

Video Article

Intra-Operative Neural Monitoring of Thyroid Surgery in a Porcine Model

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Abstract

Intraoperative injury to the recurrent laryngeal nerve (RLN) can cause vocal cord paralysis, which interferes with speech and can potentially interfere with breathing. In recent years, intraoperative neural monitoring (IONM) has been widely adapted as an adjunct technique to localize the RLN, detect RLN injury, and predict vocal cord function during the operations. Many studies have also used animal models to investigate new applications of IONM technology and to develop reliable strategies for preventing intraoperative RLN injury. The aim of this article is to introduce a standard protocol for using a porcine model in IONM research. The article demonstrates the procedures for inducing general anesthesia, performing tracheal intubation, and experimental design to investigate the electrophysiological characteristics of RLN injuries. Applications of this protocol can improve overall efficacy in implementing the 3R principle (replacement, reduction and refinement) in porcine IONM studies.

Introduction

Although thyroidectomy is now a commonly performed procedure worldwide, postoperative voice dysfunction is still common. Intraoperative injury to the recurrent laryngeal nerve (RLN) can cause vocal cord paralysis, which interferes with speech and can potentially interfere with breathing. Additionally, injury to the external branch of the superior laryngeal nerve can cause a major voice change by affecting pitch and vocal projection.

Intraoperative neural monitoring (IONM) during thyroid operations has obtained wide popularity as an adjunct technique for mapping and confirming the RLN, the vagus nerve (VN), and the external branch of the superior laryngeal nerve (EBSLN). Because IONM is useful for confirming and elucidating mechanisms of RLN injury and for detecting anatomic variations in the RLN, it can be used to predict vocal cord function after thyroidectomy. Therefore, IONM adds a new functional dynamic in thyroid surgery and empowers surgeons with information that cannot be obtained by direct visualization alone^{1,2,3,4,5,6,7,8,9,10}.

Recently, many prospective studies have used porcine models to optimize the use of IONM technology and to establish reliable strategies for preventing intraoperative RLN injury^{11,12,13,14,15,16,17,18,19,20}. Porcine models have also been used to provide practitioners with essential education and training in clinical applications of IONM.

Therefore, the combination of animal models and IONM technology is a valuable tool for studying the pathophysiology of RLN injury²¹. The aim of this article was to demonstrate the use of a porcine model in IONM research. Specifically, the article demonstrates how to induce general anesthesia, perform tracheal intubation, and set up experiments for investigating the electrophysiological characteristics of various RLN injury types.

Protocol

The animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Kaohsiung Medical University, Taiwan (protocol no: IACUC-102046, 104063, 105158).

1. Animal Preparation and Anesthesia

1. Porcine animal model

Note: This study applied the protocol described in the literature to establish a prospective porcine model of IONM^{11,12,13,14,15,16,17,18,19,22}.

1. Use KHAPS Black or Duroc-Landrace pigs (3-4 months old; weighing 18-30 kg).
2. Ensure that the experimental protocol is consistent with national/international regulations and guidelines for animal experiments, including the 3R principles (replacement, reduction, and refinement). Obtain ethical approval of the experimental protocol from the committee for care and use of experimental animals at the relevant institution.

2. Anesthesia induction

1. Pre-anesthesia preparations

1. Withhold food 8 hours before anesthesia and withhold water 2 hours before anesthesia.
2. Pre-medicate with intramuscular azaperone (4 mg/kg) at 2 hours before anesthesia. Use a 500 mL saline bottle to fabricate a face mask for each piglet. Trim as needed to ensure a secure fit to the snout.
3. Use the weighing function on the operating table to measure the net weight of each piglet (**Figure 1A**).
4. Maintain body temperature with a circulating water mattress set to 40 °C.

2. Induce general anesthesia (GA) with 2-4% sevoflurane at a fresh gas flow of 3 L/min *via* the face mask with the piglet in a prone position. An adequate depth of anesthesia is usually achieved in 3-5 minutes. Confirm the depth of anesthesia by no severe movement to pain due to peripheral venous catheterization.
3. Identify a superficial vein on the outer side of one ear and sterilize the selected region (about 6 x 6 cm²) with 75% alcohol. For maximum safety, use a 24-gauge peripheral intravenous catheter.
4. Administer intravenous anesthetic such as propofol (1-2 mg/kg) or thiamylal (5-10 mg/kg) to alleviate noxious stimulation by direct laryngoscopy.

Note: Use of neuromuscular blocking agent (NMBA) is not suggested. In subsequent experiments, NMBA may complicate intubation by depressing spontaneous breathing and may diminish electromyography (EMG) signals. Additionally, sevoflurane inhalation combined with a bolus of propofol or short-acting barbiturates is reportedly sufficient for facilitating tracheal intubation.

3. Tracheal intubation (**Figure 1B**)

1. Prepare the equipment and materials required for EMG tube intubation: a size #6 EMG endotracheal tube, a face mask for assisted ventilation, two slings to hold the mouth open, one gauze strip to pull the tongue, a blunt tip suction catheter, a veterinary laryngoscope with 20cm straight blades, an elastic bougie, a 20-mL syringe, a stethoscope, and adhesive tape.
2. Position the piglet in a prone position on the operating table. Align the head and body to ensure clear visualization of the upper airway.
3. Direct the assistant to apply traction of the upper and lower jaw to maintain an adequate mouth opening and to avoid rotation or overextension of the head. Cover the tongue with gauze and pull the tongue out to optimize the visual field.
4. Hold the laryngoscope upside down and place it directly in the oral cavity to depress the tongue.
5. Directly visualize the epiglottis and use the laryngoscope to press the epiglottis downward toward the tongue base.
6. When the vocal cords are clearly identified, gently advance the elastic bougie into the trachea. Slight rotation of the elastic bougie may be required to overcome resistance. Next, advance the EMG tube at the mouth angle to a depth of 24 cm.
7. Inflate the EMG tube cuff to a volume no larger than 3 mL. If ventilation by manual bagging reveals no obvious air leakage, *in situ* deflation of the EMG tube is feasible.
8. When the EMG tube is placed at the proper depth, confirm the free passage of fresh gas by manual bagging. Further confirm the proper tracheal intubation by end-tidal carbon dioxide (etCO₂) monitoring (capnography) and chest auscultation for early identification of inadvertent esophageal or endobronchial intubation.

