Original Article

Identifying a Safe Range of Stimulation Current for Intraoperative Neuromonitoring of the Recurrent Laryngeal Nerve: Results from a Canine Model

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Abstract

Background: Intraoperative neuromonitoring (IONM) of the recurrent laryngeal nerve (RLN) has been widely applied during thyroid surgery. However, the safe range of stimulation intensity for IONM remains undetermined.

Methods: Total thyroidectomies were performed on twenty dogs, and their RLNs were stimulated with a current of 5–20 mA (step-wise in 5 mA increments) for 1 min. The evoked electromyography (EMG) of vocal muscles before and after supramaximal stimulation were recorded and compared. Acute microstructural morphological changes in the RLNs were observed immediately postoperatively under an electron microscope.

Results: The average stimulating threshold for RLNs stimulated with 15 mA and 20 mA showed no significant changes compared to the unstimulated RLNs (15 mA group: 0.320 ± 0.123 mA vs. 0.315 ± 0.097 mA, $P = 0.847$; 20 mA group: 0.305 ± 0.101 mA vs. 0.300 ± 0.103 mA, $P = 0.758$). Similar outcomes were shown in average evoked EMG amplitude (15 mA group: 1,026 ± 268 μV vs. 1,021 ± 273 μV, $P = 0.834$; 20 mA group: 1,162 ± 275 μV vs. 1,200 ± 258 μV, $P = 0.148$). However, obvious acute microstructural morphological changes were observed in the nerves that were stimulated with 20 mA.

Conclusions: A stimulation intensity less than 15 mA might be safe for IONM of the RLN.

Key words: Canine Model; Neuromonitoring; Recurrent Laryngeal Nerve; Safe Stimulation Intensity; Thyroid Surgery

Introduction

The recurrent laryngeal nerve (RLN) injury is one of the most important complications during thyroid surgery, leading to dysfunction, and palsy of the vocal cords. For years, surgeons have attempted to prevent this complication by improving surgical techniques and optimizing surgical strategies. The identification and preservation of the anatomical integrity of the RLN during thyroid surgery is commonly accepted as a "gold standard." However, even in the most experienced hands, RLN injury occasionally occurs. In the last decade, intraoperative neuromonitoring (IONM) has been widely applied during thyroid surgery to aid in the localization and identification of the RLN. The safety and effectiveness of using IONM during clinical practice have been confirmed.[1-3]

One of the most important roles of IONM is assistance in intraoperative navigation and rapid identification of RLN distribution.[4-8] Repetitive tissue stimulation in the vicinity of the nerve allows the nerve to be localized using a stimulation probe before visual identification.[8] The reported stimulation intensity for mapping the path of the nerve ranges from 2 to 3 mA.[6,9-11] This range is higher than that used for direct nerve stimulation, as the use of stronger stimulation current would depolarize a greater sphere of tissue around the probe tip.[12] However, failure to obtain signal deflection at 3 mA during nerve localization might occasionally occur, in which case a surgeon might consider increasing the stimulation intensity. However, electrical stimulation is also a potential

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risk factor for RLN injury, and the nerve can become damaged in the case of over-stimulation.[13]

Few studies have evaluated the safety of using supramaximal stimulation in IONM. Furthermore, the safe range of stimulation intensity for neuromonitoring remains undetermined. Therefore, in this study, we developed an experimental canine model to determine whether supramaximal stimulation during IONM could induce nerve damage and investigated the safe range of stimulation current intensity.

Methods

Experimental animals

Twenty adult, male beagle dogs weighing 15–20 kg were obtained from the Animal Center of Peking University Health Science Center (Institutional Protocol No. SYXK2008-0021, Beijing, China). The dogs were randomly divided into four groups of five each and maintained under standard laboratory conditions with free access to food and water. All dogs were healthy with normal phonation. All animal experiments were approved by the Institutional Ethics Committee (Peking University People's Hospital), and all procedures were conducted according to our institutional animal care guidelines.

Preoperative preparation

The animals were fasted for 8 h and then anesthetized with intravenous 3% pentobarbital (Merck KGaA, Darmstadt, Germany) 5 ml and vecuronium (Organon Pharmaceutical Co., Ltd., Oss, The Netherlands) 0.06 mg/kg, after which a neural monitoring tube (Medtronic NIM 2 EMG ETT #6; Medtronic, Jacksonville, Florida, USA) was inserted by a single anesthesiologist. The tube was placed under direct laryngoscopy (Welch Allyn Inc., New York, USA) to ensure that the middle of its blue-marked region (the exposed electrodes) formed a good contact with the true vocal cords. The tube was then fixed by balloon inflation.

