>1, 23, 31-32</sup> In Mexico, the Clinical Practice Guide for the Implementation of IONM establishes the degrees of recommendations and levels of evidence for practice.<sup>6</sup>

Figure 1. Multimodal monitoring

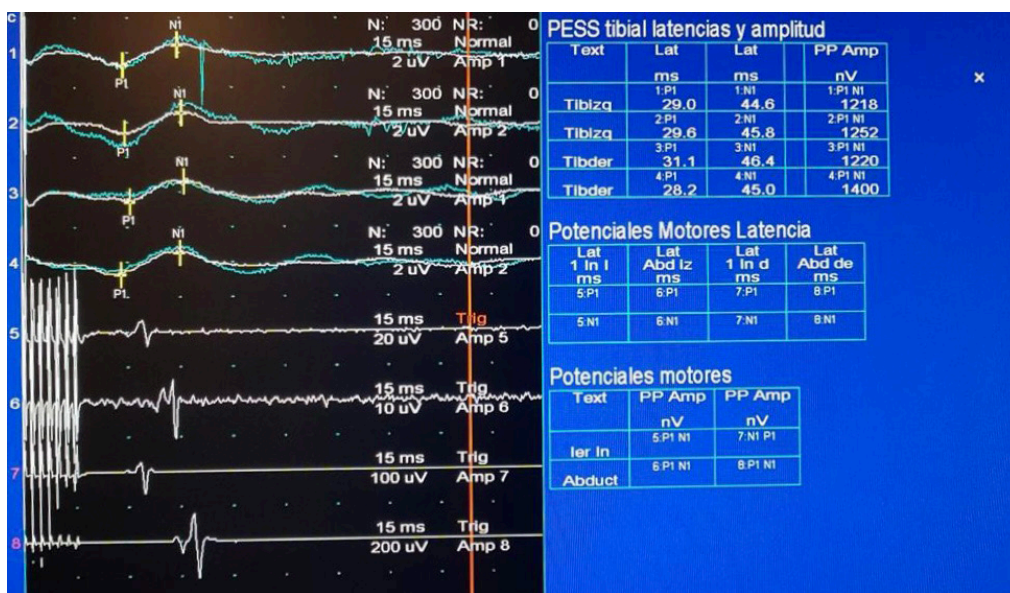


