

Experimental Setup for the Systematic Investigation of Infrared Neural Stimulation (INS)

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Abstract

Infrared neural stimulation (INS) gains growing interest both in electrophysiological research and for potential clinical applications, promising advantages like contactless operation, superior focality and hence spatial selectivity, and lack of electrical stimulation artifacts for nerve stimulation. We established an experimental setup for systematic investigation of relevant INS parameters, since little quantitative research for deeper understanding has been performed yet. Our customized setup facilitates the use of multiple fiber-based infrared laser systems of different wavelengths for remote stimulation, multi-site low-noise EMG recording and automated laser beam characterization. Hence, this setup simplifies upcoming systematic studies on both technical and physiological conditions for laser-induced neural activation. Determination of safe margins for reliable stimulation will help to understand the underlying physiological mechanisms and establish INS as alternative method for neural activation. Here, we present our experimental setup and preliminary results of our ongoing work.

Keywords

Infrared neural stimulation • Peripheral nerve Laser stimulation • Rat sciatic nerve • CMAP EMG

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1 Introduction

Infrared neural stimulation (INS) was introduced in 2005 by Wells in order to investigate alternative methods for neural activation [1, 2]. It found with work by Izzo [3] and Teudt [4] its path into auditory respectively facial nerve stimulation, yet little was published for stimulation of the central nervous system. Three different theories compete to elucidate the modus operandi of INS as there are: transient photothermal gradients [1], change of cell membrane capacitance due to heating [5] or nanoporation meaning short-time opening of the cell membrane [6] leading to depolarization of neurons. However, to the best of our knowledge no final conclusion was presented and the existing publications are hard to independently reproduce due to a lack of technical details reported. The underlying bio-physiological mechanisms remain unclear. We therefore reached out to re-evaluate infrared nerve stimulation with our experimental approach, which is not only to qualitatively reproduce INS, but also to enable novel explanation based on quantitative observations in the animal model. In contrast to literature, we try to maximize the working distance between laser fiber and illuminated tissue to maintain the touch-free properties of laser stimulation with regard to potential clinical application. Here, we depict our experimental approach to explore the promising field of INS.

2 Methods

2.1 Infrared Laser Stimulation

Laser devices for INS stimulation are commonly being operated in pulsed rather than continuous mode [1, 2, 7–9]. Illuminating a target with a pulsed laser, the *radiant exposure* H_p caused by one pulse is defined as

$$H_p = \frac{E_p}{A} = \frac{4 E_p}{\pi d^2} \quad \text{and} \quad [H] = \frac{J}{m^2}, \quad (1)$$

where E_p is the radiant energy of the pulse and A is the effective illuminated surface area assuming a circular laser spot of diameter d , commonly referred to as *spot size*. The *pulse energy* E_p depends on both average laser power and the *pulsewidth* τ_p . Although reported laser pulses for INS stimulation in the current literature are mainly described by means of the radiant exposure H , however, this does not account for the exposure time at all [1, 2, 7–9]. Considering the radiant energy E_p of a single pulse being delivered to a target within a spot of area A and a discrete exposure time $\tau = \tau_p$, we describe the radiant load using the *irradiance* I , which is given by

$$I = \frac{E}{\tau A} = \frac{4}{\pi} \frac{E_p}{\tau_p d^2} \quad \text{and} \quad [I] = \frac{J}{s \cdot m^2} = \frac{W}{m^2}. \quad (2)$$

For a pulse train of duration τ_{tr} containing a fast burst of n identical pulses, the radiant exposure and irradiance have to be modified to be H_{tr} and I_{tr} respectively:

$$H_{tr} = \frac{n \cdot E_p}{A} \quad \text{resp.} \quad I_{tr} = \frac{n \cdot E_p}{\tau_{tr} A}. \quad (3)$$

For the application of laser radiation into the rat sciatic nerve, we use an infrared diode laser system (1470 nm, 12 W, DILAS Diodenlaser GmbH, Mainz, Germany), coupled into a multi-mode fiber of 200 μm core diameter and a numerical aperture $NA = 0.22$. The laser fiber is connected to a customized focusing optics, composed of a matched set of five spherical lenses mounted on a vertical C-mount rail (Fig. 1a). Shifting of the last lens allows the variation of the laser spot size diameter down to $d = 0.9$ mm while maintaining minimum 15 mm depth of field and a fixed focus position of about 50 mm below the rail mount. The optical assembly supports the use of any other fiber-coupled laser system for future studies with different (infrared) wavelengths.