Surgical procedures

After performing routine surgical procedures such as disinfection and draping, a midline vertical cervical incision was made to expose the neck and the larynx, and total thyroidectomy was performed. The RLN was identified at the tracheoesophageal groove during dissection.

A nerve integrity monitor (NIM-Response 2.0; Medtronic, Jacksonville, Florida, USA) was applied for IONM approximately 20 min after the administration of muscle relaxant, at the moment of muscle recovery. The following parameters were set: stimulation rate, four times/s; pulse width, 100 μs; and event threshold, 100 μs. While exposing the bilateral RLN, a stimulation current was delivered to the nerves by a bipolar probe at a 30 Hz frequency. Vocal cord response was recorded by a surface electrode placed on the endotracheal tube. The electrode detected evoked electromyography (EMG) activity, the parameters of which were recorded.

After finishing the experimental procedures, the incisions were closed in multiple layers, and the animals were sacrificed by an intravenous bolus injection of potassium chloride. Aseptic technique was used during all procedures. All surgeries were performed by the same surgeon, who is experienced in thyroid surgery.

Experimental design

The stimulation threshold was defined as the lowest current required to obtain clear evoked EMG. Each nerve was initially stimulated using a 0.1 mA current, and the current was increased step-wise by 0.05 mA until EMG was observed. The current was recorded as the stimulation threshold, and the corresponding amplitude was recorded as well.

After exposure of the RLN, the stimulation threshold and amplitude at the threshold level were measured as baseline parameters before any interventions. Then, the RLNs were continuously stimulated with a high-intensity current for 1 min at a point distal from the test point. Each group of animals was exposed to a different stimulation intensity (5 mA, 10 mA, 15 mA, and 20 mA). The stimulation threshold and evoked EMG amplitude were measured 1 min later at the same point as the prior test to determine poststimulation parameters. All interventions and recordings were performed completely on one side before treating the other side.

Histological evaluation

The nerves were removed for histological evaluation 30 min after all experimental interventions. The specimens were prepared using the following steps: (1) immediate fixing in 3% glutaraldehyde (Sigma-Aldrich Co. LLC., St. Louis, USA); (2) three washes of 20 min each with phosphate-buffered solution (Zoman Bio Co., Ltd., Beijing, China); (3) fixing in osmic acid (Alfa Aesar, Massachusetts, USA) for 1.5–2.0 h; (4) two washes with distilled water (Sinopharm Chemical Reagent Beijing Co., Ltd, Beijing, China); (5) immersion in uranium (Merck KGaA, Darmstadt, Germany) for 30 min; (6) two washes with distilled water; (7) dehydration with graded ethanol (Beijing Chemical Works, Beijing, China) (50%, 70%, 90%, and 100% for 15 min each); (8) embedding in 1:1 100% acetone (Beijing Chemical Works, Beijing, China) under vacuum for 2–3 h; (9) embedding in 1:3 100% acetone: under vacuum for 2–3 h; (10) embedding and immersion at 37°C for 16–20 h; (11) polymerization at 60°C for 48 h; and (12) drying for 24 h. Finally, the myelin sheath, neuraxon, and Schwann cell nerve microstructures were observed under an electron microscope (JEOL Ltd., Tokyo, Japan).

Statistical analysis

Quantitative results are expressed as the mean ± standard deviation (SD). Data were analyzed using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL, USA). Paired *t*-tests were used to compare the average stimulation thresholds and amplitudes at the threshold level between

normal and stimulated RLNs. A value of $P < 0.05$ was considered statistically significant.

Results

Neuromonitoring outcomes

The bilateral RLNs of all 20 dogs were successfully exposed; therefore, 10 nerves per group were available for experimentation. The average stimulation thresholds of the unstimulated RLNs in the 15 mA group and 20 mA group were 0.315 ± 0.097 mA and 0.300 ± 0.103 mA, and the average amplitudes at the threshold level were 1,021 ± 273 μV and 1,200 ± 258 μV, respectively. The data from the groups stimulated with 5 mA and 10 mA were accidentally lost. After the experimental invention, no significant difference in average stimulation threshold was observed in any group [15 mA group: unstimulated 0.315 ± 0.097 mA vs. stimulated 0.320 ± 0.123 mA, $P = 0.847$; 20 mA group: unstimulated 0.300 ± 0.103 mA vs. stimulated 0.305 ± 0.101 mA, $P = 0.758$; Table 1]. Similarly, there was no statistically significant change in average evoked EMG amplitude in any group, even the group stimulated with 20 mA [15 mA group: unstimulated 1,021 ± 273 μV vs. stimulated 1,026 ± 268 μV, $P = 0.834$; 20 mA group: unstimulated 1,200 ± 258 μV vs. stimulated 1,162 ± 275 μV, $P = 0.148$; Table 2].

