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A pilot study: Infrared laser stimulation of the rat vagus nerves

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ABSTRACT

The vagus nerve originating from the brainstem in the central nervous system is a long cranial nerve that reaches the neck, thorax, abdomen, and colon. It plays a role in autonomic nervous, cardiovascular, gastrointestinal, and immune systems. Electrical stimulation of the vagus nerve has become a standard method for the treatment of neuropathic pain and epileptic conditions over the years. Infrared laser nerve stimulation (ILNS) is an evolving technique that uses infrared laser energy to stimulate cells with electrochemical capacity without the need for external agents or physical contact. This pilot study explores infrared laser stimulation of the rat vagus nerve, in-vivo. An infrared pigtailed single-mode diode laser operating at 1505 nm in continuous-wave (CW) mode was used in this study for noncontact CW-ILNS. Successful CW-ILNS of the rat vagus nerve was observed after the CN reached a threshold temperature of ~44 °C with response times as short as 10 s. With more improvement in instrumentation, better optimization of stimulation parameters, and a higher sample size, CW-ILNS may show some potential in vagus nerve stimulation for preclinical

Keywords: vagus nerves, infrared nerve stimulation, lasers, blood pressure, electrocardiogram

1. INTRODUCTION

The vagus nerve (VN) is the longest cranial nerve containing both afferent and efferent neurons. It provides a two-way connection between the brain and internal organs that reaches the neck, thorax, abdomen, and colon and hence, plays a vital role in autonomic nervous, cardiovascular, respiratory, gastrointestinal, and immune systems [1]. For example, it regulates heart rate, blood pressure, vascular resistance, breathing, and feeding [2]. The electrical stimulation of VN has become a standard method for the treatment of many diseases, including chronic pains, fibromyalgia, migraine, osteoporosis, and epilepsy. For example, it has been investigated the reorganization of the electrical activity in the brain by sending repetitive weak electrical signals to the vagus nerve [3]. However, electrical vagus nerve stimulation has many side effects, such as arrhythmia, vocal folds, headache, and depression, due to the difficulty in controlling the electrical current in the tissue [4].

Infrared laser nerve stimulation (ILNS) is an emerging technique [5] that uses infrared laser energy to stimulate electrochemically capable cells without the need for external agents or physical contact. This technique may offer three main advantages over electrical nerve stimulation. (1) A non-contact method of nerve stimulation. (2) Improved spatial selectivity. (3) Elimination of stimulation artifacts. Infrared laser nerve stimulation has recently been examined in various types of neural tissue [6, 7]. More interestingly, successful optical stimulation of the rat cavernous nerve using continuous-wave (CW) infrared laser radiation has been previously reported [8].

The objective of this pilot study to investigate the feasibility of CW-ILNS of the rat vagus nerve, in-vivo.

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2. METHODOLOGY

2.1 ILNS System

The all-single-mode fiber ILNS system, as shown in Figure 1, was composed of a visible laser, an infrared pigtailed single-mode diode laser for nerve stimulation, a fiber coupler, and a laser probe. A red diode laser (BT-VFL650-20, Beek Electronics, Ankara, Turkey) with a power of 20 mW and a wavelength of 650 nm was used to simplify the alignment of the infrared laser beam with the microscopic nerve. A pigtailed, single-mode, diode laser (BrightLock, QPC Lasers, Sylmar, CA) emitted up to 500 m W of laser power at a wavelength of 1505 nm. The infrared laser and a red diode laser aiming beam were coupled into a single-mode fiber coupler (TN1310R1A2, Thorlabs, Newton, NJ) with a 1:99 coupling ratio to combine both infrared laser and visible beams into a fiber output coupler. The fiber coupler output was connected to a laser probe consisting of single-mode fiber coupled (SMF-28e, Corning, NY) GRIN lens (50-1310A-APC, Thorlabs, Newton, NJ). The probe produced a collimated approximately 0.5-mm diameter laser beam on the nerve at a working distance of approximately 15 mm.

The 1505 nm laser was operated in CW mode during ILNS experiments, performed with an irradiation time of 90 seconds and a laser spot diameter of <0.5 mm. The incident infrared laser power was set to 35 mW. A thermal camera (PI400, Optris GmbH, Berlin, Germany) provided temperature mapping of the vagus nerve during ILNS to provide temperature feedback during the procedure (Figure 1).