Figure 2. Somatosensory evoked potentials

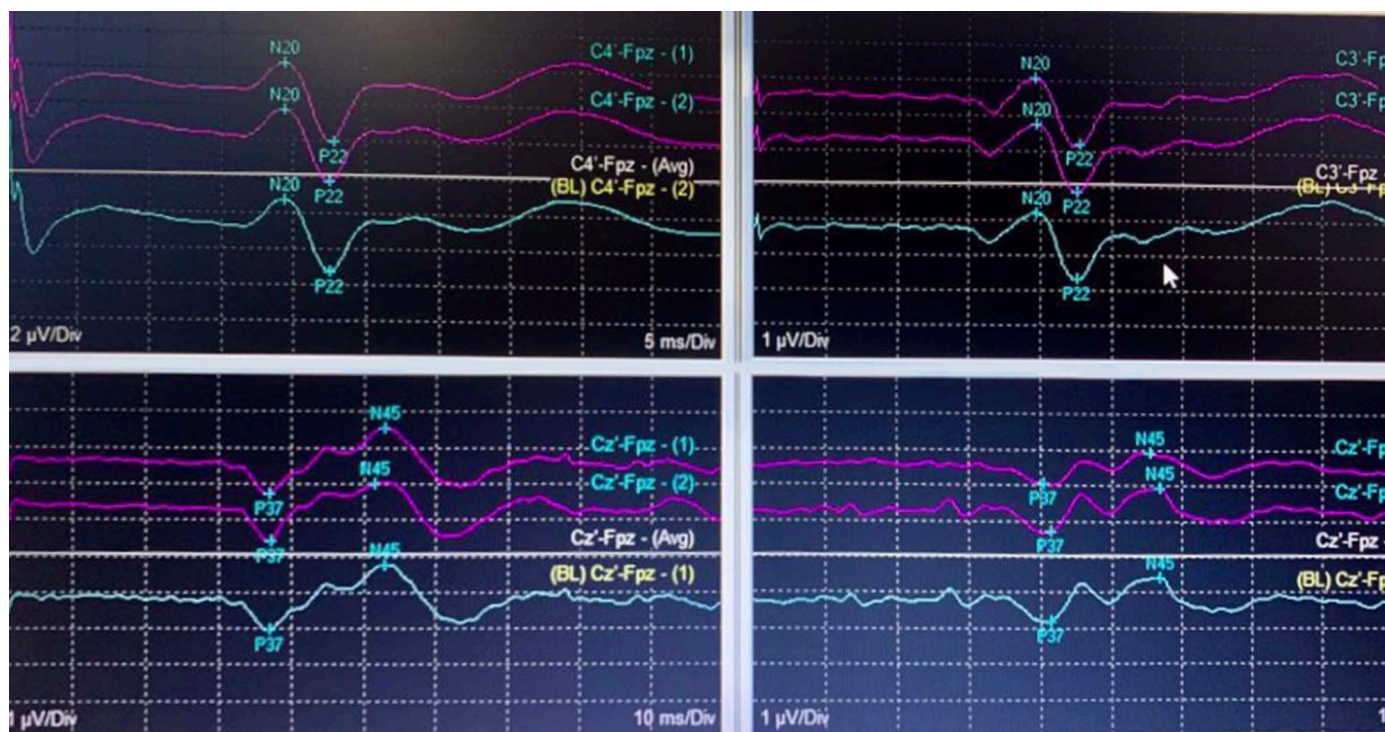
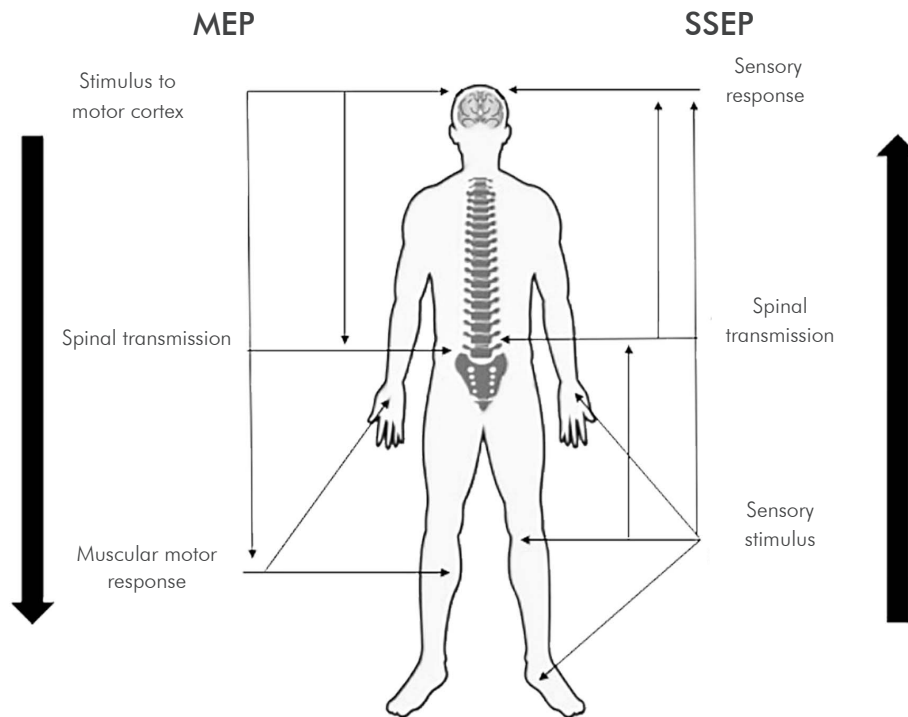


Figure 3. Motor evoked potentials



Figure 4. Direction of motor and sensory transmission pathways stimulation in motor and sensory evoked potentials



