Intraoperative neural monitoring in thyroid surgery: lessons learned from animal studies

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Abstract: Recurrent laryngeal nerve (RLN) injury remains a significant morbidity associated with thyroid and parathyroid surgery. In the past decade, surgeons have increasingly used intraoperative neural monitoring (IONM) as an adjunct technique for localizing and identifying the RLN, detecting RLN injury, and predicting the outcome of vocal cord function. In recent years, many animal studies have investigated common pitfalls and new applications of IONM. For example, the use of IONM technology in animal models has proven valuable in studies of the electrophysiology of RLN injury. The advent of animal studies has substantially improved understanding of IONM technology. Lessons learned from animal studies have immediate clinical applications in establishing reliable strategies for preventing intraoperative RLN injury. This article gives an overview of the research progress on IONM-relevant animal models.

Keywords: Intraoperative neural monitoring (IONM); recurrent laryngeal nerve (RLN); thyroid surgery; animal study; translation research

Introduction

Intraoperative recurrent laryngeal nerve (RLN) injury is a major source of morbidity from thyroid surgery. Unilateral injury can cause voice change and aspiration whereas bilateral injury can cause stridor and acute airway obstruction. Intraoperative RLN injury is a leading cause of medico-legal litigation after thyroid surgery (1-3). In the past decade, intraoperative neural monitoring (IONM) has gained wide acceptance as an adjunct technique for localizing and identifying the RLN and external branch of the superior laryngeal nerve (EBSLN) (4,5), for detecting

anatomic variations (6-8), for detecting and elucidating mechanisms of nerve injury, and for predicting the outcome of vocal cord function (9-14). Although it does not replace knowledge of surgical anatomy or excellent surgical technique, IONM adds a new functional dynamic to surgery because it empowers surgeons beyond what is available to them through visual information alone.

In recent years, many animal studies have attempted to solve common pitfalls of IONM and to investigate new applications. The use of animal models is also important for providing the education and training needed to master
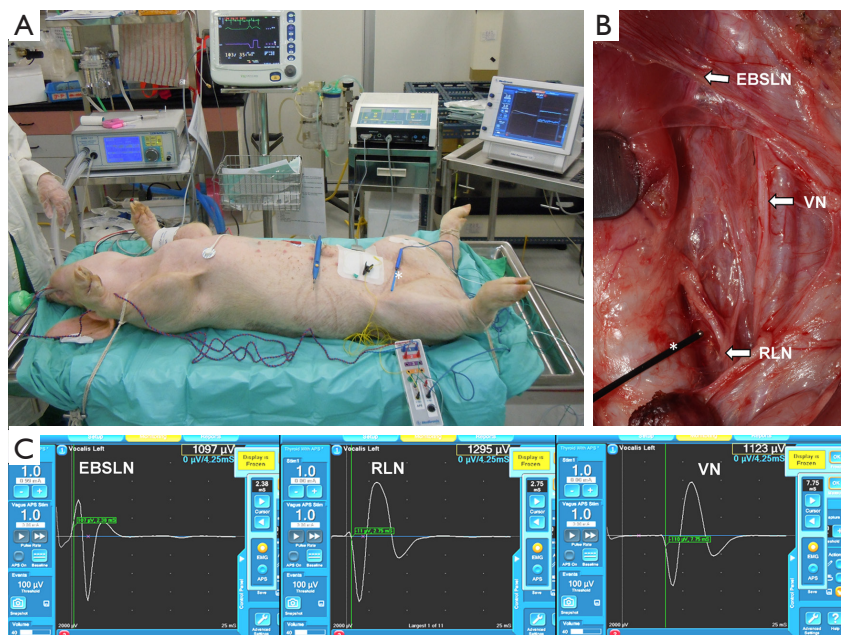
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Figure 1 Porcine model used for IONM research at Kaohsiung Medical University, Taiwan. (A) Setup for porcine model and monitoring system for IONM; (B) the porcine model is used because of its high anatomic similarity to the human laryngeal nerve. Researchers can repeatedly stimulate the external branch of superior laryngeal nerve (EBSLN), recurrent laryngeal nerve (RLN), and vagus nerve (VN) to compare the evoked laryngeal EMG response characteristics under different stimulation/recording situations; (C) the stimulation probe (*) was used to apply a 1 mA current to the EBSLN, RLN, and VN. The obtained EMG waveform, latency, and amplitude were consistent with clinical norms for electrophysiologic characteristics of the human EBSLN, RLN, and VN. IONM, intraoperative neural monitoring.

clinical skills. Specifically, the combination of IONM technology and animal models is a valuable tool for studying the pathophysiology of RLN injury. Therefore, this article reviewed the literature to determine the status of animal models relevant to IONM.

