In vivo Photonic Stimulation of Sciatic Nerve with a 1470 nm Laser

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Abstract

Photonic stimulation is a new modality of nerve stimulation, which could overcome some of the electrical stimulation limitations. In this paper, we present the results of photonic stimulation of rodent sciatic nerve with a 1470 nm laser. Muscle activation was observed with radiant exposure of 0.084 J/cm².

Key Words: photonic stimulation, nerve stimulation, rodent sciatic nerve, laser

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Today, the large majority of neurostimulators use electrical energy to activate the nervous system. However, in some applications, electrical stimulation has major drawbacks and limitations.[1,2] Stimulating electrodes must be large enough to avoid nerve damaging densities while spatial resolution is limited by the spread of the stimulating current: the stimulus activates neighboring fibers in addition to the targeted fibers, causing undesired effects such as muscle twitch or pain. Besides, large stimulation artifacts often preclude the measurement of the evoked action potentials. Photonic stimulation, i.e. stimulation of neural membranes by light energy without genetic modification of the cells or dye addition, can overcome the limitations of electrical stimulation. First, photonic stimulation allows to concentrate the stimulus power on a smaller spot, thereby increasing the spatial resolution. This opens the path to new applications. Second, since stimulation and recording are performed in different domains (respectively optical and electrical domains), no stimulation artifact will spoil the recordings. This allows for recording the evoked action potentials at short distance from the stimulated point, enabling the real-time feedback necessary to ensure proper operation of the stimulating device. Wells et al.[3] show that stimulations at wavelengths corresponding to a low tissue absorption (Tissue absorption is approximated by water absorption.), i.e. a large penetration depth, require large radiant exposures. On the contrary, stimulations at wavelengths corresponding to a high tissue absorption (i.e. a small penetration depth) require smaller radiant exposures. However, at high tissue absorption wavelengths, the stimulation threshold and the damage threshold (radiant exposure above which tissue damage starts to be observed) are very close to each other. Stimulation at such wavelengths does not allow for a safe and practical margin. Because of these findings, we chose to explore a wavelength of 1470 nm, corresponding to water absorption similar to the two wavelengths identified by Wells et al.[3] (1850 nm and 2100 nm). The range between 1450 nm and 1550 nm has already been investigated in literature.[4,5] Following McCaughey et al.,[4] Fig. 1 describes water absorption between 1200 nm and 2400. Key wavelengths used in the photonic stimulation domain are marked.

Materials and Methods

Animal care

Sciatic nerve stimulations were performed *in vivo* on sciatic nerves of mice (~ 20 g, nerve diameter around 0.8 mm) and Sprague-Dawley rats (~ 300 g, nerve diameter around 1.5 mm). All experiments were conducted at the CMMI (Center for Microscopy and Molecular Imaging, Gosselies, Belgium), an animal care facility accredited by the Animal Care Service of the Federal Public Department. This work fully complies with a protocol approved by the CEBEA (ethics committee of the Université libre de Bruxelles), in agreement with the ETS 123 European Convention.
Mice are anesthetized by a 200 µl intraperitoneal injection of ketamine (10 µl of Rompun (xylazine) sol. 2% + 20 µl of Nimatek (ketamine) 100 mg/ml + 170 µl of physiologic serum). Injections for rats are 2 ml, with the same composition, and preceded by isoflurane anesthesia to ease animal handling. To maintain sedation during the whole experiment, a second

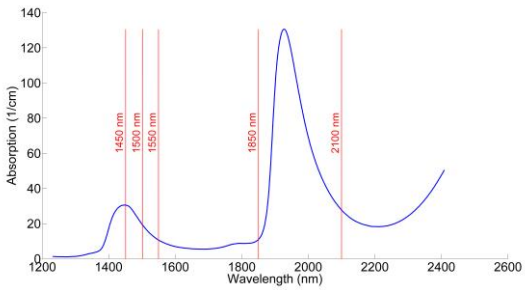

Fig 1. *Water absorption between 1200 nm and 2400 nm. The marked wavelengths are those often used in the literature to stimulate neuronal membranes (sensory or motor neurons or axons). Pertinent authors are listed here:* [4,5,9] *between 1455 nm and 1550 nm,* [10-15] *between 1844 nm and 1875 nm (laser diodes from Aculight) and* [16-18] *at 2120 nm (Ho:YAG laser). Based on McCaughey et al.*[4]

injection was sometimes necessary (50 µl for mice and 1 ml for rats) and performed with the first signs of awakening.