There are at least eight methods for monitoring SC and spinal nerve root functions during surgical procedures:<sup>33</sup>

1. SCEP: spinal cord evoked potential recorded after its stimulation.
2. SSEP: somatosensory evoked potential with cortical recording after stimulation of a peripheral nerve.
3. SSEP: Somatosensory evoked potential recorded in SC after stimulation of a peripheral nerve.
4. Spinal cord MEP: Transcranial electrical or magnetic stimulation (single pulse) in the motor cortex and recording of the response (D and I waves) in SC with an epidural or subdural electrode.
5. Transcranial motor evoked potentials (TcMEP): Transcranial electrical stimulation (motor cortex) multipulses (train of 5-7 stimuli) and response recording in peripheral muscles.
6. Spinal MEP: SC stimulation and recording in peripheral muscles.
7. Continuous electromyography: recording in rostral and caudal myotomes and muscles innervated by motor roots that emerge at procedure level.
8. Evoked Electromyography: Identifies nerve structures, confirms their integrity, conduction status and proper placement of pedicle screws. It stimulates motor roots at the procedure area, surrounding bone structures, and/or pedicle screws.

Monitoring is specific to the area related to the procedure, such as recurrent laryngeal nerve (RLN) monitoring in thyroidectomy or anterior cervical fixation/decompression surgeries, in which EMG electrodes are attached to the endotracheal tube and placed at the level of the vocal and arytenoid muscles for monitoring.<sup>2</sup>

The complete disappearance of SSEP is associated with limb paralysis and flaccidity,<sup>21</sup> an increase in latency >10% and/or decrease in amplitude >50% in SSEP and 50-100% in MEP compared to the baseline value constitute "**alarm criteria**", indicative of lesion risk in the ascending sensory or descending motor pathway, respectively, which requires timely intervention to avoid permanent damage.<sup>14,21,26</sup> These alarm criteria depend on factors such as response variability, type of anesthesia, positioning and nerve injury,<sup>26,34</sup> presence or absence of pre-existing neurological injury and surgical, metabolic and physiological events at the time of decrease or disappearance, such as mechanical or compressive trauma, or changes due to ischemia, hypoxia, hypotension, hematocrit <15%, hypothermia <32°C, hyperthermia >42°C, PCO<sub>2</sub> <20 mmHg, or decrease with ICP >25 mmHg or disappearance with ICP >30 mmHg (17, 35).

Loss of signals or reversible decreases (<30-40 min) can predict the absence of new postoperative deficits, while prolonged decreases (>40-60 min) can indicate a risk of permanent injury.<sup>18-19</sup>

In the event of changes in the IONM during surgery, the information must be shared among the multidisciplinary team and measures taken to find and eliminate the cause (LE:1C), whether surgical, due to failure or disconnection of the neuromonitoring or anesthetic equipment as a result of changes in physiological variables, due to anesthetics dose or level of hypnosis (LE: 1D).<sup>1</sup> Once the alteration is identified, it is recommended that the decision to continue the procedure is taken by the entire team. Signal improvement is a predictor of favorable neurological outcome, particularly for SC decompression surgery.<sup>22</sup>

### Somatosensory evoked potentials (SSEP)

They were first used in the 1970's to monitor SC function in scoliosis correction. Following stimulation of peripheral mixed nerves, responses are recorded at various points in the somatosensory pathway (peripheral, subcortical, and cortical responses).<sup>24,26</sup>

As an electrophysiological response to a sensory stimulus of the NS, SSEP provide functional and locational information about the somatosensory system, and are the most widely used monitoring modality.<sup>16</sup> They are indicated for any surgery that puts the proprioceptive pathway at risk and to complement the monitoring of MEP.<sup>32</sup>

The afferent pathway begins with receptors in skin, muscle, and tendons, rapidly conducting thickly myelinated afferent Ia fibers, and the first neurons (unipolar or pseudomonopolar) located in the dorsal root ganglion. Neuronal axons travel in the ipsilateral dorsal column to the cervicomedullary junction. At this site they synapse with the second neurons located in the gracile nuclei (medial), for legs, and cuneiform (lateral), for arms. Second neurons axons cross to the opposite side and subsequently travel as a medial lemniscus reaching the ventral posterolateral nucleus of the thalamus, where they synapse with the third neuron, and axons of these neurons reach the primary parietal sensory cortex.<sup>36</sup> The stimulus is performed in a mixed peripheral, median or ulnar (upper limbs) and tibial or peroneal (lower limbs) nerve and electrodes are placed at specific sites along the somatosensory pathway to record electrical responses.