Animal models for IONM research

Animal models have accelerated research and development of IONM. In the past decade, new applications of IONM in thyroid surgery have been described in animal studies, most of which have used medium-sized experimental animals such as canine/dog (15-17) and porcine/swine/mini-pig (13,18-37).

The dog larynx has been used as an animal model for more than two centuries of phonatory research. Dog models of laryngeal function and the RLN are well-established and closely mimic the anatomy, size and physiology found in humans. Canine neuroanatomy is also very similar to that in humans (15,16,38,39). After using a canine model for IONM research for several years, the Massachusetts Eye and Ear Infirmary (MEEI) group reported that the canine

model accurately mimics the human RLN and laryngeal physiology and that studies of canine RLN injury help surgeons to predict nerve function and make decisions during thyroid surgery (15-17).

The oldest animal model used in RLN research is the porcine model. During the second century A.D., Galen was the first to define the precise course and function of the RLN. His experiments in live pigs found that alterations in a section of one RLN changed the tone of squealing and that alterations in sections of both RLNs induced respiratory crisis (40,41). Another reason for the widespread use of porcine models in IONM research is their high anatomical and physiological similarity to humans (42,43). Additionally, the pig is a widely available and relatively inexpensive experimental animal. Another advantage is its medium size, which enables easy handling (44). After using the porcine model for IONM research for many years and in many studies, the Kaohsiung Medical University (KMU) group considers the porcine model a reliable and reproducible model for evaluating electrophysiologic correlates of electromyography (EMG) during IONM (*Figure 1*) (13,18-26).

Lessons learned from animal studies

Experimental studies of new applications of IONM and its common pitfalls have addressed several research issues: (I) safety considerations of electrical stimulation (18,27); (II) characteristics of laryngeal EMG (L-EMG) responses evoked by different stimuli/recordings (16,24); (III) anesthetic and EMG tube issues (19,20,25,30,31); (IV) new techniques or devices evaluation (27,32,33), and, most importantly; (V) RLN injury models and prevention strategies (13,15-17,21-23,26-29,34-36). Lessons learned from these animal models not only improve understanding of current IONM technology, but also guide future research in the appropriate surgical strategies for preventing RLN injury during thyroid and parathyroid surgery. The real strength of animal modeling is in models of RLN injury which occur rarely in humans and can't be accurately quantified in the human.

Safety considerations of electrical stimulation

In addition to RLN stimulation, vagus nerve (VN) stimulation is a recommended standard procedure during IONM to ensure that neural testing is not performed distal to the site of RLN injury (4,11). Thus, IONM requires repeated direct electrical stimulation of the RLN and the VN throughout the operation. However, there are safety concerns about the repetitive RLN and VN electrical stimulation.

Wu *et al*. (18) used a prospective porcine model to investigate the optimal intensity and safety of electrical VN and RLN stimulation during IONM. They found no untoward electrophysiological or cardiopulmonary effects after continuous pulsatile VN and RLN stimulations (3 mA, pulse width 100 µs, frequency 4 Hz, and duration 10 minutes). They also reported that 1 mA is a stable and safe intensity for evoking maximal EMG and suggested that regular stimulation with 1mA during IONM is the optimal intensity in terms of minimizing nerve damage risk and minimizing false-positive shunt stimuli. In Schneider *et al*. (27), a pig study to confirm the safety and feasibility of a new vagal anchor electrode for continuous IONM (C-IONM) revealed no vagal side effects during or after continuous VN stimulation (1.0 mA, pulse width 200 µs, frequency 3 Hz, mean duration 280 minutes). Pathologic evaluation of the VN biopsy specimen also showed that the nerve was intact with only slight epineurial edema. For surgeons, these animal studies are extremely valuable for reducing safety concerns about repetitive nerve stimulation

and facilitating the formulation of valid guidelines for electrical stimulation during IONM.