Surgery and the whole experiment are performed on top of a heating plate to avoid hypothermia. The animal is placed in ventral position. Hairs are shaved over the whole leg. An incision is made along the thigh. Tissues and muscles above the sciatic nerve are carefully split, avoiding muscle cut, and held aside by medical clamps in order to expose the nerve. The wound is regularly moistened with Hartmann liquid (perfusion solution from Baxter, Lessines, Belgium). At the end of the experiment, the animal is euthanized either by cervical dislocation (mice) or in a CO_2 gas chamber (rats).

Room and wound temperatures are regularly checked. Room temperature varies between 21 and 23°C. The wound temperature is maintained between 28 and 32°C.

Electrical stimulation

A custom-made laboratory neurostimulator (NDP_V1) is used to electrically stimulate the nerve in order to verify its viability (see Fig. 2 for the whole experimental set-up). This neurostimulator is controlled through a user interface implemented in Matlab. Two silver hook electrodes (self-made from 0.5 mm wires) are placed on the nerve surface, separated by a distance of 5 mm.

Optical set-up

The optical source is a neoV1470 from neoLaser (Caesarea, Israel), originally developed for endovascular applications. This laser is continuous-wave, with an operating wavelength of 1470 nm and can reach up to 10 W output power. Its user interface allows for setting the

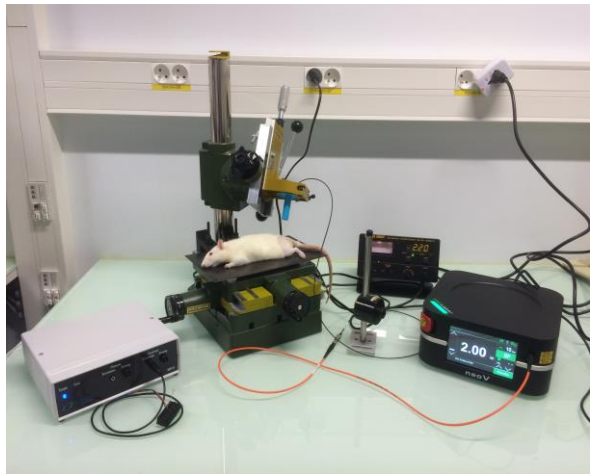

Fig 2. *Photonic stimulation set-up - From left to right: the NDP_V1 with two stimulating electrodes, the milling support with a rat on the heating plate and the cannula pointed towards its leg, the power meter and the neoV laser.*

power, the pulse duration and the interval duration between two pulses. All optical components were acquired at Thorlabs (Dortmund, Germany). The output fiber of the laser is an SMA- SMA patch cord M25L01, connected via a hybrid mating sleeve ADAFCSMA1 to an SMA-FC/PC patch cord M83L01. This later is a cable designed for optogenetic applications. It is connected via an ADAL2 connector to a CFML12L10 cannula. The ADAL2 connector is fixed to a 3D printed piece designed in-house to be held in place by a milling support (ProxxonMicromot BFB 2000 Mill/Drill Unit). On this support, an XY compound table (ProxxonMicromat KT 150) with the heating plate fixed on it allows for fine positioning of the animal with respect to the cannula. A power meter, MellesGriot 13PEM001, is used to quantify the actual laser power delivered at the cannula output, given the losses at each connector. As the power meter is limited to 2 W, higher values were extrapolated after assessing the linearity of the output power. The total power transmission is around 35%.