Note: Capnography showed both the etCO₂ waveform and the digital value in mmHg. When esophageal intubation occurred, etCO₂ was absent or near zero after 6 breaths. When the EMG tube was in the correct place, the typical etCO₂ waveform and adequate value (usually >30 mmHg) was noted. Furthermore, the breathing sound of a bilateral lung filled is clear and symmetric as determined by chest auscultation.

9. Use medical tape to fix the EMG tube at the mouth angle. Since the tube usually requires adjustment during IONM experiments, do not fasten the tube to the snout.
10. Connect the EMG tube to the ventilator. Continuous capnography is mandatory for monitoring the etCO₂ value and curve throughout the experiment.

4. Anesthesia maintenance (**Figure 1C**)

1. After the EMG tube is fixed, position the piglet on its back with the neck extended (**Figure 1C**). Maintain general anesthesia with 1-3% sevoflurane in oxygen at 2 L/min.
2. Ventilate the lungs in volume-control mode at a tidal volume of 8-12 mL/kg, and set the respiratory rate to 12-14 breaths/min.
3. Begin physiologic monitoring, including capnography, electrocardiography (ECG) and monitoring of oxygenation (SaO₂).

2. Equipment Setting and Animal Operation (Figure 1D)

1. Equipment Setup
 1. Connect the channel leads from the EMG tube to the monitoring system.
 2. Set the monitoring system to run a 50 ms time window. Set pulsed stimuli to 100 μ s and 4 Hz. Set the event capture threshold to 100 μ V.
2. Surgical procedure
 1. Wear sterile surgical gloves and use povidone iodine with cotton swabs to disinfect the neck surgical site.
 2. Make a transverse collar incision about 10-15 cm in length with a scalpel to expose the neck and the larynx.
 3. Raise the subplatysmal flap 1 cm cranially from the clavicle to the hyoid bone.
 4. Remove the strap muscles and visualize the tracheal rings and nerves. Use monopolar and bipolar electrocautery to assist the surgical dissection and hemostasis.
 5. Localize, identify, and carefully expose the EBSLN, RLN, and VN with a handheld stimulation probe.
 6. Position an automated periodic stimulation (APS) electrode on one side of VN for stimulating during continuous IONM (CIONM). Connect the APS electrode with the monitoring system. Set pulsed stimuli to 1 Hz, 100 μ s, and 1 mA.
3. At end of experiments, euthanize all piglets by the veterinarian.

3. Electrical Stimulation

Note: To apply the 3R principle in porcine IONM studies, always perform repeatable electrophysiology studies that do not cause nerve injury before performing experiments that may cause nerve injury. This can be used to study the intensity, safety, and cardiopulmonary effects^{11,17}. The IONM equipment can be classified as stimulation equipment or recording equipment (**Figure 2A**).

1. Evaluate the baseline EMG responses of the target nerves, including the EBSLN, RLN, and VN (**Figures 2B, 2C**).
 1. Start with an initial stimulation current of 0.1-mA current and increase stimulation in 0.1-mA increments until an EMG response is detected and recorded.
 2. Further increase the current until the maximal EMG response is obtained.
 3. Record the baseline amplitude, latency, and waveform of the EMG response.
 4. Define the minimal stimulus level as the lowest current (mA) that clearly evoked EMG activity of >100 μ V. Define the maximal stimulus level as the lowest current that evoked the maximal EMG response.
2. Evaluate the Safety of electrical stimulation^{11,19}
 1. Apply a continuous 1-minute stimulus at the fifth tracheal ring level of the VN or RLN.
 2. Progressively increase the stimulus current from 1 mA to 30 mA.
 3. During VN stimulation, evaluate hemodynamic stability by monitoring of heart rate, ECG, and invasive arterial blood pressure.
 4. Finally, evaluate nerve function integrity by comparing EMG responses proximal to the nerve stimulation site before and after each level of stimulation is applied.
3. Effect of anesthetics (muscle relaxants and their reversals)^{12,20}

Note: Improper use of NMBA is a potential cause of unsuccessful IONM. The proposed animal model was used to compare recovery profiles among different depolarizing NMBA (e.g., succinylcholine) and nondepolarizing NMBA (e.g., rocuronium) at varying doses and to identify the optimal NMBA for use in IONM. The animal model can also be used to evaluate the effectiveness of NMBA reversal drugs (e.g., sugammadex) for rapidly restoring neuromuscular function suppressed by rocuronium.

 1. Firstly, apply C-IONM and use the automatically calibrated baseline latencies and amplitudes of EMG as control data.
 2. Administer a bolus injection of 0.3 mg/kg rocuronium in a volume of 10 mg/mL and observe the real-time EMG changes.
 3. Three minutes after injection, perform one injection of 2 mg/kg sugammadex in a volume of 100 mg/mL as a rapid bolus. Record the recovery profile of laryngeal EMG for 20 minutes.
4. Stimulation electrodes (Stimulation probes/dissectors) (**Figure 3**)¹⁷

Note: There are different types of stimulation electrodes that can be used for nerve stimulation during IONM, e.g., monopolar probes (**Figure 3A**), bipolar probes (**Figure 3B**), and stimulation dissectors (**Figure 3C**).