Histological outcomes

No significant pathological changes were observed among the specimens from the 5 mA, 10 mA, and 15 mA groups compared to the normal group under electron microscopy [Figure 1]. The nerve fibers were regularly arranged with well-organized myelin sheaths. Aligned microfilaments, evenly distributed matrix, and normal organelles were clearly observed without Schwann cell degeneration or proliferation [Figure 2]. However, there were acute microstructural morphological changes in the 20 mA group specimens. Released lamellar myelin and demyelination were observed with macrophage phagocytosis. Schwann cell degeneration was occasionally observed [Figure 3].

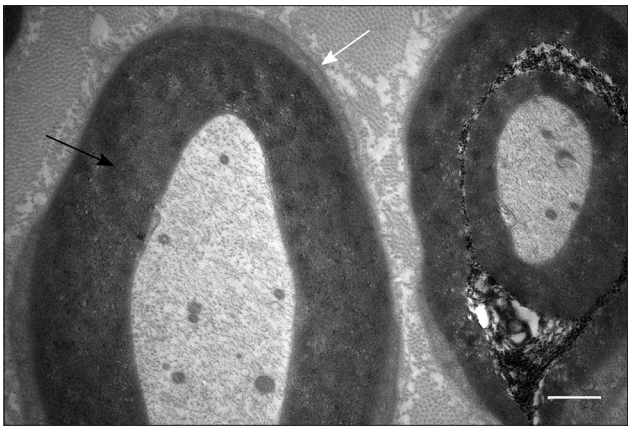

Figure 1: Electron microscopic image of a normal recurrent laryngeal nerve (Original magnification, ×10,000). The black arrow indicates myelin, and the white arrow indicates a Schwann cell.

Discussion

RLN injury is a major complication of thyroid surgery. Such injury results in temporary or permanent recurrent laryngeal nerve palsy (RLNP), which manifests as hoarseness in unilateral injury or life-threatening acute airway obstruction in bilateral injury. Temporary RLNP occurs in 1.4–38.4% of cases while permanent palsy occurs in 0–18.6% of cases.[14] Substantial effort has been made to avoid this complication. The strategy used for thyroid surgery has advanced to routine capsular dissection and direct visualization of the RLN, which is accepted as the gold standard due to its association with a significantly lower RLNP rate.[15,16] IONM was recently introduced to aid in the localization and identification of the RLN and as a prognostic measure of postoperative nerve function.[17]

One major benefit of RLN monitoring is assistance in nerve identification. Early and definite identification of the RLN during thyroid surgery, especially in difficult cases,

Table 1: Stimulation threshold changes for recurrent laryngeal nerves after electrical stimulation at different intensities

Stimulation intensity (mA)	Status	Values (mA), mean ± SD	*n*	*t*	*P*
15	Unstimulated	0.315 ± 0.097	10	−0.198	0.847
	Stimulated	0.320 ± 0.123			
20	Unstimulated	0.300 ± 0.103	10	−0.318	0.758
	Stimulated	0.305 ± 0.101			

SD: Standard deviation.

Table 2: Changes in evoked electromyography amplitude of the recurrent laryngeal nerve after electrical stimulation at different intensities

Stimulation intensity (mA)	Status	Values (μV), mean ± SD	*n*	*t*	*P*
15	Unstimulated	1021 ± 273	10	−0.216	0.834
	Stimulated	1026 ± 268			
20	Unstimulated	1200 ± 258	10	1.581	0.148
	Stimulated	1162 ± 275			

SD: Standard deviation.

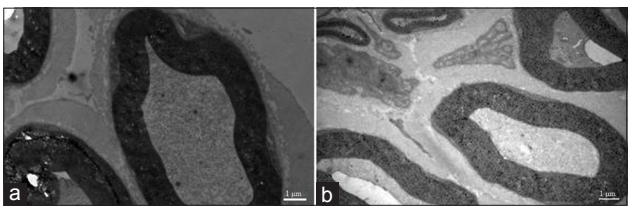

Figure 2: Electron microscopic image of a recurrent laryngeal nerve continuously stimulated with a 15 mA current for 1 min (Original magnification ×10,000). No obvious pathological changes appeared.

