IRREVERSIBLE BLOCKING OF POLAR EXCITATIONS ON FROG SCIATIC NERVE USING SEMICONDUCTOR PULSE LASER IRRADIATION

Yuya Hirayama, Takeshi Yamakawa, Satoru Ishizuka, Zimin Lev Grigorievich, Toshitaka Yamakawa

Abstract:

The final purpose of our research is to cripple the epileptogenic focus by laser irradiation. However, the effect of laser irradiation on brain nerves is not well known. In this paper, we observed laser irradiation to the transmission of action potential of a bullfrog sciatic nerve in the experiment. In addition the marginal amplitude of laser energy is obtained. The bullfrog nerve preparations were stimulated supramaximal intensity pulse. The pulse width and the pulse interval are 1 msec and 1 sec, respectively. A semiconductor pulse laser irradiation (the wavelength, the pulse width and the frequency are 808 nm, 990 µsec and 50 Hz, respectively) was employed until when the amplitude of the action potential decreases to 10 %. The laser light was irradiated to the portion between two electrode pairs for recording. The energy of the laser is variable between 13.9-202.5 mJ/cm^2. The laser irradiator is connected to an optical fiber whose diameter is 1 mm. The action potential of the sciatic nerve is observed at 1 day after in order to confirm irreversibility. The experiment was delivered by the laser pulse, the energy of which over 32.9 mJ/cm^2. The peak of action potential was decreased gradually by laser irradiation with depending on the time of irradiation. The time to blocking is reciprocally proportional to laser energy. At a day after the experiment, the action potential was not recovered. This result shows the laser irradiation give irreversible blocking to polar excitations. We observed the frog sciatic nerve is damaged by laser irradiation. The results show a possibility that the epileptogenic focus can be crippled by laser irradiation. We are going to have more experiments and obtain important laser parameters. In the future, the experiment and the result are expanded using rat brain and applied human surgery.

Keywords: *irreversible blocking, semiconductor pulse Laser Irradiation, frog sciatic nerve.*

1. Introduction

1.1. Epilepsy

The epilepsy is one of brain diseases [1]. It has some symptom called epilepsy seizures. The epilepsy seizure caused by abnormal fire of brain neurons. The portion of generation source of epilepsy seizure is called epileptogenic focus.

Epilepsy treatment are two approaches the one is a drug therapy (using AED: Antiepileptic drug), the other is a surgery. Normally, the drug therapy is chosen. When the epilepsy seizure is not controlled by AED, adapting surgery is considered. However, the epilepsy surgery has three risks. One is a very long time (about 8-10 hours) craniotomy. Two is resection of epileptogenic focus when the healthy lesion is also resected because of making it to certain treatment. Three is complicating diseases after epilepsy surgery.

From above, epilepsy patients are asking new epilepsy surgery which is more convenient, more minimally invasive, and more high accuracy.

1.2. Medical Treatments using laser irradiation

Medical treatments using laser irradiation have attracted increasing attention in recent years. Especially, low energy laser irradiation is applied to dental treatment. Some reports said low energy laser has a effect of healing acceleration to canker sore, gingivitis, and injury of dental extraction [2],[3]. Effects of laser irradiation to cells and nerves are some reports. These reports said the effect is depended on laser wavelength, laser energy, and etc. For example, low energy laser irradiation can block the conduction of nerve impulses [4]–[7] and improve recovery from nerve injuries in both experimental rat models [8]-[10] and clinical investigations [11]-[13]. The conduction block of action potentials induced by diode laser irradiation was observed without almost no change in tissue temperature in isolated rat spinal nerves [14], [15]. He-Ne laser acting on membrane resting potential to increase hyperpolarization has been reported in pyramidal cells of a rat hippocampal slice preparation, suggesting direct effects on potassium channels [16].

From thing above, there are a lot of numbers of these reports. However, causal relation of change in cells and nerves that laser irradiation gives is not well known. In addition, some laser parameters threshold of changing cells and neurons are also not well known. Our purpose of this paper is searching for laser energy threshold of giving damage to nerve cells. We use to a frog sciatic nerve and it give semiconductor pulse laser irradiation.