The dorsal proprioceptive pathway conveys discriminative touch, vibration, and proprioception, while the anterolateral exteroceptive pathway conveys pain, temperature sensation,

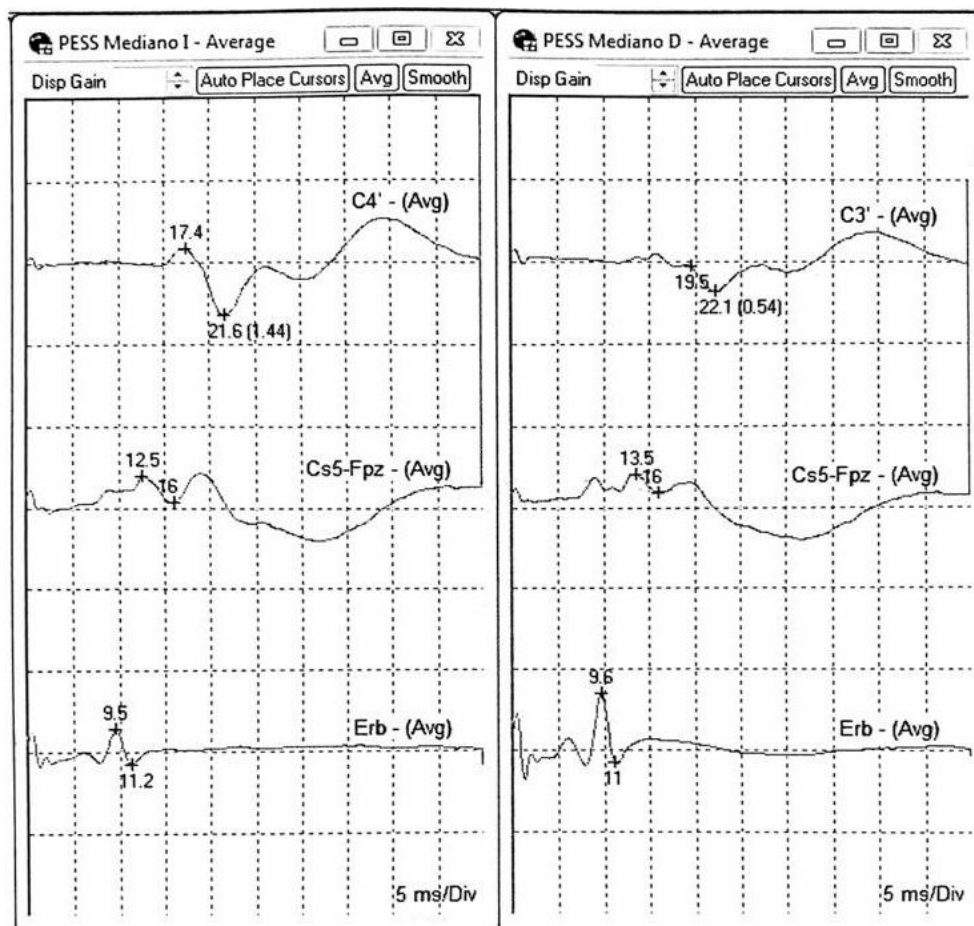
and superficial touch. This technique does not evaluate the anterolateral system because the axons are thinner, with higher thresholds, and slower and more variable conduction. The nerves stimulated will depend on the location of the surgical site.<sup>32</sup>

The nomenclature to designate the peaks and troughs of the SSEP waveforms is by means of a letter that represents its polarity; an ascending deviation means negative polarity (N), a descending one is positive (P); a number is also assigned based on the latency (time between the stimulus and the response), which is measured in milliseconds (ms);<sup>17</sup> similarly, the amplitude represents the number of functional fibers —these last two, amplitude and latency, are used to demonstrate changes in neuronal activity. The peaks called N20 and P22 result from the stimulation of the median nerve, of thalamic and cortical origin, whereas the cortical responses are P37 and N4536 correspond to the posterior tibial nerve or the peroneal nerve (Figure 5).