 1. To mimic direct stimulation of nerves during surgery, apply 1 mA stimulation to the EBSLN, RLN, and VN without overlying fascia.
 2. To mimic indirect mapping and localizing of the nerve position before visual identification during surgery, apply 1 mA stimulation at a 1- and 2-mm distance away from the nerves at overlying fascia.
 3. Record and compare the EMG responses between different types of stimulation electrodes.
5. Recording electrodes (EMG tubes/needle electrodes/pre-gelled skin electrodes) (**Figure 4**)
 1. Use the animal model to evaluate how rotation or upward/downward displacement of the EMG tube electrode (**Figure 4A**) affects the stability of the EMG signal. Additionally, use the animal model to compare the EMG responses between different electrode types (e.g., needle electrodes and adhesive pre-gelled electrodes, **Figure 4B**) and different recording approaches (e.g., transcutaneous/percutaneous and transcartilage approaches, **Figures 4C and 4D**) in terms of feasibility, stability, and accuracy during IONM.
 2. For a feasibility study, apply a 1 mA stimulus current to bilateral EBSLNs, VNs and RLNs. Record and compare EMG responses evoked by each electrode tested (i.e., EMG tube, transcutaneous, percutaneous, and transcartilage electrodes).
 3. For a stability study, evaluate and compare EMG signal stability in C-IONM under experimentally induced cricoid/tracheal cartilage displacement.

4. For an accuracy study, evaluate and compare the accuracy of the tested electrodes in C-IONM for identifying EMG signal degradation under RLN injury.

4. RLN injury study (Figure 5)

1. In accordance with the 3R principle, perform RLN injury experiments in the porcine model after all repeatable electrophysiology studies are completed. Perform tests of nerve segments from proximal nerve segments to distal nerve segments (*i.e.*, proceed from the caudal part of the RLN to the cranial part of the RLN).
2. Use C-IONM to confirm and compare patterns of real-time changes in evoked laryngeal EMG signals during and after acute RLN injuries with different injury mechanisms (*e.g.*, traction, clamping, transection, or thermal injuries) (**Figures 5A and 5B**). Use C-IONM for continuous real-time display and recordation of EMG changes and sequential recoveries throughout the experiment (**Figure 5C**).
3. Collect injured RLN segments for histopathological analysis of morphological alterations caused by the nerve injury experiments.
4. Traction compression/stretch injury
Note: Traction compression or stretch injuries are the most common intraoperative RLN injuries. Experimentally induce traction stress and observe the resulting electrophysiological EMG changes and histopathological changes.
 1. Traction compression injury¹³
 1. Wrap a thin plastic loop (*e.g.*, a vascular loop 1.3-mm wide) around the RLN and use a force gauge to apply retraction with 50 g of tension (**Figure 5A**). This scheme mimics an RLN trapped against a dense, fibrous band or a crossing artery at the region of Berry's ligament during medial traction of the thyroid lobe.
 2. Traction stretch injury¹⁶
 1. Wrap the RLN with a wider elastic material (*e.g.*, a 10-mm wide silicone Penrose drain), and use a force gauge to retract the RLN with 50 g of tension) This scheme mimics an RLN adhered to or encased in the goiter capsule and stretched forward during medial traction.
5. Clamping injury
Note: Intraoperative mechanical trauma to the RLN usually results from poor exposure or visual misidentification of the RLN.^{13,16}
 1. After the traction compression RLN injury experiment, pinch the distal segment of the RLN with hemostatic forceps for one second. This scheme mimics the nerve being inadvertently clamped owing to visual misidentification as a vessel during the operation. Record the accompanying EMG signal change for comparison with further histopathological findings of the nerve specimen.
6. Thermal injury
Note: Most intraoperative RLN thermal injuries result from thermal spread when electrocautery devices and various energy-based devices (EBDs) are used to induce hemostasis near the RLN. Like traction injury, thermal injury is rarely visible to the naked eye. Therefore, perform animal IONM experiments to determine the best model for evaluating the pathophysiology of RLN thermal injury and to test the thermal tolerance¹⁴ and the safety of EBDs^{15,18}.
 1. Use C-IONM to register the EMG changes continuously throughout the experiment.
 2. For the activation study, investigate how energy-based devices (EBD) can be safely applied for hemostasis and dissection near the RLN during surgery (**Figure 5B**).
 1. Activate the EBD (electrothermal bipolar vessel sealing system, set power at level 2, and the energy discontinues automatically by 2 to 4 seconds) at a 5-mm distance away from the RLN.
 2. If EMG signals remain stable after several tests, perform a further test at the narrower distance (*e.g.*, 2-mm, and followed by 1mm distance).
 3. If any substantial EMG change occurs after any test the experiment is complete and followed by continuous real-time EMG recording for at least 20 minutes.
 3. For the cooling study, evaluate the cooling time to determine post-activation optimal EBD cooling parameters.
 1. Contact the activated EBD on the RLN directly after a 5 second cooling time.
 2. If the EMG signals remain stable after three tests, test the shorter cooling time (*e.g.*, 2 seconds, and followed by 1 second).
 4. If the EMG remains stable after repeated tests, confirm the safety of the EBD by touching the RLN immediately after activation.