2. Material and Methods

All experiments were performed under appropriate conditions in accordance with the Declaration of Helsinki and the Bioethical Standards of Animal Experiments in KIT.

2.1. Tissue Preparation

This study used 20 bullfrogs weighing 200-300 g. Frogs were anesthetized and bilateral sciatic nerves were removed from the border of the spinal cord to the knee portion in accordance with conventional methods for practical neurophysiology. Extracted nerve preparations were set on electrodes in the experimental chamber and stimulated supramaximal intensity pulse.

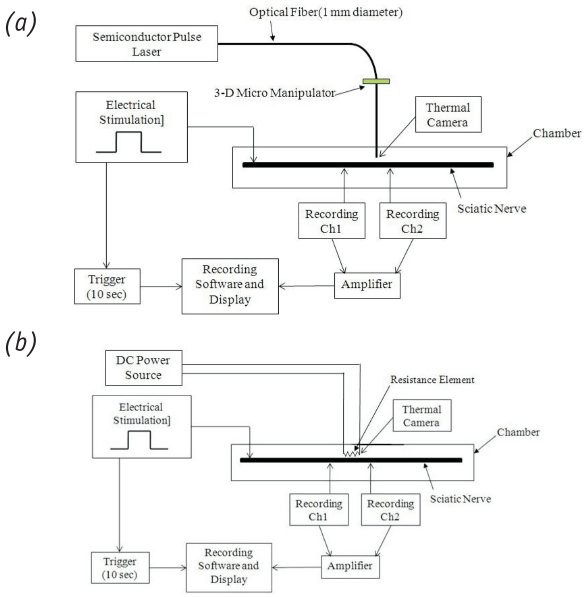

Fig.1. Experimental Equipment.

2.2. Experimental Equipment

Fig.1 shows the experimental equipment. We have two experimental equipments. (a) is experiment of laser irradiation. (b) is experiment of heating. The chamber has several electrodes for stimulating and recording. The electrodes are made of silver and the diameter is 0.65 mm. The stimulation pulse width and the pulse interval are 1 msec and 10 sec, respectively. The thermal camera (NEC Avio, H2640) is set in a direction perpendicular to laser direction and records the temperature of the sciatic nerve at the position of laser irradiation. The laser light was focused to the portion between recording Ch 1 to Ch 2. The recording software pClamp (Axon Instruments) was used. The frog sciatic nerve has several types of nerve fiber. We recorded the action potential of the myelinated nerve and the unmyelinated nerve in order to check the difference in laser irradiation effects between myelinated and unmyelinated nerves. A semiconductor pulse laser's wavelength, pulse width and frequency were 808 nm, 990 µsec and 50 Hz, respectively. The energy of the laser is variable between 13.9-202.5 mJ/cm^2. The laser irradiator was connected to an optical fiber whose diameter is 1 mm. The head of the optical fiber was touched the sciatic nerve.

2.3. Experimental Procedure

Our experimental sequence consists of two types; the decreasing type and the non-decreasing type. In the decreasing type, the laser irradiation was started and the action potential began to decrease. The decreasing was meant that the laser blocked the conduction of the polar excitation. The laser irradiation was employed until when the amplitude of the action potential decreases to 10 % of original. The time, which the action potential decreases to 10 % of original, was defined dysfunction time. In the non-decreasing type, the action potential was not decreased even after when the laser irradiation was continued for 10 minutes. Then the laser irradiation is stopped and the action potential was recorded for 10 minutes. The action potential of the sciatic nerve is observed at 1 day after in order to confirm irreversibility.

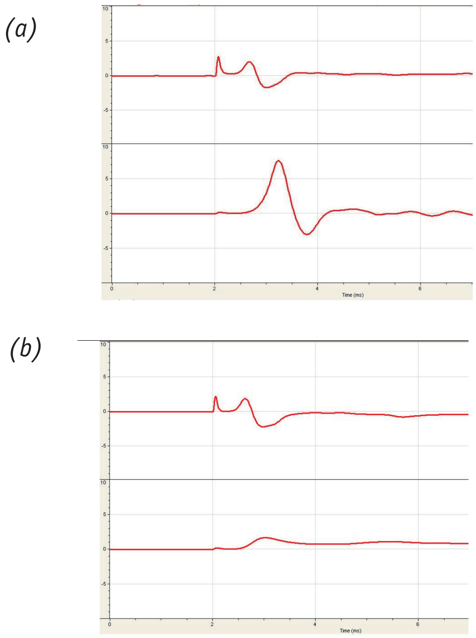

Fig. 2. Example of the action potential.