Evoked L-EMG response characteristics under different stimulation/ recording conditions

As in IONM of a facial nerve during skull base, temporal, and parotid surgery, IONM of the VN/RLN, and EBSLN in thyroid surgery requires a stimulation electrode to depolarize the nerve and a recording electrode to record the evoked EMG response (4,5). Initially, IONM recordings were obtained by laryngoscopic or transcutaneous placement of needle electrodes into the vocal folds. However, the difficulty of inserting the needle electrode accurately and safely led to a search for alternative means such as placing surface electrodes in the postcricoid area or attached to an endotracheal tube (ETT) for direct contact with the vocal cord. In the past several decades, various stimulation techniques for IONM of the facial nerve have been described by Møller and Jannetta (constant-voltage stimulation) (45), Prass and Lüders (flush-tip insulation) (46), and Kartush *et al*. (bipolar and insulated stimulus dissectors) (47-50) and are still currently used for IONM in thyroid surgery.

In animal studies, researchers and surgeons can repeatedly stimulate or intentionally injure laryngeal nerves to compare the relative strengths or shortcomings of different stimulation or recording techniques and different IONM devices. For example, the MEEI group (16) used a canine RLN injury model to compare different recording electrode configurations (ETT surface electrodes/ bipolar needle electrodes) and different methods of RLN stimulation (transesophageal/percutaneous/direct open stimulation). They confirmed that a neural integrity monitoring (NIM) system can detect typical patterns of nerve injury in intraoperative L-EMG. Additionally, IONM can be used for quantitative measurements of amplitude, latency, and wave duration in healthy and injured canine RLNs.

Wu *et al*. (24) used an experimental porcine model to compare L-EMG characteristics evoked by stimulation of the EBSLN/RLN/VN with ten different stimulation monopolar/bipolar probes and dissectors. The relative strengths and shortcomings of the different electrodes were also compared. They confirmed that, for all stimulation probes/dissectors, a 1-mA stimulus could evoke typical EMG waveforms from the EBSLN/VN/RLN. Notably, the stimulus-response curve showed that EMG amplitude

increased as the stimulation current increased. To elicit the maximum EMG, monopolar probes and stimulation dissectors required less than 1mA whereas bipolar probes required a higher current. All stimulation electrodes recorded lower evoked EMG amplitudes (with no change in latency) when the nerve was stimulated with overlying fascia or when the distance between the probe/dissector and the nerve was increased. Improved understanding of distinguishing EMG characteristics of EBSLN/VN/RLN and normative data for different stimulation devices, different recording devices, and different conditions serves to optimize the efficacy of IONM performed by thyroid surgeons, but would also improve their ability to predict pathologic neural states.

Anesthetic management and EMG tube issues

For successful IONM of RLN, the anesthesiologist has essential roles in application of the neuromuscular blocking agent (NMBA) and in placement of the EMG tube (4). The use of NMBA is considered standard practice for safe and optimal ETT in general anesthesia. Neuromuscular blockade is needed to reduce airway trauma during tracheal intubation and to optimize mechanical ventilation (51). However, NMBA use can diminish EMG signals and interfere with intraoperative interpretation of IONM results (52-54). Lu and the KMU group (19,20) designed and performed a series of porcine studies to investigate how NMBAs affect IONM. Specifically, they compared laryngeal muscle recovery profiles in animals treated with depolarizing NMBAs (e.g., succinylcholine) and animals treated with nondepolarizing NMBAs (e.g., rocuronium) and concluded that 1 ED95 of rocuronium (0.3 mg/kg) is the optimal dose for IONM (53). Recently, they used the same porcine model to investigate the feasibility of a protocol for increasing the speed of recovery from NMBA: a standard dose of 2 ED95 rocuronium (0.6 mg/kg) combined with sugammadex. Their initial animal model and subsequent clinical study showed that sugammadex 2 mg/kg rapid and effectively restores neuromuscular function suppressed by rocuronium at the beginning of thyroid surgery (25).

In thyroid surgery, IONM of the laryngeal nerve is almost always performed by attaching a recording electrode to ETT-based surface electrodes. Therefore, an ETT that is rotated or is too deep, too shallow, or too small to contact the vocal folds can cause false negatives and other errors that can increase the risk of RLN injury (55,56). Kim and the Korean Intraoperative Neural Monitoring Society (KINMoS) (30) used a porcine model to investigate how

a change in the position of the ETT can affect the EMG response. Their animal experiments revealed that rotation and depth of the ETT were significantly associated with EMG amplitude but not with EMG latency. Therefore, they proposed the concept of using "combined events" to increase reliability in tracking neural injury, i.e., using both amplitude and latency (14) to differentiate amplitude changes that were not associated with ETT malpositioning.