Representative Results

Electrophysiology study

Baseline EMG data, minimal/maximal stimulus level, and the stimulus-response curves

Using a standard monopolar stimulating probe, the obtained minimal stimulation level for VN and RLN stimulation ranges from 0.1 to 0.3 mA, respectively. In general, the stimulus current correlated positively with the resulting EMG amplitude response^{11,17}. The EMG amplitude plateaued at the maximal stimulation levels of 0.7 mA for VN stimulation, and 0.5 mA for RLN stimulation¹¹.

Electrical stimulation (intensity, safety, and cardiopulmonary effect)

In the safety study, there is no unwanted effects on EMG signal or hemodynamic stability observed after continuous pulsatile VN and RLN stimulations in the setting of 1 mA to 30 mA. In addition, baseline EMG amplitudes and latencies of the VN or RLN were relatively unchanged after the nerves was stimulated by a high-current. Therefore, it was suggested that an intermittent high stimulus current during IONM was not harmful to the VN or RLN¹⁹.

Effects of anesthetics (muscle relaxants and their reversals)

Experimental comparisons of NMBA of this animal model showed that different types and doses of muscle relaxants have different natural recovery profile. For example, recovery times for succinylcholine (1 mg/kg) and low-dose rocuronium (0.3 mg/kg) were significantly shorter than that for standard dose rocuronium (0.6 mg/kg). The experiments for NMBA reversals confirm that sugammadex (reversal of rocuronium) effectively and rapidly restores neuromuscular function suppressed by rocuronium²⁰.

Stimulating electrodes (stimulation probes and dissecting stimulators)

Typically, IONM is performed with a commercially available ETT-based surface recording electrode system (*i.e.*, a so-called EMG tube). However, a limitation of the clinical use of EMG tubes is the need to maintain constant contact between the electrodes and vocal cords during surgery to obtain a robust EMG signal. False IONM results can result from an EMG tube that is mispositioned during intubation (*e.g.*, due to incorrect insertion depth, incorrect tube size, or rotation of the electrode) or from an EMG tube that is displaced during surgical manipulation or neck retraction (*e.g.*, causing rotation or upward/downward displacement of the electrode).

Experimental comparisons of stimulating electrodes showed that the stimulation probes/dissectors evoked typical EMG waveforms from the EBSLN/RLN/VN with 1 mA current. The stimulating current correlated positively with the resultant EMG amplitude. In monopolar probes and stimulating dissectors, maximum EMG was elicited by <1 mA. In bipolar probes, maximum EMG required a higher current. In all groups, evoked EMG amplitudes decreased as the distance from the probe/dissector to the nerve increased. Evoked EMG amplitudes also decreased in stimulated nerves that had overlying fascia. Therefore, the animal model confirmed that both stimulation dissectors and conventional probes are effective to evoke EBSLN, RLN, and VN waveforms to monitor real-time nerve function during surgery¹⁷. Various stimulation probes/dissectors are now available in IONM system for specific stimulation requirements, surgical monitoring application and the preference of the users.

Recording electrodes (EMG tubes, needle electrodes, and pre-gelled skin electrodes)

The feasibility study confirmed that the EMG tube electrodes on the vocalis, the transcutaneous/percutaneous needle electrodes, and the transcutaneous/transcartilage pre-gelled electrodes were effective for recording typical evoked laryngeal EMG waveforms from the VN and RLN under 1 mA stimulation. **Figure 6** shows that transcutaneous/transcartilage pre-gelled electrodes generally recorded lower EMG amplitudes compared to EMG tube and needle electrodes.

In the stability study, real-time EMG tracings were compared before and after tracheal displacement was experimentally induced. **Figure 7** shows that the change in contact between EMG tube electrodes and vocal folds after tracheal displacement significantly changed the recorded EMG signals. However, tracheal displacement had no apparent effect on electrode contact quality or on EMG signal quality from the transcutaneous or transcartilage electrodes.

The accuracy study evaluated the accuracy of real-time signals in reflecting adverse EMG degradation during RLN stress experimentally induced by continuous VN stimulation with the APS electrode. When RLN traction stress was experimentally induced, the EMG tube electrodes on the vocalis muscle and the transcartilage/percutaneous/transcutaneous electrodes recorded similar patterns of progressive degradation in EMG amplitude (**Figure 8**).

RLN injury study

Traction injury

Typical real-time EMG changes during RLN traction revealed a progressive amplitude decrease combined with a latency increase (the so-called "combined event"). In addition, the EMG signals gradually recovered after release of traction (**Figure 9A**). The histopathology study showed that morphological changes occurred mostly in outer nerve structures such as the epi- and peri-neurium. Structures in the endoneurium remained relatively intact^{13,16}.

Clamping injury

All RLNs showed an immediate LOS (within less than 1 s) after acute mechanical injury was experimentally induced. In addition, no gradually EMG recovery can be observed in a short period of time after the injury (**Figure 9B**). The histopathology study showed that distortion of the epineurium and perineurium was greater in the clamping injury group compared to the traction injury group^{13,16}.

Thermal injury

During the thermal injury study, the real-time EMG reveals a combined event, which then rapidly degrades to LOS (**Figure 9C**). The reaction time before LOS and the severity of electrophysiologic injury may be related to the dose of thermal stress¹⁴. Studies of EBDs reveal that the safe activation distance to the RLN and the cooling time vary by EBD type. For example, the safe activation distances and cooling times are 5 mm and 1 second for monopolar electrocautery (15 watts), 3 mm and 1 second for bipolar electrocautery (30 watts), 2 mm and 3 to 10 seconds for the Harmonic scalpel, and 2 mm and 2 to 5 seconds for the Ligasure system, respectively. Notably, the Harmonic scalpel should be cooled for more than 10 seconds or cooled by a quick (2 seconds) muscle touch maneuver before it touches the RLN. The Ligasure system should be cooled for more than 2 seconds or cooled by a quick muscle touch maneuver before it touches the RLN^{15,18}. The histopathological examination of the thermal injured nerves showed relatively severe damage to the inner endoneurium with less distortion of the outer nerve structure¹⁶.