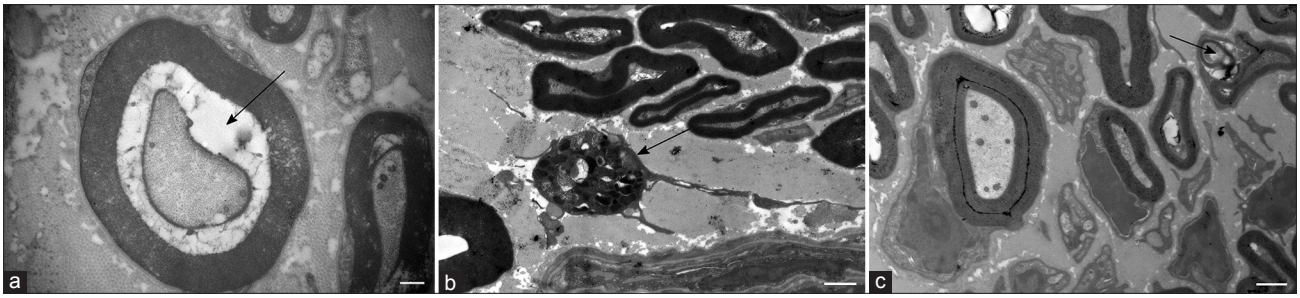

Figure 3: Electron microscopic image of a recurrent laryngeal nerve stimulated with a 20 mA current for 1 min. Acute microstructural morphological changes were observed. (a) The arrow indicates axon edema (Original magnification ×20,000). (b) The arrow indicates macrophage phagocytosis (Original magnification ×10,000). (c) The arrow indicates released myelin sheath (Original magnification ×10,000).

is important for preventing RLN injury.[4,6,8] Previously reported stimulus intensities used to localize the nerve before visualization has ranged from 1 to 3 mA.[6,9-11] Many studies have confirmed that the stimulation current that is routinely used is safe and meets the demands required for surgery most of the time.[3,6,9,18,19] However, it has been clearly demonstrated that over-stimulation induces tissue damage, which may result from the passage of current through the tissue.[13] The range of stimulation current intensity that can be safely applied during clinical practice remains to be determined.

In this study, we used twenty dogs to evaluate the functional and histological outcomes of applying supramaximal electrical stimulation to the RLN. The baseline electrophysiologic parameters were similar to those reported in previous studies.[20,21] The safety of stimulation with 3 mA has been widely confirmed in clinical and animal experiments.[3,9,19] In a study reported by Ulmer *et al*.,[22] the maximal current used for stimulation was 5 mA, and no postoperative RLNP was detected. However, stimulation with 5 mA during IONM has rarely been used in the clinic. In the current study, we initially treated animals with a current of 5 mA and observed no functional and histological changes after the intervention, verifying its safety. Previous studies in canine or porcine models that have characterized EMG changes, such as amplitude decrements and increases in stimulation threshold level after neural injury, have suggested that these parameters can be used as predictors of nerve injury.[20-23] We next increased the experimental current in a stepwise manner. Even after stimulation with 20 mA, there was still no functional abnormality in the nerve based on the stimulation thresholds and evoked EMG amplitudes observed. The RLNs were harvested immediately after the operation to evaluate histological changes. Normal morphology was observed after stimulation with 15 mA, but acute microstructural morphological changes occurred after stimulation with 20 mA. The anatomical and functional correlations of our canine model might be translatable into a safe range of stimulation intensity for IONM during human thyroid surgery. Despite losing the data from the groups stimulated with 5 mA and 10 mA, based on our results, we postulate that a stimulation intensity less than 15 mA will not induce any functional or histological changes to RLNs during IONM.

We chose to evaluate a canine model because dog thyroid glands have similar anatomy to human thyroid glands and therefore can be used to mimic human thyroid surgery. The canine thyroid is a dark red, elongated structure that is attached to the fascia along the ventrolateral surfaces of the proximal trachea, ventrally covered by the sternocephalicus and sternohyoideus muscles, and laterally covered by the sternothyroideus muscle. The caudal laryngeal nerves in dogs, termed RLNs in humans, are dorsal to the thyroid lobes.[24]

One limitation of the current study is the loss of the data from the groups stimulated with the low-intensity current. Future studies with larger sample sizes and better experimental design are required. Another limitation was the lack of exclusion of influencing factors and the absence of functional evaluation of the nerves, such as the observation of vocal cord movement by video laryngoscopy. However, this study provides evidence for the safety of using electrical stimulation during IONM in clinical practice.

In conclusion, our study indicates that a stimulation intensity less than 15 mA could be safely applied during IONM.

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Conflicts of interest

There are no conflicts of interest.

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