2.2 Laser Characterization

To determine the radiant energy E_p of single laser pulses and fast pulse trains of short (< 2 ms) duration, we use a pyro-electric energy meter (ES145C, Thorlabs, Newton, New Jersey, US). For beam profiling, we employ the commonly known knife-edge method [10]: a razor blade is moved orthogonally into the continuous beam by a motorized micromanipulator (Luigs und Neumann, Ratingen, Germany), while measuring the downstream radiant power (S310C, Thorlabs) with regard to the blade position (Fig. 1a). Beam diameter $d(z)$ as a function of the position z along the

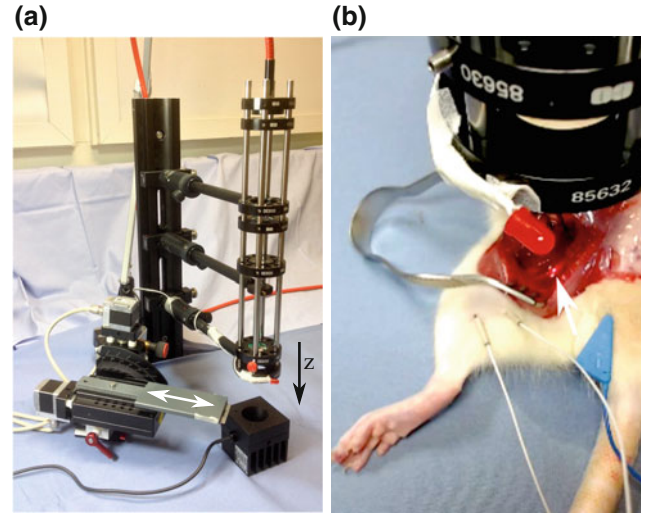


Fig. 1 Experimental setup. **a** Vertically mounted focusing optics for INS studies. Automated beam characterization (knife-edge) with micromanipulator and power meter. **b** Rat sciatic nerve under stimulation with laser pulses (white arrow). EMG is recorded by means of intramuscular needle electrodes (Color figure online)

optical axis can be extracted, the beam profile characterized. We use a laser beam profiler (LBP-1-usb, Newport Corporation, Irvine, California, US) to rapidly determine the beam diameter or *spot size* during experiments.

2.3 Parametric Laser Control

The ability of our setup to cover a wide range of defined stimulation conditions like *pulse energy* E_p , *exposure time* τ and the irradiated area A (*spot size*), holds great potential for detailed research of INS. To this end, we use a set of parameters for pulse train definition, which are depicted in Fig. 2. Assuming a fixed average laser power \bar{P} , the pulse energy E_p scales linearly with the *pulsewidth* τ_p . Considering n identical pulses as shown in Fig. 2, the period between two pulses is set by the Interval time τ_i . Hence, the pulse train is defined and can be iterated specified by the *Repeat* time and number of *Runs* to execute.

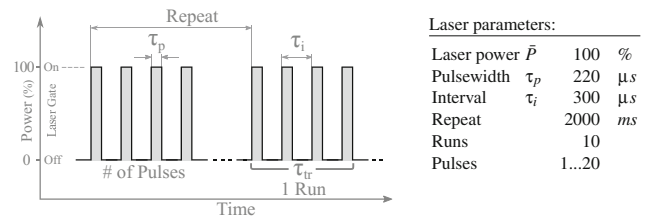


Fig. 2 Laser parameters for INS stimulation: Definition and experimental settings

The free choice of those parameters allows arbitrary laser stimulation sequences and ensures maximum versatility for upcoming studies. With regard to Eq. (3), differently composed pulse trains that result in identical irradiance I_{lr} , however, may still feature different internal patterns, which might be physiologically relevant when optically activating nerves.