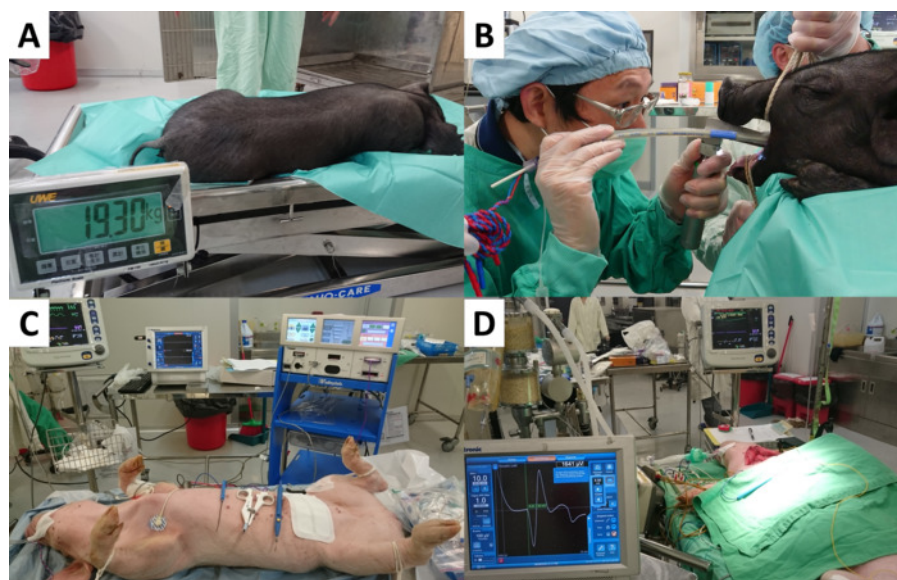


Figure 1. Preparation and anesthesia of KHAPS Black/Duroc-Landrace Pigs for IONM research. (A) Net weight of each piglet was measured before anesthesia. (B) An assistant maintained an adequate mouth opening while traction was applied to the upper and lower jaw. A laryngoscope was then used to press the epiglottis downward toward the base of the tongue. When the vocal cords were clearly identified, the elastic bougie was gently advanced into the trachea. The EMG tube was then inserted to a depth of 24 cm at the appropriate mouth angle. (C) The piglet was placed on its back with the neck extended. The channel leads from the recording electrodes were connected to the monitoring system. Physiologic monitoring was performed during the study. (D) The neck and the larynx were exposed for experiments. [Please click here to view a larger version of this figure.](#)

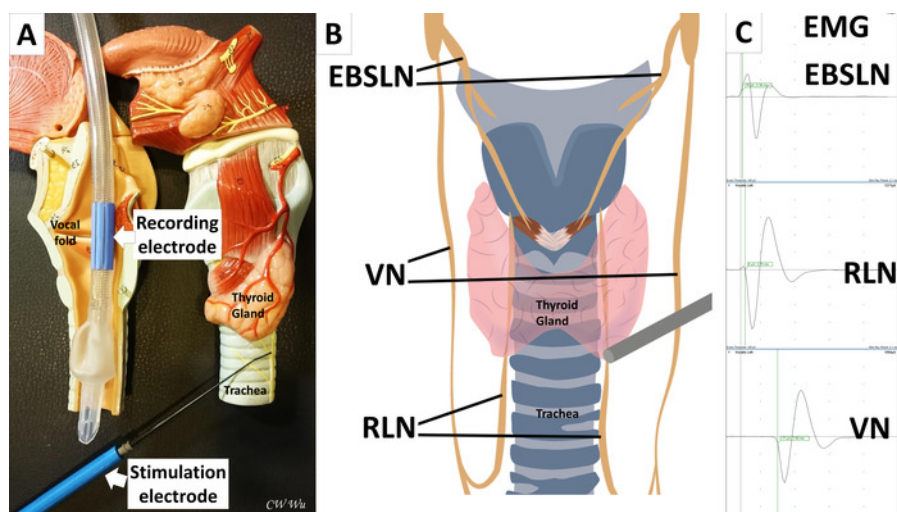


Figure 2. The multifaceted electronic equipment and principle of the IONM system. (A) The basic equipment included the neural stimulating electrodes (stimulator) and the recording electrodes (connected to the ETT). (B) The stimulating electrodes can be used to determine the location and functional status of the EBSLN, RLN, and VN during IONM. (C) The evoked EMG response is displayed on an LCD screen. [Please click here to view a larger version of this figure.](#)



Figure 3. The various stimulation electrodes available for use in IONM. (A) Monopolar probes (B) bipolar probes, and (C) stimulation probes/dissectors. The selection of stimulation probes/dissectors used for IONM depends on the specific stimulation requirements, the specific application desired and the preference of the surgeon. [Please click here to view a larger version of this figure.](#)

