MECHANISMS OF OPTICAL STIMULATION ON RAT HIPPOCAMPAL NEURON: FROM WHOLE-CELL TO SINGLE-CHANNEL RECORDINGS

Jia Liu[1, 2], Xinyu Li[1], Lijia An [2], Changsen Sun[1*]

[1]Lab of Biomedical Optics, College of Physics and Optoelectronic Engineering, Dalian University of Technology, Dalian 116023, China.
[2]Department of Biological Science and Technology, Dalian University of Technology, Dalian 116023, China.
E-mail: suncs@dlut.edu.cn

Since the emergence of laser at 1960s, scientists began to search for remote methods to influence the normal electrical activity of biological membranes with various lasers. Novel optical methods of spatially and temporally precise on neural control are required by clinic. The primary achievement was that the low-intensity infrared pulsed light was employed to elicit action potentials (AP) in both the sciatic muscle cell of leopard frog[1, 2] and pyramidal neurons in rat [3], respectively. Wherein, the infrared lasers were employed as an effective means to fully excite the cells. The AP is shaped by the permeability increase of voltage-gated sodium (Na^+) channel protein, which is closed at the peak of the AP, and potassium (K^+) ion continues to leave the cell to decrease the membrane potential. The rapid influx of Na^+ ions determines the generation of AP on cell membrane and produces an inward electric Na^+ current. The outward K^+ current plays roles in repolarization phase [4]. The most exciting fact was that a pulsed-infrared diode laser with a wavelength at 1.875μm was employed to pace the embryonic heart [5]. The heart rate was increased from 0.634 Hz to 2 Hz when the stimulation laser pulses were started and decreased to 0.693 Hz by the end of the trace. The biophysical mechanism underlying these phenomena become interesting due to it connects to the photophysical process in laser therapy [6].

Patch clamp technique provided a tool for scientists exploring the function of excitable cell membrane at single-channel level. And our previously study calculated and detected the photothermal response of extracellular solution to the near-infrared laser irradiation using an open pipette method [7-9], and quantitatively described the changes of Na current in hippocampus neuron primary cells [9]. We found that there was a positive correlation between the acceleration of Na current kinetics and the temperature rises in the extracellular solution [9].

In this work, we continued the topic on the mechanism underlying the optical stimulation of cell membrane. When the cell membrane was irradiated by a single-mode laser at a wavelength 980nm and in power of 72mW, the Na+ current amplitude, steady state activation, inactivation, recovery and single-channel characteristics were changed based on the whole-cell and single-channel experimental observations. Then, the single-channel experiments were done to confirm the role of photothermal effect in the optical stimulation experiments. Our results showed that the major mechanism for laser-neuron interaction is a photothermal effect mediation process underlying the near infrared 980nm irradiation.

Tools and Methods

Spraque-Dawley rats were provided by the Animal Center, Dalian Medical University. All animal experiments were conducted at the Lab of Biomedical Optics, Dalian University of Technology, in accordance with the standards set by the Institutional Animal Care and Use Committee. Hippocampus were isolated on an ice block from brains rapidly excised 7-10 day-old Spraque-Dawley rats of both sexes which were decapitated under deep alcohol anesthesia. The neuron cells were processed by potassium channel blocker tetraethylammonium (TEA) to get rid of the effect of potassium current.

Electrophysiological recordings: data were acquired by the Patch Clamp EPC-10 amplifier hardware and PULSE software (HEKA, Germany) in whole-cell and single-channel recordings modes respectively. In whole-cell recording, neurons were bathed in solution. The membrane

potential was held at -70 mV, and filtered at 2 kHz and digitized at 2-3 kHz in the software interface. In single-channel recording mode, cells were tested by cell-attached mode and the holding potential was chosen at -110 mV.

Data acquisition and analysis: All the experiment data were acquired and analyzed by PULSE 8.78, Clampfit 10.0 (Axon Instruments), IgorPro 5.01 (Wavemetrics, Lake Oswego, OR), and Origin 7.5 (Microcal, Northampton, MA). Data are plotted as mean ± SE.