Electrical recording

Effectiveness of the stimulation is ensured either by observation of the muscle movements or by recording electromyography (EMG) signals from needle electrodes inserted subcutaneously, one in each leg. The NDP_V1 system is also equipped with a fully floating recording circuitry allowing to capture the muscle action potentials.

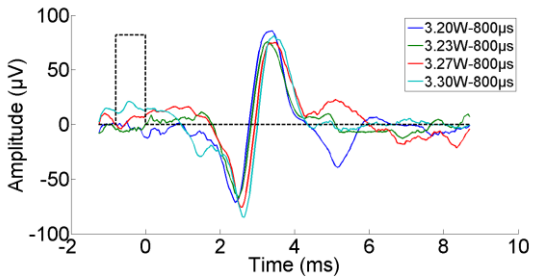

Fig 3. *Measurements of muscle action potentials triggered by photonic stimulation in a mouse. The black dotted line represents the photonic pulse duration of 800 µs. The signals are smoothed by a moving average filter (Matlab function 'smooth').*

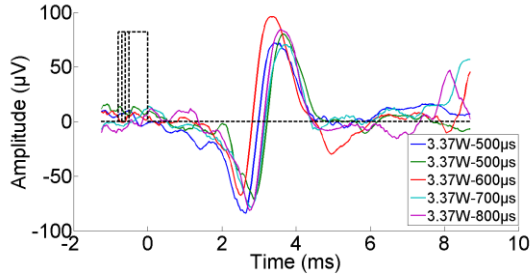

Fig 4. *Measurements of muscle action potentials triggered by photonic stimulation in a mouse. The black dotted lines represent the photonic pulse duration, which varies from 500 µs to 800 µs. The signals are smoothed by a moving average filter (Matlab function 'smooth').*

Results

We were able to stimulate both mouse and rat sciatic nerves with photonic pulses of power values comprised between 0.8 and 3.37 W. Leg movements were observed with pulse durations as short as 300 µs for a power of 2.2 W. For the visual detection of muscle movements, a sound emitted by the laser while firing prevented blind observation. However, videos were taken during the experiments and watched in mute mode afterwards. In addition to visual observation, muscle action potentials were recorded. Typical results are shown in Fig. 3 and Fig. 4. These figures show very repetitive results. These preliminary experiments were needed to appreciate the range of stimuli required to obtain a response. Unfortunately, in the range of interest, our apparatus did not provide an adequate stimulation resolution for recording motor recruitment curves. In Fig. 4, the last three recordings (600, 700 and 800 µs) seem to show a second response after a delay of 8 ms. A priori, this delay is too long for an H-reflex, which would be expected to occur around 2 ms or at most 3 ms as found in the literature.[6] If estimating the distance between the stimulation site and the spinal cord to be 2 cm and the conduction velocity in the sensitive fibers (group I) to be 60 m/s while the conduction velocity in the motor fibers would be 40 m/s and the synaptic delay at the spinal cord to 1 ms, the total calculated delay for H-reflex amounts to 1.83 ms. However, some authors record latencies of 8 ms in mice.[7] Additional experiments taking into account temperature, precise distance measurements and progressive recruitment need to be conducted to investigate this possible second response. Visually, photonic stimulation seemed to activate less muscle fibers than electrical stimulation because of the smaller muscle response. Depending on the stimulation site, a single toe could be activated selectively by the photonic stimulus. This could not have been obtained with electrical stimulation, even by decreasing the stimulus amplitude to the threshold. Some experiments of repetitive stimulation were conducted by sending laser pulses at a frequency around 0.5 Hz. This frequency is only an approximation because the stimuli were individually triggered using a foot-pedal. Each pulse of the repetitive stimulation did not always yield one muscle twitch, especially for long duration pulses (i.e. above 30 ms). The longest photonic stimulus duration for which two consecutive pulses both yielded an individual muscle twitch was 30 ms. For pulses shorter than 1 ms, each stimulus was always followed by a single response, up to our test limit of 30 consecutive stimuli. It is difficult to estimate the stimulating radiant exposure because the spot size is not known precisely. The infrared beam is coupled with an aiming beam but this visible spot size is larger than the infrared spot size (i.e. the numerical aperture of a fiber is larger for visible wavelengths). Moreover, the spot size varied from one trial to another because the distance between the fiber output and the nerve varied. However, we can approximate our spot size by a circle 1 mm in diameter. A pulse duration of 300 µs with a power of 2.2 W on a spot size of 0.79 mm^2 corresponds to a radiant exposure of 0.084 J/cm^2.