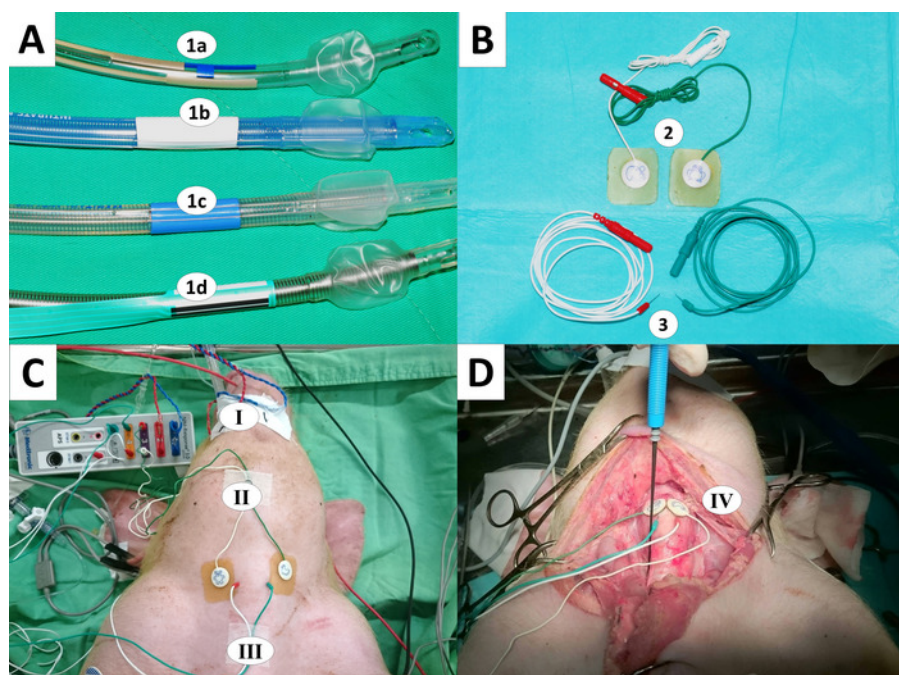


Figure 4. Various recording electrode types are available for use in IONM. (A) The EMG ETT electrodes include (1a) Trivantage (1b) Contact Reinforced (1c)-Standard Reinforced, and (1d)- FLEX EMG Tubes; (B) (2)-adhesive pre-gelled electrodes and (3)-needle electrodes. (C and D) The EMG tube is designed to touch the vocal fold through intubation (I), and the adhesive pre-gelled or needle electrodes can be used in transcutaneous (II), percutaneous (III), or transcartilage (IV) approach for EMG recording during IONM. [Please click here to view a larger version of this figure.](#)

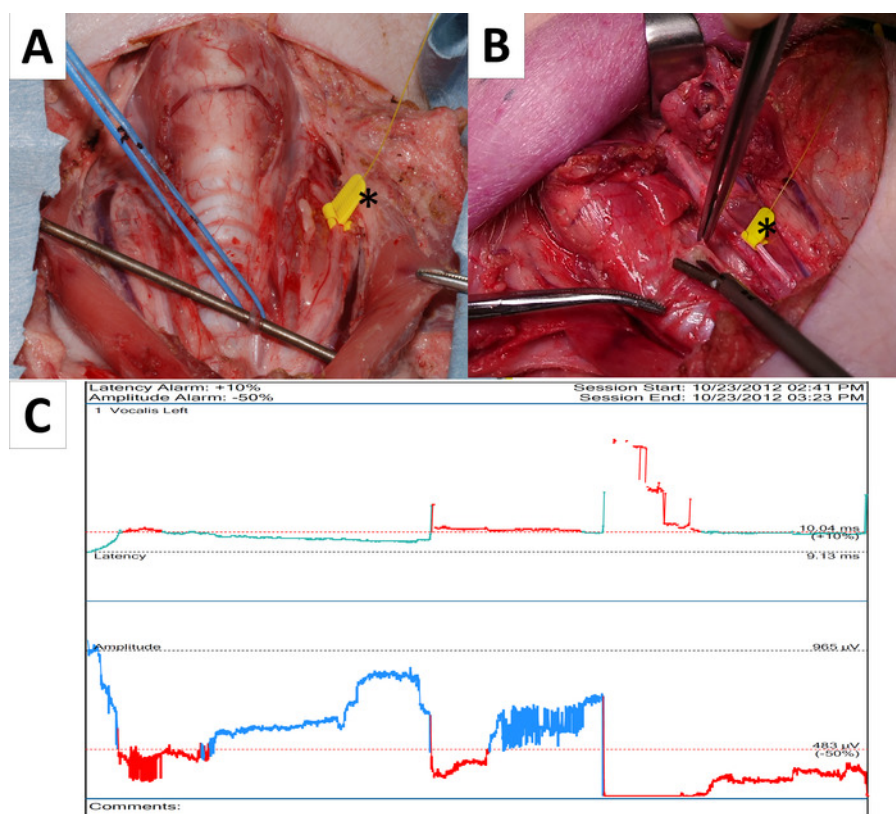


Figure 5. Continuous IONM was performed via APS of the VN (*) to investigate real-time EMG changes in the RLN during (A) traction and (B) thermal injury. (C). Throughout the experiment, the C-IONM system displayed and continuously recorded the induced EMG changes and sequential recoveries in real time. [Please click here to view a larger version of this figure.](#)