With the goal of developing a method to investigate the influence of optical irradiation to neurons, we designed a protocol which was showed in Fig.1(A) and composed of three segments as control, laser-on and laser-off, respectively. The control and laser-off segments were under the normal electrical stimulating condition. The laser irradiation segment represented that the laser irradiation was applied right after the control segment and turned off after lasting 500ms and in turn start an electrical recording, which named as "Laser-on" segment. The on or off of laser was triggered by the output of the EPC-10 amplifier and controlled by the PULSE software with an accuracy of 0.1ms.

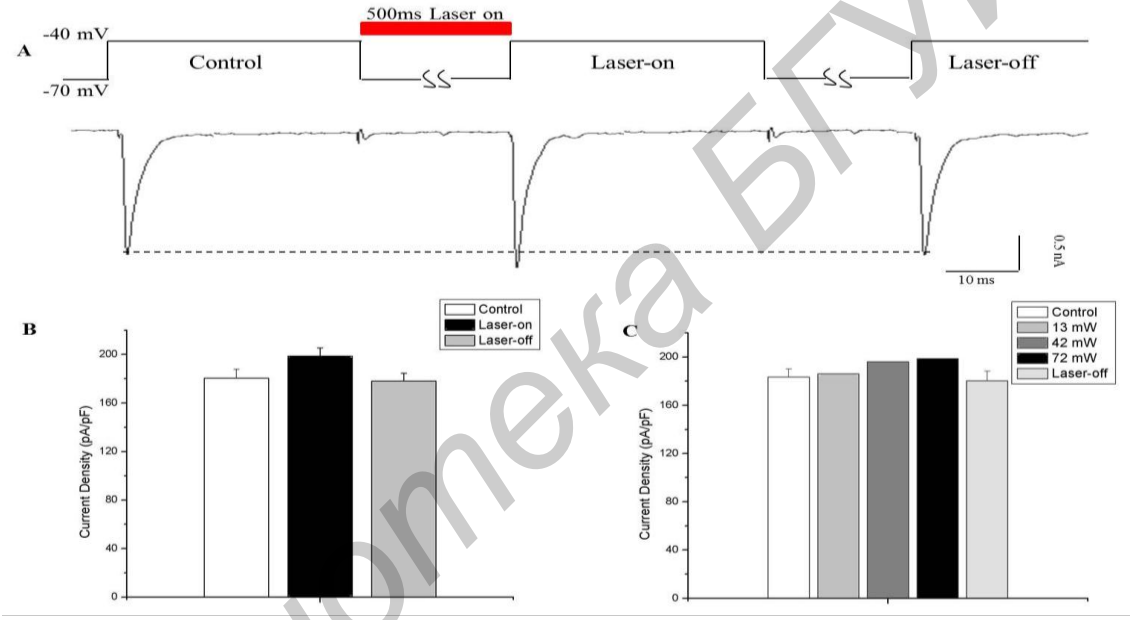

Figure 1 - The whole-cell sodium current of an acutely isolated rat hippocampal neurons (AIRHN). (A) The protocol design; (B) Comparison of mean amplitude of Na^+ currents of the control, laser-on and laser-off segments, respectively; (C) Comparison of mean amplitude of Na^+ currents affected by the laser power of 13, 42, 72mW, respectively.

Then we found a well-repeated phenomenon that an increases in the amplitude of whole-cell current after 500ms duration laser irradiation was presented. This increase was reversible after turned off the laser. The current density showed in the Fig. 2(B) was 180.63±7.05, 198.71 ±6.79, and 178.16±6.73pA/pF (n=8) corresponding to control, laser-on, and laser-off segments, respectively, underlying an infrared laser power of 72mW. We can found an obvious increase in the Na^+ current peak changes from 180.63±7.05 to 198.71 ±6.79. The comparison of mean amplitude of Na^+ currents affected by the different laser power of 13, 42, 72mW, respectively, was presented in Fig.2(C). And a nearly linear relationship was observed between the increases in Na^+ current peaks and the applied infrared laser powers.