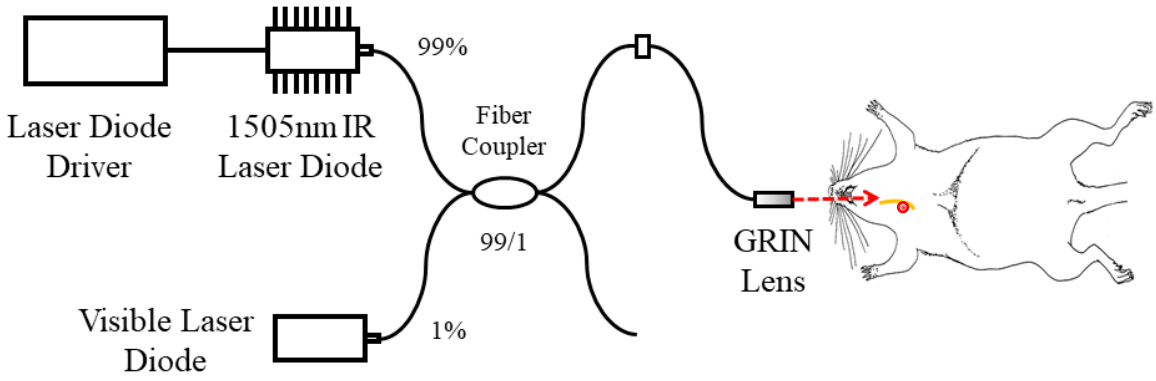

Figure 1. A schematic drawing of the all-single-mode fiber infrared laser nerve stimulation system.

2.2 Animal Experimental Setup

Figure 2 shows the photography of rat surgical preparation. All studies were performed under an animal protocol (protocol number: 04/2019) approved by the Multidisciplinary Laboratory Animal Experiments Local Ethics Committee at Dokuz Eylul University. One male Sprague Dawley rat (450 g) was anesthetized by intraperitoneal injection with ketamine (90 mg/kg) and xylazine (10 mg/kg). The rat was placed on a surgical table in a supine position and secured with self-adhesive dressing. The jugular area of the animal was shaved and then sterilized with an iodine antiseptic solution. A four to five-centimeter incision was practiced to the leftmost part of the trachea from the hyoid bone clavicles. Excessive tissues such as salivary glands, mandibular glands, and connective tissue were removed until they reached the sternomastoid, sternohyoid, and omohyoid muscles. The small slit between the sternomastoid and sternohyoid muscles was enlarged to identify the carotis communis located just behind the sternomastoid muscle using blunt dissection. The left vagus nerve was separated from the carotid artery. The right carotid artery was isolated to measure systemic blood pressure (BP).

Similar to standard methods for electrical stimulation of the VN [9, 10], the left VN placed on stainless-steel stimulation electrodes (FGT35, Commat Ltd, Ankara Turkey). The electrodes attached to a MP36 stimulator (BSLSTMB, Biopac Systems Inc., Santa Barbara, CA). Stimulation parameters were 3.5 V for 40 s at a repetition rate of 1 Hz with a with a square wave duration of 0.5 ms.