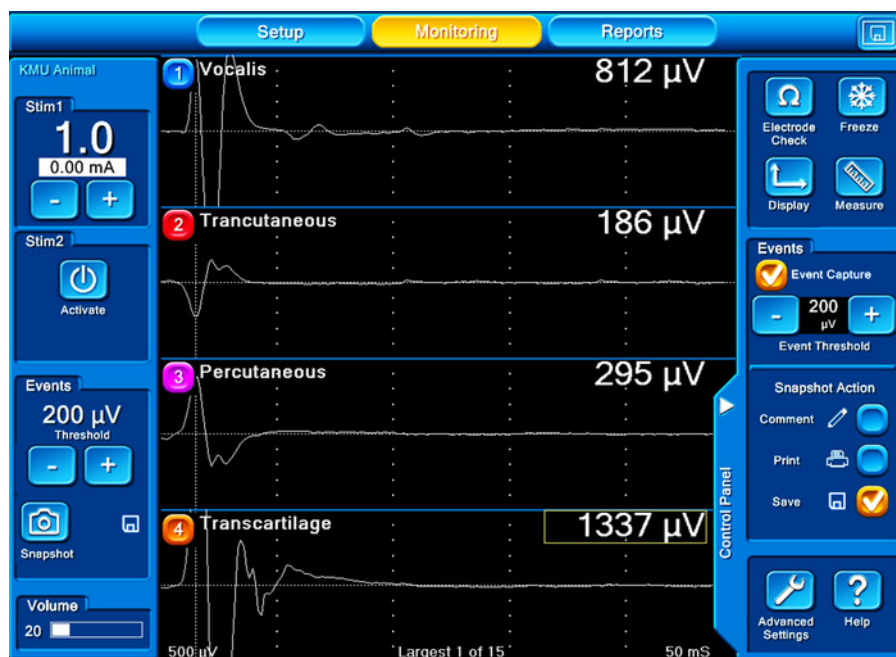


Figure 6. Comparison of evoked EMG responses between four different types of recording electrodes. The feasibility studies indicated that all electrode types (*i.e.*, EMG tube, transcutaneous, percutaneous, and transcartilage electrodes) accurately recorded typical evoked laryngeal EMG waveforms from the RLN under 1 mA stimulation. [Please click here to view a larger version of this figure.](#)

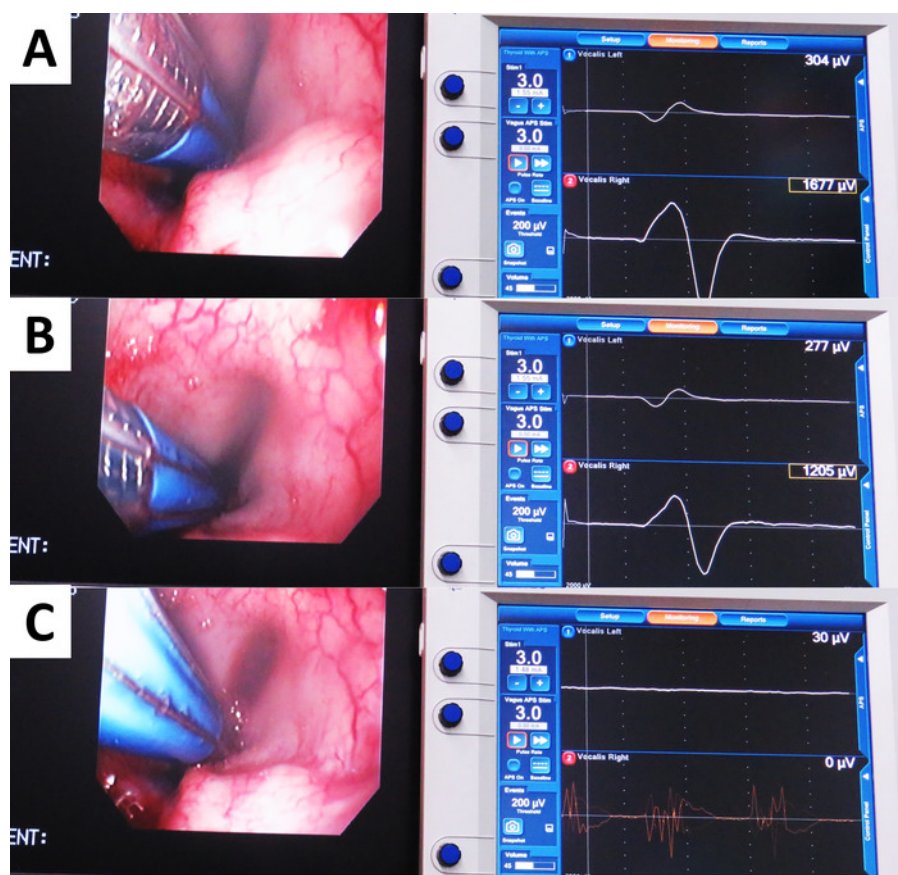


Figure 7. Comparison of real-time EMG tracings before and after experimental tracheal displacement. For stability study, tracheal displacement was experimentally induced. Changes in contact between the EMG tube electrodes and vocal folds caused significant variation in recorded EMG signals. (A) Electrodes in the normal position recorded strong EMG signals. (B) Electrodes with slight upward displacement (1 cm) recorded relatively weaker EMG signals. (C) Electrodes with moderate to severe upward displacement (2 cm) showed an EMG LOS. [Please click here to view a larger version of this figure.](#)