In order to understand this enhancement at more precision level, we operated the EPC-10 in a single channel recording mode and a normal electrophysiological Na^+ current was showed in Fig. 2(A), which was elicited in a single AIRHN by depolarizing voltage steps from -70 to -40mV at room temperature. To examine the sensitivity of this response to the infrared laser irra-

diation, the protocol was designed as three equal 500ms segments along the 1500ms time span in one record. The first segment is without laser irradiation and the second one turns the laser on simultaneously. Then these two segments can be used to check out what's the difference happening in the second light-irradiated segment. During the third segment the laser was turned off to monitor the recovery property from the laser irradiating in the second segment. These experiments were performed on 8 neurons (n=8) which were also treated with potassium channel blocker tetraethylammonium (TEA) to get rid of the effect of potassium current. And an evident enhanced in their excitabilities when the infrared laser irradiation was applied. Fig. 2B was a record about single-channel activities of an AIRHN influenced by the laser irradiation.

Fig. 2(B) presented the Na^+ current, which was elicited underlying an infrared laser irradiated neuron *in vitro* at the wavelength of 980nm. A tiny bias current δ was observed during the laser irradiation.

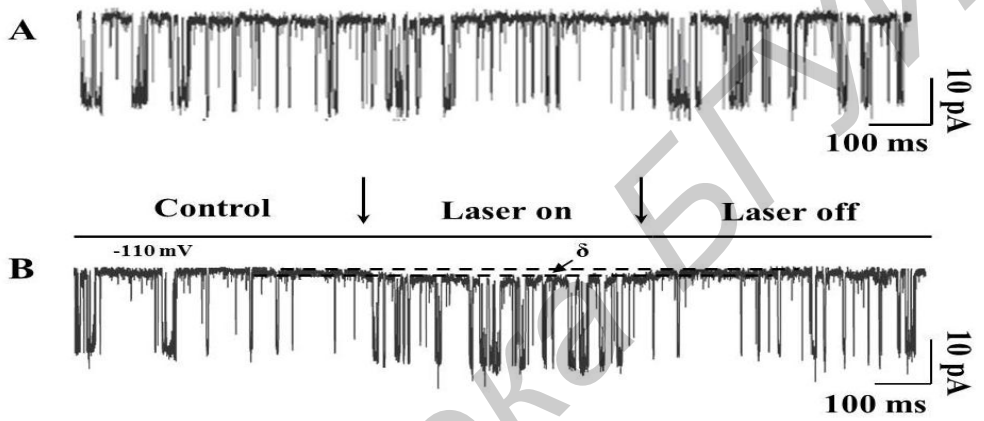

Figure 2 - Effect of laser irradiation on single Na^+ channel currents. (A) The normal electrophysiological currents of an AIRHN; (B) laser on group, single-channel currents treated by laser irradiation during the middle 500 ms (n=8), where δ means the magnitude of bias of baseline caused by laser irradiation.

Here we analyzed the influence of the infrared laser irradiation on neuron from four different aspects, including number of current transients (n), dwell time, open probability (P_0), and amplitude of single currents. The results were shown in Fig. 3(A), (B),(C) and (D), respectively. As the Fig. 3(A) shown, the number of current transients increased evidently during the laser applied. The dwell time increased by 0.87ms (about 53.4%) during laser irradiating and then decreased to a level that a little bit larger than (about 1.8%) its control state, as shown in Fig. 3(B). And P_0 in Fig. 3(C) was increased compared with the control when laser was applied, and then it returned when laser was turned off. At last, we examined the change of the amplitude of single-channel currents induced by laser, while in the laser-on group the amplitude was a little increased by the laser irradiation Fig. 3(D).