The inherent constraints of the laser's proprietary control software require a more sophisticated approach for external laser control to facilitate advanced flexibility. Hence, a customized LabVIEW user interface operates the entire experiment. All parameters and commands are passed to an Arduino Nano microcontroller and electronic circuit (Fig. 3). On the Arduino, a local program interprets the parameters to render pulse trains and—after a firing command—accordingly generates pin output voltages, which are then fed into the circuitry and drive the laser gate. On the left side in Fig. 3, a PWM output voltage is stabilized using a combination of two integrating capacitors and an operational amplifier to provide a steady analog voltage (0...10 V) for setting the power value \bar{P} as a fraction of full laser power (12 W). Analog inputs constantly monitor the laser status for safety and prevent unintended firing. On the right side, the laser's internal gate voltage is controlled by means of a digital output controlling a set of two serial fast switching transistors (Darlington) to supply a gate voltage above 18 V. Further, a trigger signal envelopes each pulse train according to Fig. 2 for data synchronization.

2.4 Data Acquisition

Laser-induced nerve activation by means of INS can result in visible muscle activity like twitching [11]. The elicited neural signal travels along the nerve leading to muscle

contraction, termed compound muscle action potential (CMAP). Depending on the strength of the neural signal, limb or muscle movement can be weak and therefore remain unobserved. For the reliable detection of any emerging muscle activity, we record the electromyogram (EMG) by means of intramuscular needle electrodes attached to a commercial EMG recording system (ISIS, inomed Medizintechnik GmbH, Emmendingen, Germany). For each experiment, all laser parameters are saved into a text file.

2.5 Animal Preparation and Electrode Placement

For preliminary studies of INS on the rat sciatic nerve in vivo, we used female Sprague-Dawley rats ($n = 11$, 240–350 g). All animal experiments conducted in this study were performed with approval from the locally responsible Animal Welfare Committee with the Regierungspraesidium Freiburg in accordance with the guidelines of the European Union Directive 2010/63/UE (permit G17/80). Rats were initially anesthetized with Isofluran prior to intraperitoneal injection of a mixture of ketamine (100 mg/kg) with xylazine (8 mg/kg) and accordingly readministered depending on the depth of anesthesia. The skin was shaved and removed over both thighs. The sciatic nerves were then exposed by incision of the muscular fascia and mostly blunt dissection of *m. gluteus superficialis* and *m. biceps femoris*. For INS, the rat was placed under the laser setup and the wound was held open using a surgical retractor and moistened frequently with saline solution. A reference electrode was placed close to the tail and a pair of EMG electrodes was inserted into *m. biceps femoris* for preliminary studies. Proper nerve function was verified