New techniques and devices

The number of newly developed endoscopic procedures for neck surgery has increased in the past decade. In 2005, Grunebaum et al. (32) used a porcine model to demonstrate that RLN monitoring and stimulation can be simultaneously and routinely performed during endoscopic neck surgery without major complications. They contended that RLN identification and monitoring should be considered an essential component of endoscopic neck surgery in humans. In 2009, Witzel et al. (33) used a porcine model to demonstrate the feasibility of using IONM in transoral thyroid resection.

In continuous IONM (CIONM), various stimulation electrode designs developed in recent years now enable seamless monitoring of functional integrity throughout the course of the nerve. Schneider et al. (27) used a pig model to test the feasibility of a new vagal anchor electrode for CIONM before its use in an ongoing clinical trial.

RLN injury models and prevention strategies

Elucidating the mechanisms of RLN injury not only enables further refinement of surgical techniques, but may also reduce RLN palsy rates (9-13). Researchers and surgeons can induce RLN injuries of varying severity and with varying causal mechanisms. Real-time EMG evolutions can then be recorded and correlated with vocal cord function outcome and histopathology results. Therefore, combining IONM technology with animal models provides a valuable tool for studying electrophysiology, severity, and recovery in various RLN injuries (*Figure 2*).

Many animal studies have already investigated the use of IONM for various RLN injuries, including traction (13,21,27,34-36), clamping and crush (13,15-17,28), thermal or cautery (13,21-23,26,27), and transection (15,16,21,29) injuries. Traction injury is an important line of research in the literature on animal IONM model. This injury commonly occurs when the thyroid lobe is
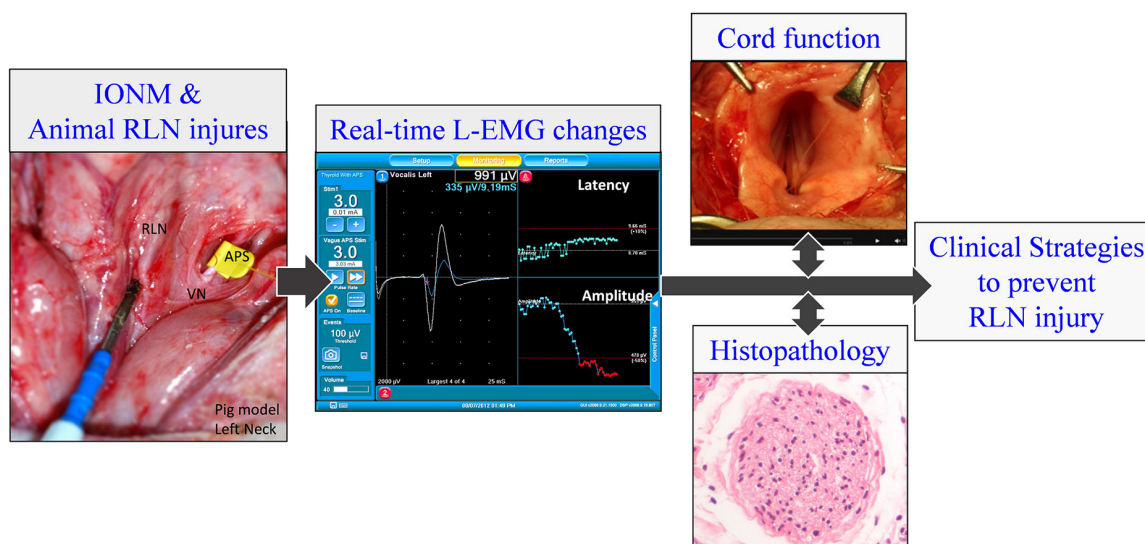
Figure 2 Animal IONM model for research of RLN injury. Animal experiments using CIONM enable researchers to induce different mechanisms and degrees of RLN injuries and to record real-time EMG evolutions. These data can then be correlated with vocal cord function outcome and results of histopathologic analysis. The data also have clinical applications for establishing reliable strategies for preventing RLN injury during thyroid surgery. CIONM, continuous intraoperative neural monitoring; RLN, recurrent laryngeal nerve; VN, vagus nerve; APS, a circumferential clip electrode that is placed around the vagus nerve for "Automated Periodic Stimulation".

mobilized and retracted during thyroidectomy. During surgical manipulation, the RLN can be over-stretched and compressed on the trachea by a dense fibrous band or a crossing artery (especially within the Berry's ligament region) (9). Therefore, several animal studies have applied a small sustained compressive or tensile (13,21,27,34-36) stress on the RLN to simulate the clinical scenario of RLN traction injury during thyroid surgery.