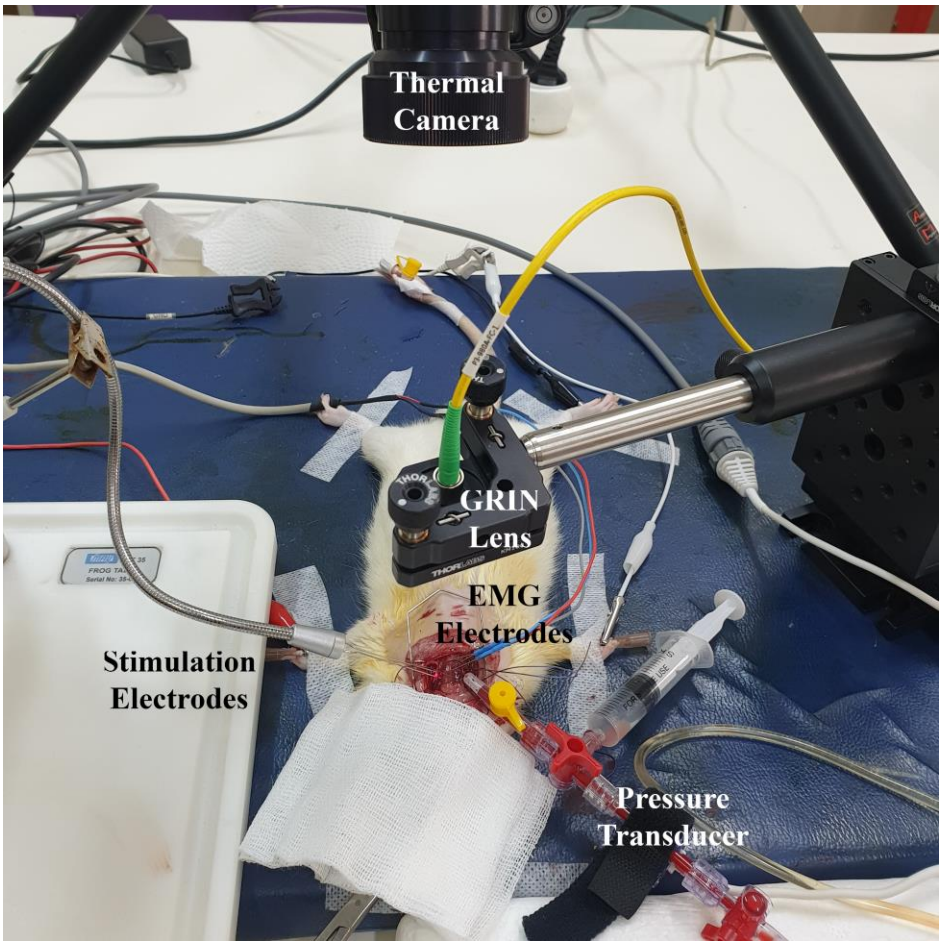

Figure 2. Experimental setup used for CW-ILNS in a rat vagus nerve model, in vivo.

2.3 Physiological Recordings

An MP36 four-channel data acquisition system with appropriate accessories (Biopac Systems Inc., Santa Barbara, CA) and Biopac Student Lab 3.7.7 software for life science research detected all physiological recordings. The sample rate was 25000 samples per second. The response parameters were analyzed with MATLAB programming platform.

2.3.1 Blood Pressure (BP) Recordings

The right carotid artery was cannulated with a 24G intravenous cannula (Bicakcilar Medical, İstanbul, Turkey) and connected to a pressure transducer (SS13L, Biopac Systems Inc., Santa Barbara, CA) with a three-way stopcock to measure BP. The entire system was filled with heparinized saline solution to prevent clotting [11].

2.3.2 Electromyogram (EMG) Recordings

The stainless-steel needle electrodes (Natus Neurology Inc., Middleton, WI) were modified to fit the MP36 system by combining the standard 9-pin connector. Three needle electrodes were inserted into the sternohyoid muscle to obtain EMG recordings. The system amplified signals 1000 times and applied 5-250 Hz notch bandpass filtering.

2.3.3 Electrocardiogram (ECG) Recordings

Three stainless steel needle electrodes were attached to the SS2L lead set (Biopac Systems Inc., Santa Barbara, CA). Electrodes were placed subcutaneously on the left foreleg, right foreleg, and right hindleg to obtain ECG recordings [12]. The system amplified signals 1000 times and applied 0.05 - 35 Hz notch bandpass filtering.

3. RESULTS

Figure 3 shows blood pressure measurements in the rat vagus nerve model before, during and after both electrical nerve stimulation (A) and CW infrared laser nerve stimulation (CW-ILNS) (B). Electrical stimulation of the vagus nerve caused a 32-mmHg decrease in blood pressure. On the other hand, CW-ILNS reduced blood pressure 20 mmHg. The electrical stimulation of the vagus nerve also resulted in an average heart rate drop of 110 beats per minute. Besides, cardiovascular responses rapidly recovered after electrical stimulation. However, no strong decrease in heart rate was observed during CW-ILNS. This may be because the possible mechanism of infrared laser stimulation (i.e., photothermal effect) does not provide a rapid response.