Intraoperative injury causes acute neuronal or axonal disruption that primarily reduces the amplitude of the SSEP and has less effect on latency. Demyelination increases latency and produces less effect on amplitude; amplitude is the main parameter to consider in intraoperative monitoring.<sup>32,36</sup>

Cortical SSEP responses of the median nerve are generated by the primary somatosensory cortex supplied by the middle cerebral artery and are useful for detecting ischemia associated with aneurysm clipping during temporary internal carotid occlusion. The tibial nerve has been used in ischemic events associated with anterior cerebral artery aneurysms.<sup>15</sup> Cortical SSEP amplitude decreases with regional CBF <20 ml/min/100 g and is completely lost with <15 ml/min/100 g.<sup>17</sup> An abrupt loss of the cortical SSEP response (<1 min after clipping)<sup>15</sup> or decrease in MEP amplitude >50% can predict postoperative neurological dysfunction.<sup>14</sup>

Figure 5. SSEP of upper limbs



In posterior fossa surgery there is a high risk of brainstem injury; in this regard, SSEP are useful for monitoring the integrity of the medial lemniscus.<sup>16</sup> In carotid endarterectomies, the use of electroencephalogram (EEG), MEP, and SSEP of the median and tibial nerve is recommended for arterial bypass decisions, intraoperative brain neuroprotection, and risk reduction of cerebral ischemia.<sup>5</sup>

Unilateral amplitude decrease in upper extremity SSEP responses with or without MEP impairment detects peripheral nerve or brachial plexus conduction abnormality in 2% to 3% of scoliosis surgeries.<sup>27</sup> Descending aortic procedures have a high risk of SC infarction and paraplegia by temporarily or permanently interrupting blood flow; in this cases IONM detects incipient ischemia prior to permanent damage.<sup>17</sup>

### Motor Evoked Potentials (MEP)

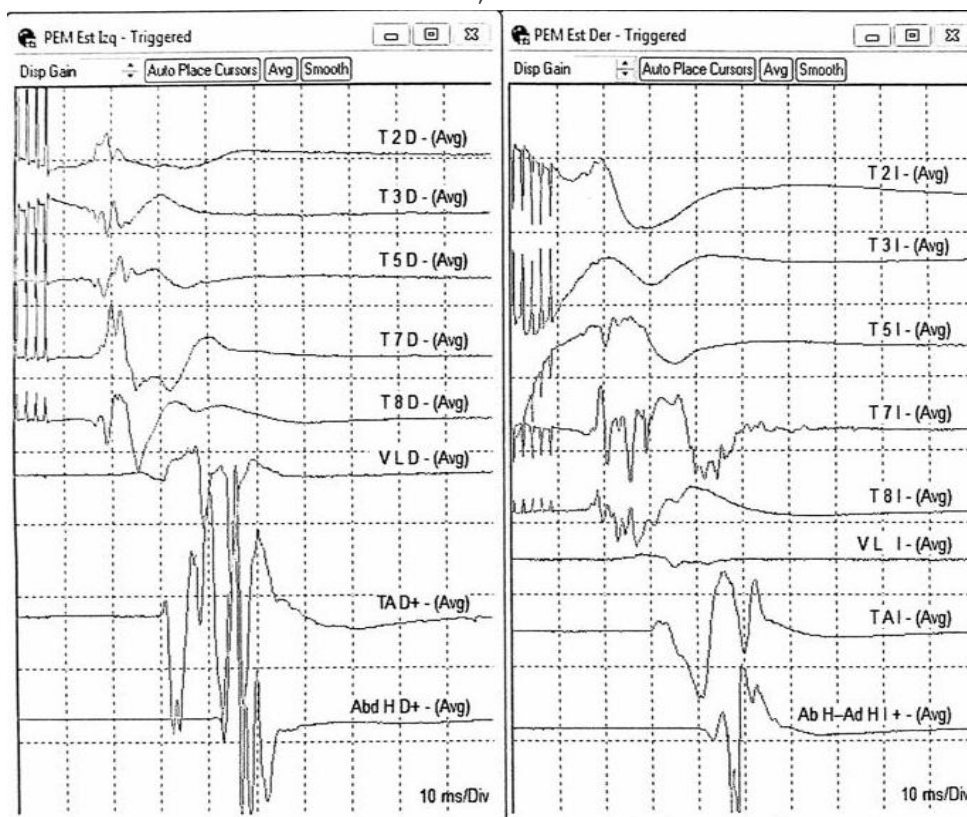
They evaluate functional integrity of the motor pathway, through transcranial electrical stimulation and depolarization of corticospinal neurons; descending impulses are recorded in SC and extremities<sup>2</sup> (Figure 6).