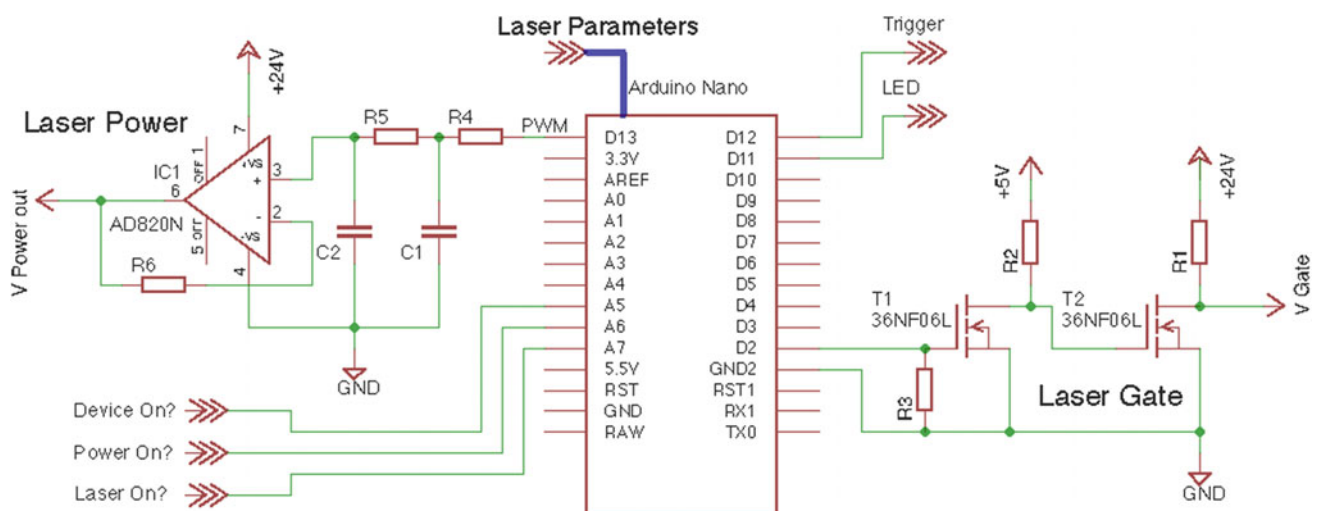


Fig. 3 Schematics of the customized circuit for laser operation with Arduino Nano controlling laser power and gate voltages according to laser parameters