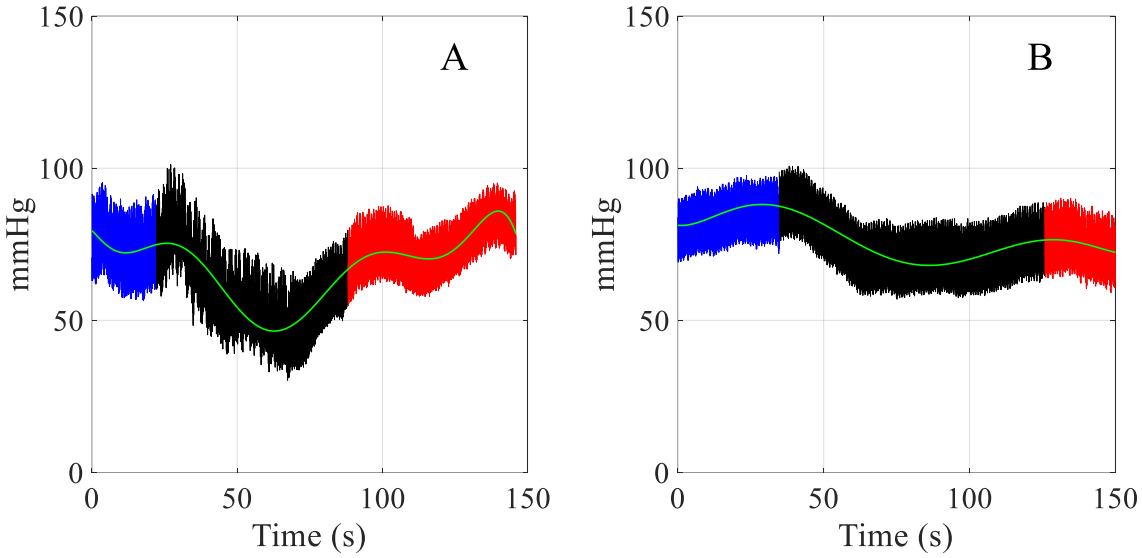

Figure 3. Blood pressure measurements in the rat vagus nerve model before, during and after both electrical nerve stimulation (A) and CW infrared laser nerve stimulation (CW-ILNS) (B). The black line indicates the stimulation time. The blue and red lines indicate the time before and after stimulations, respectively. The green line indicates the trend of variance.

Figure 4 shows the electromyogram results during stimulations of the rat vagus nerve. During electrical stimulation, the stimulating artifact and laryngeal electromyogram response can be clearly measured, as shown in Figure 4A. Unlike electrical stimulation, ILNS did not produce any electromyogram signal. The VN mainly innervates cricothyroid muscle. However, finding out the location of cricothyroid muscle is very problematic in rat model.

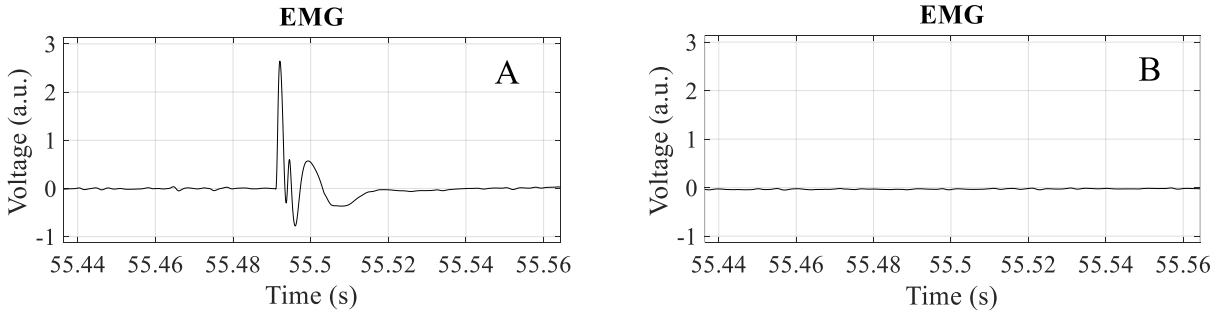

Figure 4. EMG signals of (A) electrical stimulation and (B) ILNS from sternohyoid muscle. Stimulus artifact and laryngeal EMG can be observed in electrical stimulation of the VN.

Thermal images of the rat VN before ILNS and at peak temperature during ILNS are provided in Figure 5, for the same stimulation parameters and results as shown in Figure 3 and Figure 4. It should be noted that the baseline nerve temperature was not at normal body temperature (37 °C), but rather a few degrees cooler (30 °C) due to the open surgical model used in these studies (Figure 2).