In 1980, Merton and Morton recorded muscle action potentials after transcranial motor cortex stimulation in humans, prompting the development of SC monitoring.<sup>10</sup> Subsequently, in the 1990s, muscle motor evoked potentials (mMEP) and epidurals (eMEP or D wave) were introduced.<sup>24</sup>

MEP can be spinal, neurogenic and muscular, they facilitate a selective and specific evaluation of the integrity of the motor pathway, from the cortex to the peripheral nerve fibers and muscles,<sup>24</sup> and allow detecting the functional integrity of the corticospinal pathway with high sensitivity and specificity.<sup>25</sup> Monitoring of the motor system can be performed by short-train transcranial stimulation (5-7 stimuli) or single stimulation with recording in SC.<sup>33</sup> Lesions of axons, neurons or motor support systems show a threshold that ranges from the reduction of amplitude until its disappearance.<sup>32</sup>

The disappearance of the mMEP response does not always indicate permanent motor deficit; the sensitivity of PEM monitoring to detect decreased cerebral blood flow (CBF) in aneurysm surgery has already been validated,<sup>14</sup> but its sensitivity

Figure 6. Lower extremity motor evoked potentials, T2, T3, T5, T7, T8, vastus lateralis, tibialis anterior, abductor hallucis



to ischemic aggression and spinal cord compression is high, so false positives increase if the trial is based solely on this potential.<sup>33</sup> A >50% decrease in eMEP amplitude (D wave) correlates with postoperative motor deficit; variations in mMEP and eMEP amplitude allow predicting postoperative motor function.<sup>24</sup>

### Spinal cord evoked potentials

Developed in Japan in the 70's, they consist of the stimulation of the SC with an epidural electrode, recording a proximal or distal response, which corresponds to the sum of neuronal activities originating in ascending and descending tracts near the recording electrode.<sup>33</sup>

Stimulation can also be performed cranially and recorded caudally or vice versa. Due to the different conduction properties (speeds) of the SC pathways, the recorded potentials take the form of two different waves. The recorded potentials are very robust and represent combined activity of the dorsal columns (DC), corticospinal tracts, and other spinal cord pathways. This method is largely abandoned, but retains value in severe pre-existing neuropathies, research, or when determining the degree of conduction is important.

### Electromyography

It is the real-time graphic recording of muscle electrical activity, in order to evaluate the integrity of nerve structures. Two variants are used intraoperatively with different purposes: continuous and evoked EMG; two subdermal needles are placed 2-3 cm apart into the muscle innervated by the peripheral nerve, cranial nerve, or nerve root involved in the procedure.

Continuous EMG: Myogenic activity is recorded to show if a nerve structure is being damaged (nerve irritation or injury). It is based on the property of the motor nerve to respond to thermal or mechanical injury with the consequent activation of the muscles innervated by said nerve.