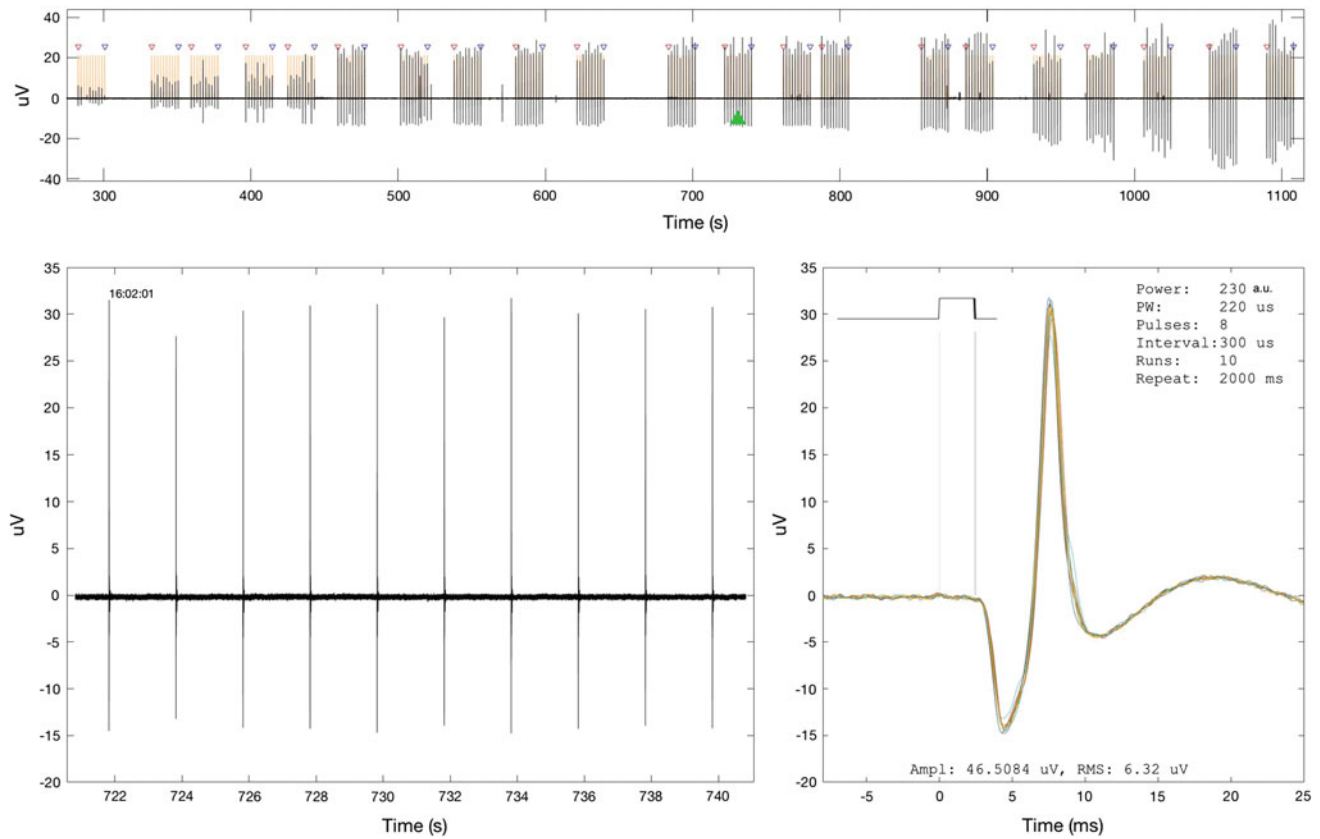


Fig. 4 Preliminary results. Top: long-term EMG recording under INS with increasing number of stimulation pulses. Left: Detailed extract (top green arrow, 8 pulses) of distinct response to laser stimulation with parameters according to Fig. 2. Right: Overlay of the 10 laser-evoked

CMAPs with regard to the stimulation onset (trigger). CMAPs were highly consistent and do not show stimulation artifacts (Color figure online)

using an electrical nerve stimulation device (Neurostimulator, inomed Medizintechnik GmbH, Emmendingen, Germany). After experiment termination, both sciatic nerves were extracted for histological analysis.

2.6 Experimental Procedure

For proof of concept, the laser was focused at various locations on the main trunk of the sciatic nerve, up to about 15 mm proximal to the first branching point (Fig. 1b). Data recording was started and the laser was fired according to the pulse parameters (see Fig. 2). Overall radiant exposure per pulse train H_{lr} was then successively increased with the number of pulses n .

3 Results

In all of $n = 11$ preliminary experiments, we were able to evoke muscle activity from the rat sciatic nerve. However, successful stimulation occurred primarily at specific

locations susceptible for INS and is therefore considered to be highly location-dependent. At locations that did not reveal a clear response to INS at low to medium radiation levels, the increase of radiant exposure for stimulation partially led to visible thermal damage. Tissue damage by INS will therefore be addressed in upcoming dosimetry histo-pathological studies.

In Fig. 4, an exemplary data set is shown. The top graph depicts a recorded EMG under INS. The laser trigger signals are shown in yellow. The bottom left graph shows one selected pulse train with laser-evoked CMAP signals, in the right graph an overlay plot is depicted. In general, the majority of the elicited CMAP responses rated “successful” occurred in a synchronous, rhythmical manner according to the laser stimulus, within 3–20 ms after stimulus onset ($t = 0$). Also, muscle twitching was observed at higher CMAP amplitudes. Apart from few outliers, the shape of the evoked responses was highly consistent. No stimulation artifact was observed through all experiments.

When considering the approximation for the train time $\tau_{lr} \approx n \cdot \tau_i$, we note that the irradiance I_{lr} remains constant with fixed single pulse energy which was determined to be

$E_p = 1.9 - 2$ mJ. In upcoming studies, different stimulation patterns, and therefore alteration of the irradiance, will be performed by variation of laser power \bar{P}_i , exposure times τ (τ_p, τ_i) and different spot sizes.

4 Conclusion

In this paper, we presented our novel experimental setup, that allowed us to consistently reproduce infrared nerve stimulation in all preliminary experiments on the rat sciatic nerve. In the future we will continue our research on different optical stimulation parameters and wavelengths, exposure thresholds and limits for reliable and safe stimulation, and bio-physiological aspects of INS.

Conflict of Interests The authors declare that they have no conflict of interests. This work was funded by the German Federal Ministry of Education and Research (BMBF), project *NeuroPhos*, FKZ 13GW0155C.

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