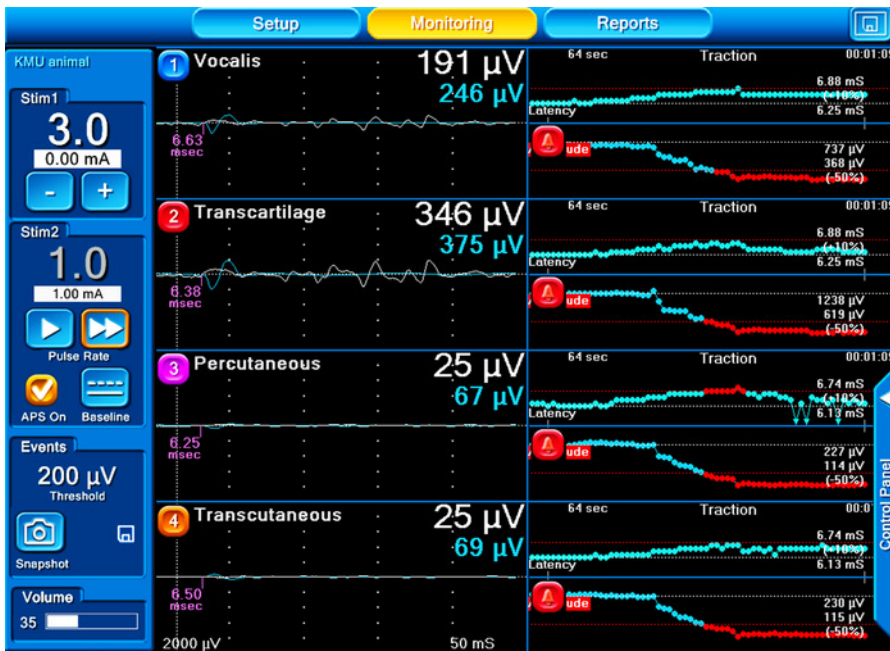


Figure 8. Comparison of real-time EMG tracings during experimental RLN experimental RLN traction injuries between four different types of recording electrodes. The accuracy studies showed that, when RLN traction stress was experimentally induced, all electrode types (i.e., EMG tube, transcutaneous, percutaneous, and transcartilage electrodes) recorded similar patterns of progressively degrading EMG amplitude. [Please click here to view a larger version of this figure.](#)

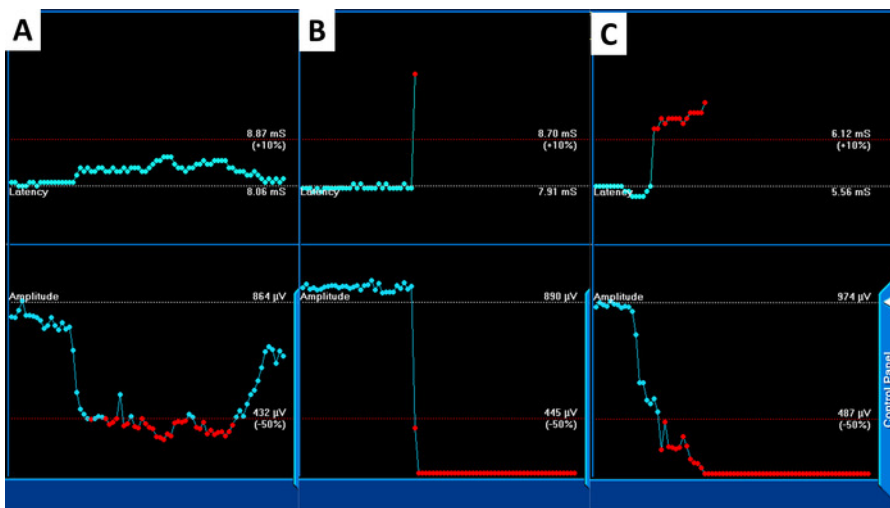


Figure 9. Comparison of real-time EMG changes and sequential recoveries after different RLN injury types. (A) In traction injury, the EMG signals gradually degraded under nerve stress and gradually recovered after release of traction. (B) In clamping injury, the EMG signals showed an immediate LOS and no recovery. (C) In thermal injury, the EMG signals revealed a combined event and then rapidly gradually degraded to LOS with no recovery. [Please click here to view a larger version of this figure.](#)

Discussion

Injury to the RLN and EBSLN remains a significant source of morbidity caused by thyroid surgery. Until recently, nerve injury could only be identified by direct visualization of trauma. The use of IONM now enables further functional identification of the RLN by applying stimulation and recording the contraction of the target muscles. Currently, however, both conventional intermittent and continuous IONM systems have some technical limitations in false-positive and false-negative interpretations. Hence, suitable animal models are necessary to these clinical issues.

Recently, plenty of animal experimental studies have tried to overcome pitfalls of IONM and to investigate new applications. Most of these studies have used medium-sized animals such as canine/dog^{23,24,25} and porcine/swine/mini-pig^{11,12,13,14,15,16,17,18,19,22,26,27,28,29}. Canine models of the RLN and laryngeal function are well-established and highly mimic human anatomy, size and physiology. The porcine model is the oldest animal applied in RLN research^{30,31}. The first experiments in live pigs performed by Galen in the second century A.D. demonstrated functional alterations in a transected RLN. Currently, the porcine model is most commonly used for IONM research because its anatomy and physiology are

very similar to those in humans. Experimental pigs have a medium size that enables easy handling and are widely available at a relatively low cost²¹.

This article demonstrates standard protocols for using the porcine model in IONM research, including protocols for general anesthesia and tracheal intubation. The 3R principle is implemented in the design of experiments for investigating electrophysiological characteristics of RLN injuries. Key issues in the use of the proposed porcine model include (1) EMG parameter characteristics and safety considerations when applying electrical stimulation^{11,17,19}, (2) the use of muscle relaxants and reversals^{12,20,32}, (3) stimulating and recording electrodes¹⁷, and most importantly (4) models of RLN injuries^{13,14,15,16,18} that cannot be accurately quantified in humans. The protocols were set up to induce different severity and types of RLN injuries. Recorded real-time EMG data were correlated with postoperative vocal cord function and histopathology examinations. Although some data from experimental studies are inapplicable to clinical practice, our porcine model provides a valuable research platform not merely in understanding technology of IONM, but also in guiding future experiments to improve surgical strategies for lesser RLN injuries during thyroid surgery.

Disclosures

The authors have nothing to disclose.

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