Evoked EMG: Direct or indirect electrical stimulation is used to identify a nerve structure, demonstrate its integrity and/or estimate the degree of injury; it allows corroborating the adequate placement of the pedicle screws (PS) by means of indirect stimulation of a nerve root. When the PS is poorly placed, medially or laterally, or there is a fissure in the pedicle, the electric current in the head of the PS spreads and stimulates the adjacent root, obtaining a compound muscle action potential at low amperage. If the PS is well placed, it will be surrounded by bone resistant to the passage of current, it will not stimulate the adjacent root and there will be no responses. Safety values vary depending on the spinal segment involved.

### Nerve action potential (NAP)

It is one of the most useful techniques in peripheral nerve procedures, since it provides fast and reliable information on the status of peripheral nerves during surgery. It is useful for detecting regenerating peripheral nerve axons and determining the corresponding surgical action in a procedure.

It can be recorded with stimulation and recording electrodes placed directly on the nerve. Stimulation is done with a tripolar electrode that allows the current to be concentrated, minimizing its spread; recording is done with a bipolar electrode.

### Anesthetics and their influence on intraoperative neurophysiological monitoring

Most anesthetics increase the activity of inhibitory pathways, decreasing neuronal activity and attenuating IONM responses, specially neuromuscular blockers (NMB) and inhaled halogenated drugs;<sup>1,3,7</sup> for an optimal IONM, it is important to understand the influence of drugs and other variables on the records<sup>21, 35, 37, 38</sup> (Table 1).

**Table 1.** Anesthetic drugs and their effects on Somatosensory Evoked Potentials and Motor Evoked Potentials.

Drug	Effect on SSEP	Effect on MEP
Propofol(*)	Minor decrease	Minor decrease
Etomidate	Increase	No effect
Midazolam	Decrease	No effect
Ketamine	Increase	Little effect - Decrease
Fentanyl	Little effect - Decrease	Decrease
Remifentanyl	Little effect - Decrease	Little effect - Decrease
Sufentanyl	Little effect - Decrease	Decrease
Dexmedetomidine	Little effect - Decrease	Little effect - Decrease
Sevoflurane	Larger decrease	Larger decrease
Desflurane	Larger decrease	Larger decrease
Rocuronium	No effect	No response
Succinylcholine	No effect	No response



NMB block the transmission of signals from motor nerves to muscle fibers at the level of the neuromuscular plate; when monitoring the functional integrity of a nerve structure is through myogenic activity, it is imperative that the muscle group be sensitive to changes in root, peripheral nerve, or cranial nerve function when pulled or compressed, therefore it is recommended not to use them or to use NMB with a short half-life and always monitor their activity, communicating any decision with the neurophysiologist.<sup>14</sup>

Benzodiazepines at induction doses greater than 30 mcg/kg cause depression in cortical, subcortical, and peripheral SSEP. They also have depressant effects in MEP.<sup>7</sup> Despite not being contraindicated, they are rarely used in neurosurgery due to their impact on postoperative evaluation.

Inhaled halogenated anesthetics have a significant dose-dependent effect on IONM,<sup>1,3</sup> decreasing MEP amplitude and increasing the possibility of false positives,<sup>38</sup> cortical SSEP are also affected to a lesser extent.<sup>7</sup> Isoflurane causes a greater reduction in amplitude and dose-dependent increase in latency, followed by sevoflurane and desflurane. At molecular level, they cause a reduction in neuronal excitability due to its effect on potassium channels and by means of inhibiting the activation of lower motor neurons by upper motor neurons.<sup>7,33</sup>

Opioids cause mild decrease in amplitude and prolongation of latency of cortical potentials; their depressant effect is not comparable to those inhaled, making them a useful alternative in IONM.<sup>35</sup>

Adjuvants are recommended to generate synergy, post-anesthetic analgesia, and to reduce doses of hypnotics and anesthetics; perfusion lidocaine 1.5-6.0 mcg/kg/min generates hemodynamic stability, less need for opioids and postoperative analgesia; magnesium sulfate 1-5 mg/kg/h is an analgesic adjuvant, provides hemodynamic stability and lower opioid requirement; dexmedetomidine has little effect or decreased EP at doses <0.5 mcg/kg/hr and with adequate infusion stability they can be continued for analgesia in the postoperative period in continuous infusions, single or associated, for 24-48 hours depending on the type of surgery or number of levels operated. Ketamine increases amplitude of SSEP and MEP of SC and is a useful alternative in patients with previous neurological damage,<sup>35</sup> it is recommended in spinal surgery as well to improve the recording of EP, less use of opioids, and as postoperative analgesia in infusions of 0.3 mg/kg/hr.