3. Results

Fig. 2 shows a recording example. The upper is a recording ch1 and the bottom is a recording ch2. (a) shows action potentials of before laser irradiation and (b) shows action potentials of after laser irradiation. Ch2's action potential is decreased after irradiation. Fig. 3 is a temporal response of ch2's peak of action potential. The laser energy of this experiment is 100 mJ/cm^2.

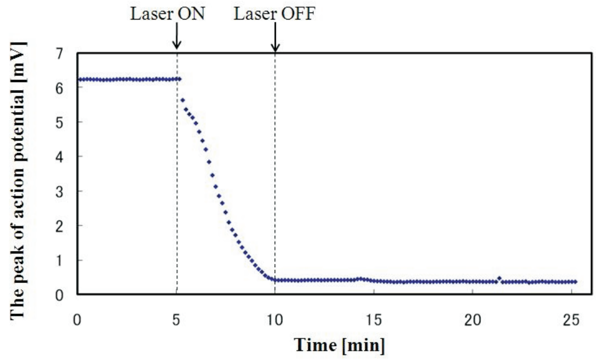

Fig. 3 . The temporal response of action potential.

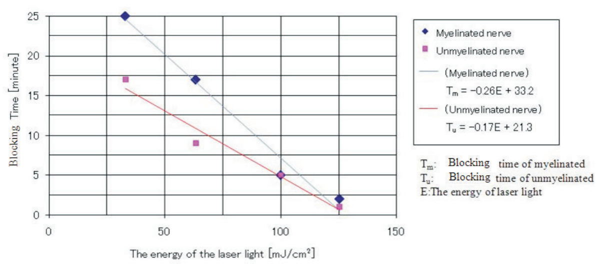

Fig. 4. The relationship between laser energy to blocking time.

Fig. 4 shows the relativity between the energy of the laser light and dysfunction time of the sciatic nerve. When the laser energy was lower than 32.9 mJ/cm^2, the peak of action potential was not decreased and the temperature of the sciatic nerve was low (nearby human average temperature). When the energy of laser light was higher than 32.9 mJ/cm^2, the peak of action potential was decreased gradually by laser irradiation with depending on the duration of irradiation. The dysfunction time was proportional to laser peak power. The dysfunction time of the unmyelinated nerve was shorter than that of myelinated nerve. At a day after the experiment, the action potential was not recovered. The energy of laser light was 137.9 mJ/cm^2 when the temperature wasincreased to nearby 100 degree C.

This result shows that the laser irradiation causes plastic dysfunction to polar excitations. In order to compare the effectiveness of the optical energy and the high temperature for nerve dysfunction, the action potential of the sciatic nerve was recorded in different temperatures. During recording, the sciatic nerve was heated by a resistance element. When the temperature of the sciatic nerve reached to 44 deg. C, the action potential began to decrease. The peak of action potential was almost disappeared after continuous heating for 10 min. These results suggest that the heat charged in the nerve cell is more effective for nerve dysfunction than the optical energy.

4. Conclusion

We observed that the frog sciatic nerve was damaged by laser irradiation. The results show a possibility that the epileptogenic focus can be crippled by laser irradiation. However, we guess the conclusive factor to damage nerve cells is high temperature. We going to determine more effective laser parameters (the laser energy, the wavelength, the irradiation time) for heating brain cells and focusing only affected region. In the future, we decide best method of crippling the nerve cells and the method is expanded using rat brain and applied human surgery.

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References

[1] Kanemoto K., *Epileptology Handbook; 2nd Ed.* (in Japaneses), Igaku-Shoin Ltd. , Japan, 2006.