Wu *et al.* (21) use their well-established porcine model of CIONM performed via automated periodic VN stimulation to study the EMG signal evolution during and after acute RLN traction. Detailed analyses of real-time EMG changes during RLN traction revealed a progressive amplitude decrease combined with a latency increase (the so-called "combined event"). Notably, EMG signals gradually recovered completely or at least partially after release of traction. The EMG recovery was almost complete when traction stress was relieved by 50% amplitude reduction, but the recovery was worse if the traction was relieved after loss of signal (LOS) or after prolonged and repeated application of traction to the nerve.

Several other studies have used animal CIONM models to investigate L-EMG signal changes in RLNs under traction stress. Schneider *et al.* (27) showed that CIONM by VN stimulation is technically feasible for predicting imminent

nerve failure. Lee *et al.* (34) reported that traction injury of swine RLNs causes LOS at a power of 2.83 MPa. Brauckhoff *et al.* (35) reported that latency increase may be the first warning of RLN stretch injury during CIONM and proposed that a 50% amplitude loss can be taken as an appropriate alert limit. Lamadé *et al.* (36) showed that certain RLNs were 4.3 times more vulnerable to tensile force compared to the contralateral nerve. Thus, the right and the left nerves cannot be assumed to have equal sensitivity to trauma. The findings of these animal studies can help thyroid surgeons to interpret adverse EMG signals that indicate impending RLN traction injury and to use the IONM as a risk minimization tool for postoperative RLN paralysis.

Another common mechanism of intraoperative RLN injury is thermal injury, which often results from thermal spread of electrocautery and various energy-based devices (EBDs) used for hemostasis or dissection near the RLN. Lin *et al.* (22) used a porcine CIONM model to demonstrate that the critical temperature at which RLN thermal injury occurs is approximately 60 ℃. They concluded that using CIONM to detect early stages of acute thermal stress can help to avoid further severe or repeated injury to the RLN caused by EBDs.

Since EBDs are widely used for hemostasis and dissection, their safety must be carefully evaluated, especially

their effects on the RLN. In a porcine CIONM model used to evaluate the safety parameters of the Harmonic Focus (HF; Ethicon, Cincinnati, OH, USA), Wu *et al.* (23) found that the HF can cause unexpected iatrogenic RLN injury and recommended that its use should be standardized. The same porcine CIONM model has also been used to improve the safety of several other EBDs such as THUNDERBEAT (TB; Olympus Co Inc., Tokyo, Japan) (37) and the LigaSure™ Small Jaw (LSJ; Medtronic, Minneapolis, MN, USA) (26). The findings of these animal studies have immediate clinical applications in establishing efficient and safe strategies for using EBDs in thyroid surgery.

The main limitation of a clinical study of RLN injury is the limited availability of specimens for histopathology analysis. A clear understanding of the mechanisms and expected outcomes of RLN injuries is important not only for prognosis, but also for making correct intraoperative decisions about the extent and timing of contralateral thyroidectomy and the need for nerve repair. In Dionigi *et al.* (13), an electrophysiologic and histopathologic analysis of RLN injuries experimentally induced in a porcine CIONM model revealed that lesions associated with traction and mechanical injury only showed distortion of the outer nerve structure whereas lesions associated with thermal injury showed severe damage in the inner endoneurium. They calculated correlations with the clinical data for 281 RLN injuries from 6,093 at-risk nerves and defined, classified, and ranked the RLN injuries by severity. Clinicians can use this valuable combination of clinical data and data from animal studies to evaluate the severity of RLN injuries, formulate intra- and post-operative treatment, and predict recovery.

Conclusions

The IONM technology is an adjunct, not a replacement for anatomical knowledge and surgical skill. Additionally, data from animal studies may not be totally applicable to humans. Nevertheless, animal models are essential for improved understanding of IONM technology and the electrophysiology of RLN injury; data obtained from animal studies can be used to improve the efficacy of clinical techniques and strategies for preventing RLN injury during thyroid surgery.

Currently, both conventional intermittent IONM and CIONM systems have technical and interpretive limitations that can cause false-positive and false-negative errors. Therefore, future animal and clinical studies must continue to address these issues and develop innovative applications

until IONM is established as a safe and easily performed standard procedure in thyroid surgery.

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Footnote

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