Etomidate and ketamine generate a dose-dependent increase in EP amplitude.<sup>7</sup> Bolus doses of etomidate 0.3 mg/kg increases cortical SSEP amplitude, without changes in subcortical and/or peripheral responses; a slight decrease in the amplitude of the MEP is observed, with no changes in latency.<sup>35</sup>

### The technique of choice

In addition to the selection of the anesthetic protocol for the baseline and control recording, measures to maintain a constant level of hypnosis and muscle relaxation are crucial to immediately detect changes in EP responses during or after surgical manipulation (LE:1C).<sup>1</sup>

Both desflurane and sevoflurane decrease SSEP and MEP amplitudes and prolong SEP latencies in a dose-dependent manner; when used, <0.5-0.6 CAM<sup>3,7</sup> and adjuvants are recommended.<sup>38</sup> Intravenous bolus administration or abrupt changes in the CAM of halogenated drugs can compromise records.

TIVA with propofol allows better recording of MEP and SSEP (LE: 2C);<sup>6</sup> the dose recommendation is 3.0-4.5 mcg/ml PC based on hemodynamics and anesthetic depth monitor with processed or unprocessed EEG of the patient; its effect on latency is minimal, which, along with the pharmacokinetic profile that allows infusions at constant concentrations and less depressant effect than inhaled ones, makes it the hypnotic of choice.<sup>1</sup> Most studies agree on the use of infusions or TIVA-TCI with intravenous anesthetics such as propofol as a hypnotic, remifentanyl TCI, sufentanil TCI or ketamine, as an anesthetic and short-acting NMB or for intubation, but not during surgery.<sup>4,14,22,26,35</sup>

The level of hypnosis and muscle relaxation must be kept constant; the use of neuromuscular and anesthetic depth monitors, ideally EEG, is recommended.<sup>1</sup>

Physiological variables such as temperature, blood pressure, heart rate, blood oxygen concentration, partial pressure of carbon dioxide, should be maintained without significant changes; the neuroanesthesiologist must ensure adequate positioning of the patient and sustain clear objectives of hypnosis and metabolic and physiological homeostasis, maintaining adequate saturation and systemic oxygenation, hematocrit, normotension, normothermia and normocapnia, avoiding modifications that alter the recordings.<sup>14</sup>

Stimulation of the motor cortex can cause involuntary movements of the hand, jaw and other parts of the body

with the risk of self-biting and other injuries. Therefore, it is important to anticipate and prevent possible adverse events associated with IONM. Informed consent should include not only the goals and methods of monitoring, but also the risk of associated adverse events.<sup>1</sup>

### Conclusions

Multimodal IONM is a useful method that allows the functional state of the motor and sensory systems to be evaluated in real time with the aim of preventing postoperative injuries in procedures that put the nervous system at risk and pose important challenges for the neuroanesthesiologist.

It is essential to understand the clinical bases of the different IONM techniques and interpret alerts and alarm criteria in a timely manner, through close cooperation between the neurosurgeon, neurophysiologist and neuroanesthesiologist, for early decision-making and better surgical results.

In turn, the neuroanesthesiologist must ensure adequate positioning, anesthetic depth status, hemodynamic stability, and cerebral/spinal cord perfusion pressure, avoiding changes that alter the recordings.

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### Conflict of interest

The authors declare that they have no conflict of interest.

### Ethical Responsibilities

The authors state that no experiments have been performed on humans or animals for this research, and that no patient data appears in this article.

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