Discussion

Through the experiment described in this paper, we show that mouse and rat sciatic nerves can be stimulated with a 1470 nm laser designed for endovascular applications. The whole experimental set-up is affordable and easy to reproduce. The chosen wavelength, 1470 nm, is within a wavelength range used in telecommunication applications and, therefore, benefits from optical technology easily available at a reasonable price. The NDP_V1 can be replaced by another commercially available data acquisition system (such as the Biopac MP150 system). The chosen animal models, mainly mice, have several advantages, including their price, availability and handling ease. We are not aware of any previous similar *in vivo*

experiments in mice. Rats, gerbils and rabbits seem to be the only mammals used in *in vivo* photonic stimulation.

Our results can be compared to the results of McCaughey et al.[4] Amongst the four sources used in their article, the most comparable to ours is the Yb:glass laser (1495 nm). With an output power of 2.3 W, a pulse duration of 2 ms and a spot diameter of 0.4 mm, the stimulating radiant exposure is around 3.7 J/cm^2. At such a radiant exposure, the EMG signal amplitudes recorded were around 600 µV. Our radiant exposure is approximately 40 times smaller. Their EMG signals are 3 times larger than ours but EMGs cannot be compared because of different electrode positions. Their spot size is smaller than ours but the main difference between both sets of parameters is the pulse duration, which is way shorter in our case. We believe that short pulse durations may be the key towards success for photonic stimulation in research and clinical applications. With an experimental set-up as the one described in this paper, which allows a large range of parameters to be explored (except for the wavelength), it should be possible to gain deeper insight into the mechanisms behind photonic stimulation. So far, some hypotheses are put forward in the literature. Shapiro et al.[6] show evidence for a reversible alteration of the cell membrane capacitance by rapid water heating. Beier et al.[7] propose cell membrane nanoporation as a possible mechanism. Albert et al.[8] identify membrane heat sensitive channels that could be activated by infrared irradiation. It is important to note that all these authors work with wavelengths around 1850 nm. The mechanism behind stimulation at a wavelength of 1470 nm could differ. Moreover, several mechanisms could combine their effect, depending on the wavelength, on pulse duration or on power intensity. Once the mechanism is understood, the next step would be to establish safe limits for long term repetitive stimulation. This is essential for future clinical and research implementation of photonic stimulation. Harmlessness of light in the wavelength range 1450 nm - 1550 nm on living tissue has still to be proven. The results presented here are the main outcomes of a preliminary work. A systematic analysis of stimulation parameters (power, pulse duration, wavelength and spot size) will follow, in order to better understand the mechanism underlying photonic stimulation and identify an optimal set of parameters for safe and repetitive stimulation.

Contributions

Marie Dautrebande and Pascal Doguet designed and performed the experiments and anaylysed data. Marie Dautrebande wrote the manuscript. The other coauthors made a critical revision of the manuscript. Pascal Doguet and Yohan Botquin helped in the experiments and in designing the electrical set-up. Simon-Pierre Gorza gave his expertise for the optical set-up. Jean Delbeke gave his expertise for the intepretation and analysis of data. Pascal Doguet and Antoine Nonclercq supervised the work.

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Conflict of Interest

The author declare no potential conflict of interests.

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