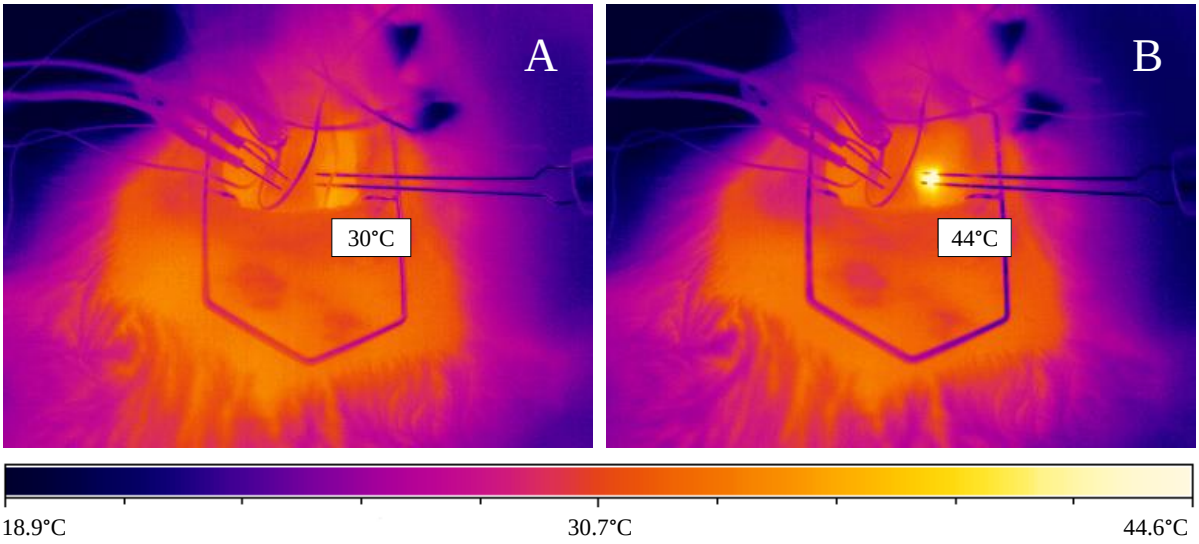

Figure 5. Thermal camera image of the rat vagus nerve before stimulation (A). Thermal camera image of the rat vagus nerve during infrared laser nerve stimulation. The nerve temperature reaches 44°C (B).

4. DISCUSSION

The Vagus Nerve (VN) is a bundle of neural fibers that contain both afferent and efferent neurons that transmit signals in both directions. The efferent type neurons of VN are responsible for certain motor functions such as the stimulation of muscles in the pharynx, larynx, and heart. Therefore, VN plays a critical role in the body in controlling physiological events such as heart rate, blood pressure, vascular resistance, breathing, and nutrition.

On the other hand, afferent sensory neurons, which make up 60 to 80% of the vagus nerve bundle, carry information signals from visceral organs to the brain via type C fibers [2]. Moreover, if the VN is stimulated correctly, it can re-regulate the interrupted electrical activity of the brain [3]. For example, electrical stimulation of the vagus nerve despite some side effects has shown potential as an effective technique for epileptic patients with drug-resistant seizures. Therefore, new approaches that eliminate or reduce the side effects of electrical current can be used as leverage that can provide a better quality of life for patients.

The CW-ILNS technique, which has recently been demonstrated in rat cavernous nerve [13], may offer a potential with its advantages, including a noncontact method of stimulation: The infrared laser energy is delivered in a noncontact mode. Improved spatial selectivity: The laser beam can be focused to a spot smaller than the dimensions of a typical electrode. Elimination of stimulation artifacts: The nerve is stimulated optically but measurement is performed electrically or by other means. This pilot study investigates the feasibility of CW-ILNS of the vagus nerve in an in-vivo rat model.

The results of this pilot study suggest that blood pressure and electrocardiogram recordings may be a clue to successfully measuring vagus nerve stimulation using infrared laser power. Electrical stimulation of the VN resulted in a 32 mmHg decrease in blood pressure, while ILNS measured a 20 mmHg decrease in blood pressure. Electrical stimulation might have caused a systemic stimulation that can lead to a dramatic decrease in blood pressure and heart beat more than laser stimulation. However, in electromyogram measurements, no signal for laser stimulation could be measured. Therefore, further studies investigating the motor outputs of the vagus nerve with type A and type B neurons should be performed to appreciate possible selective stimulation.

The main limitation of this study is the lack of iterative trials for reliable laser stimulation parameters. For example, in this study, the surface temperature of the nerve increased to 44 degrees in Celsius by inducing a 35-mW laser power. This nerve temperature was within the threshold presented previously for rat cavernous nerve activation. However, no such limit value has yet been determined for the rat vagus nerve. Overall, by performing a larger number of rigorous experiments, statistically significant results should be produced.

5. CONCLUSIONS

This pilot study has demonstrated the stimulation effects of 1505 nm infrared laser radiation at 35 mW power on the rat vagus nerve in blood pressure, electromyogram, and electrocardiogram recordings. CW-infrared laser nerve stimulation (CW-ILNS) of the rat vagus nerve with improvement in instrumentation, reliable optimization of stimulation parameters, and a higher sample size may be an important model for preclinical studies of epilepsy.

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