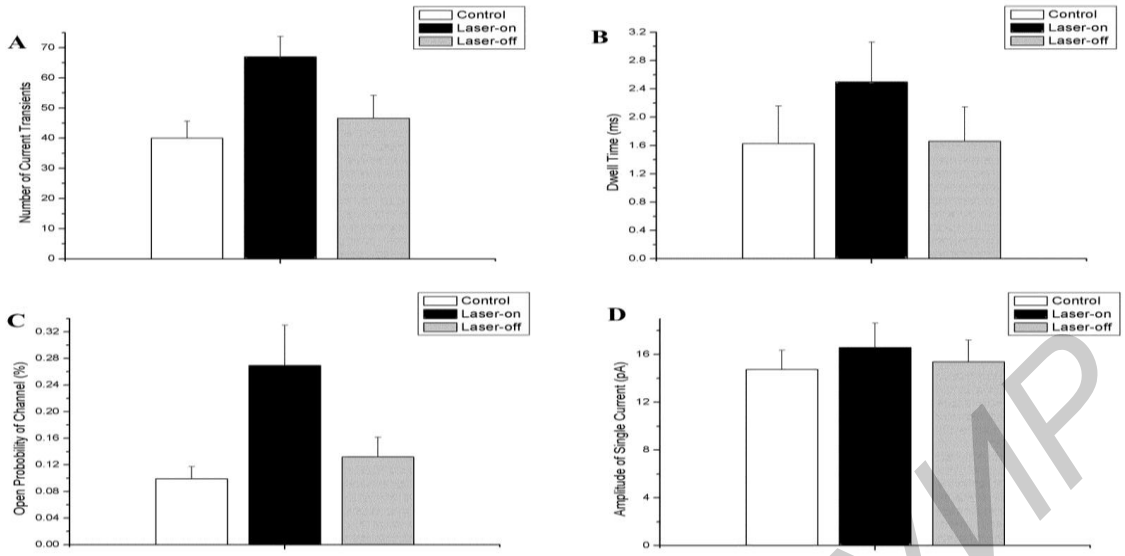

Figure 3 - Comparison of the mean value of different aspects of single channel property before, under, and after laser irradiation. (A) number of current transients; (B) dwell time of open channel; (C) open probability of channel; (D) amplitude of current transients (n=8).

The results presented that an enhancement on excitability of AIRHN stimulated by an infrared laser occurs. The laser-induced enhancing in excitability was observed from whole-cell level to single-channel level. Data analysis revealed that the infrared laser excited the AIRHN by advancing the opening and a little weakening the rate of closure of sodium channel and the recovery from its inactivation. The results demonstrated that the underlying mechanism of the laser-induced effect was closely related a photothermal transient effect that was expected to be verified by the more precision temperature experiment than the open pipette method.

References

1. **J. Wells**, C. Kao, K. Mariappan, J. Albea, , E. D. Jansen, , P. Konrad, and A. Mahadevan-Jansen, "Optical stimulation of neural tissue in vivo," Opt. Lett., 30(5), 504-506 (2005).

2. **J. Wells**, P. Konrad, C. Kao, E.D. Jansen, and A. Mahadevan-Jansen, "Pulsed laser versus electrical energy for peripheral nerve stimulation," J Neurosci Methods, 163(2), 326-337 (2007).

3. **J. Wells**, C. Kao, E.D. Jansen, P. Konrad, and A. Mahadevan-Jansen, "Application of infrared light for in vivo neural stimulation," J Biomed Opt, 10(6), 064003 (2005).

4. **B. Hille**, Ion channels of excitable membranes (Sinauer Sunderland, MA, 2001).

5. **M. W. Jenkins**, A. R. Duke, S. Gu, Y. Doughman, H. J. Chiel, H. Fujioka, M. Watanabe, E. D. Jansen and A. M. Rollins, Optical pacing of the embryonic heart, Nature photonics, 4, 623-626 (2010).

6. **Boulnois, J. L**. "Photophysical processes in recent medical laser developments: a review", Lasers in Medical Science, 1, 47–66 (1986).

7. **K. W. Guan**, X.Y. Li, J. Liu, and C.S. Sun, "Photothermal response of extracellular solution to the near-infrared laser irradiation determined by its optical absorption properties," Acta Phys.Sin, 62(5), 58702 (2013).

8. **S. S. Liang**, F. Yang, C. Zhou, Y. Wang, S. Li, C. K. Sun, J.L. Puglisi, D. Bers, C. S. Sun, and J. Zheng, "Temperature-dependent activation of neurons by continuous near-infrared laser,". Cell Biochem Biophys, 53(1), 33-42 (2009).

9. **Xinyu Li**, Jia Liu, Shanshan Liang, Kuiwen Guan, Lijia An, Xuefei Wu, Shao Li, and Changsen Sun, "Temporal modulation of sodium current kinetics in neuron cells by near-infrared laser," Cell Biochem Biophys, 67(3), 1409-1419 (2013).