[2] Kasai S., Yamamoto Y., Kotani H., Ando N., Kamiya K., Kono T., "The basis of the Low Power Laser Irradiation for Wound Healing", *The Journal of Japan Society for Laser Medicine*, vol. 18, no. 1, 1997, pp. 27-34.

[3] Mezawa S., "The Effect of Low-Power Laser Irradiation on Analgesia and New Bone Formation", *The Journal of Japan Society for Laser Medicine*, vol. 17, no. 3, 1996, pp. 43-50.

[4] Matsuda Y., Niwa M., Iwai H., Kogure S., Honjoe N., Komatsu M., Ishii Y., Watanabe K., "Effects of Argon Laser Irradiation on Polar Excitations in Frog Sciatic Nerve", *Lasers in Surgery and Medicine*, vol. 38, 2006, pp. 608-614.

[5] Whitters C.J., Hall A., Creanor S.L., Moseley H., Gilmour W.H., Strang R., Saunders W.P., Orchardson R., "A clinical study of the pulsed Nd:YAG laser-induced analgesia", *Journal of Dent.*, vol. 23, 1995, pp. 145-150.

[6] Orchardson R., Peacock J.M., Whitters C.J., "Effects of pulsed Nd:YAG laser radiation on action potential conduction in isolated mammalian spinal nerves", *Lasers Surg. Med.*, vol. 21, 1997, pp. 142-148.

[7] Orchardson R., Peacock J.M., Whitters C.J., "Effects of pulsed Nd:YAG laser radiation on action potential conduction in nerve fibers inside teeth in vitro", *Journal of Dent.*, vol. 26, 1998, pp. 421-426.

[8] Rochkind S., Nissan M., Alon M., Shamir M., Salame K., "Effects of laser irradiation on the spinal cord for the regeneration of crushed peripheral nerve in rats", *Lasers Surg. Med.*, vol. 28, no. 3, 2001, pp. 216-219.

[9] Rochkind S., "Central nervous system transplantation benefited by low-power laser irradiation", *Lasers Med. Sci.*, vol. 7, 1992, pp. 143-151.

[10] Anders J.J., Vorke R.C., Woolery S.K., Merwe W.P.V., "Low power laser irradiation alters the rate of regeneration of the rat facial nerve", *Lasers Surg. Med.*, vol. 13, no. 1, 1993, pp. 72-82.

[11] Midamba E.D., Haanaes R.H., "Low reactive-level 830 nm GaAlAs diode laser therapy (LLLT) successfully accelerates regeneration of peripheral nerves in human", *Laser Therapy*, vol. 5, 1993, pp. 125-129.

[12] Iijima K., Shimoyama N., Shimoyama M., Mizugundchi T., "Evaluation of analgesic effect of low-power He:Ne laser on postherpetic neuralgia using VAS and modified McGill pain questionnaire", *Journal of Clin. Laser Med. Sur.*, vol. 9, no. 2, 1991, pp. 121-126.

[13] Khullar S.M., Vrodin P., Barkvoll P., Haanaes H.R., "Preliminary study of low-level laser for treatment of longstanding sensory aberrations in the inferior alveolar nerve", *Journal of Oral Maxillofac Surg.*, vol. 54, no. 1, 1996, pp. 2-8.

[14] Tsuchiya K., Kawatani M., Takeshige C., Sato T., Matsumoto I., "Diode laser irradiation selectively diminishes slow component of axonal volleys to dorsal roots from the saphenous nerve in the rat", *Neurosci. Lett.*, vol. 161, 1993, pp. 65-68.

[15] Tsuchiya K., Kawatani M., Takeshige C., Sato T., Matsumoto I., "Laser irradiation abates neuronal responses to nociceptive stimulation of rat-paw skin", *Brain Res. Bull.*, vol. 34, no. 4, 1994, pp. 369-374.

[16] Kataoka Y., Oda-Mochizuki N., Tamura Y., Cui Y., Yamada H., "Activation of potassium channels is possibly involved in inhibition of neurotransmission of the central nervous system by low-level near-infrared laser irradiation", *Neurosci. Res.*, vol. 46, no. s.1, 2003, p. 143.