Heat accumulation during infrared stimulation impacts the response of *ex vivo* rat sciatic nerve*

Louis VANDE PERRE, Javier CHÁVEZ CERDA, Maxime VERSTRAETEN, Romain RAFFOUL, Jean DELBEKE, Riëm EL TAHRY, Antoine NONCLERCQ, *Senior Member, IEEE*, and Simon-Pierre GORZA

***Abstract* — Infrared neural stimulation (INS) is a neuromodulation technique that involves short optical pulses delivered to the neural tissue, resulting in the initiation of action potentials. In this work, we studied the compound neural action potentials (CNAP) generated by INS in five *ex vivo* sciatic nerves. A 1470 nm laser emitting a sequence of 0.4 ms light pulses with a peak power of 10 W was used. A single 4 mJ stimulus is not capable of eliciting a nerve response. However, repetition of the optical stimuli resulted in the induction of CNAPs. Heat accumulation induced by repetition rates as high as 10 Hz may be involved in the increase in CNAP amplitude. This sensitization effect may help to reduce the pulse energy required to evoke CNAP. In addition, these results highlight the importance of investigating the role of the slow nerve temperature dynamics in INS.**

I. Introduction

Neurostimulation modulates the nervous system's activity to treat wide varieties of pathologies, such as deafness, chronic pain, neurodegenerative diseases and epilepsy [1]. Among the various techniques for neural activation, electrical stimulation has been historically and remains the most common method in use. However, electrical neuromodulation suffers from shortcomings. Stimulation artifacts pollute the electrophysiological nerve recordings, the stimulation lacks spatial precision due to electrical current spread in the body, the neurostimulators are not compatible with Magnetic Resonance Imaging and the order in which the nerve fibers are activated is not physiological [2]. The use of light to stimulate nerve could solve most of these limitations [3].

Infrared neural stimulation (INS) consists in nerve activation resulting from a transient infrared light exposition. Compared to optogenetic techniques, this method does not require any prior genetic modification of the targeted tissue [4]. In the last decade, theoretical developments and *in vitro* experiments have provided a better insight of the mechanism of INS, although its precise mode of action is not yet fully understood [5], [6]. At the same time, in vivo experiments have provided feasibility data [7]–[9]. Overall, researchers have gradually accepted the role of a temperature gradient in the underlying nerve activation mechanism, but the effect of a slow gradient is not well established. INS has been achieved with pulse durations in the range of microseconds [10], milliseconds [11], and seconds [8]. Moreover, the accumulation of heat caused by a sequence of light pulses at a repetition rate higher than 3 Hz could result in nerve responses of greater amplitude [12], [13]. However, former studies did not report an impact of the stimulation rate on the recorded compound action potential amplitude [7], [14].

The present work aims to evaluate heat accumulation as a function of the repetition rate and its effect on the amplitude of compound neural action potential (CNAP). The combination of thermal imaging with electrophysiological recording of a single nerve will help to understand what specific spatiotemporal temperature gradient best triggers CNAPs. The latter information could help both to find a more efficient therapeutic application of INS and to understand the underlying mechanisms.

II. Material and Methods

Fig. 1 is a schematic representation of the experimental setup developed to record CNAP in an excised rat sciatic nerve. The setup is composed of an acquisition chain to record the electrophysiological activity of the nerve, an electrical stimulator, and an optical stimulator. These are contained in an isothermal box equipped with a temperature regulation system. A thermal camera captures the sciatic nerve sample surface to monitor the spatiotemporal temperature gradients induced by INS. The experimental setup used for the experiments is extensively detailed in [15].

A. Electrical Recording and Stimulation

A custom-made acquisition chain has been developed to record the electrical activity of the nerve with higher modularity and higher sensitivity than commercial systems. One pair of electrodes provides bipolar recording of the nerve's electrophysiological activity (see Fig. 1). A reference electrode is placed between the recording and the stimulating electrodes to minimize the amplitude of the stimulation artifact. Electrodes are made of 99.99% silver wire with a diameter of 250 µm (Sigma Aldrich, Belgium). The

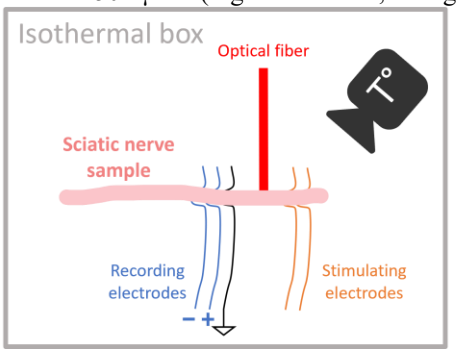

Figure 1. Schematic view of the experimental setup.

*Research supported by the Fonds Emile Defay. L.VP acknowledges the support of the Fonds pour la formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA, FNRS, Belgium).

L. Vande Perre, J. Chàvez Cerda, M. Verstraeten, R. Raffoul, and A. Nonclercq are with the Bio-Electro- and Mechanical-Systems (*BEAMS)*, Université libre de Bruxelles, Brussels, Belgium. (Corresponding author e-mails: louis.vande.perre@ulb.be, antoine.nonclercq@ulb.be).

J. Delbeke and R. El Tahry are with Institute Of NeuroScience (*IONS*), Université catholique de Louvain, Brussels, Belgium.

S.-P. Gorza is with OPERA-photonics, Université libre de Bruxelles, Brussels, Belgium. (e-mail: simon.pierre.gorza@ulb.be).

acquisition chain is composed of a preamplifier (gain: 40 dB), an amplifier (gain: 28 dB), an isolation stage (gain: -10 dB), and a filtering stage (High-pass of first-order), with a 10–8000 Hz bandwidth. The overall resolution of this channel is 0.20 µV with a dynamic range of ±3.46 mV, both referred to the input. The measured root mean square noise is 0.43 µV, also referred to the input. The amplified output is digitized at a 40 kHz sampling frequency by a DAQ (USB-6212, National Instrument Corp., USA) and processed using MATLAB (MathWorks, Natick, USA). This recording setup is described in more details and has been used in [13], [15]–[17].

The DAQ is also interfacing with the current-controlled stimulator. The stimulator generates biphasic square current pulses (negative pulse of 0.2 ms, then an interphase delay of 10 µs, and finally a recovery pulse of 0.2 ms with opposite amplitude) ranging from 10 to 500 µA. The same electrical stimulator has been used in [13], [15].

B. Infrared Laser and Thermal Camera.

The infrared laser used to optically stimulate the nerve is a NeoV 1470 nm from NeoLaser (Caesara, Israel). It is a compact diode laser able to send trains of light pulses at variable peak power (1-10 W) and variable stimulation rate (0-50 Hz). During the experiment, the pulse duration can be changed from 100 µs to 10 ms to adjust the energy delivered to the nerve. A multimode fiber (numerical aperture 0.22, core diameter 400 µm, NeoLaser) gently touching the surface of the nerve delivers the light to the sample (see Fig. 1).

The thermal camera is a GOBI-640+ camera (Xenics, Leuven, Belgium) with a maximum framerate of 62 fps.

C. Surgical Procedure

The experiments were conducted on rat sciatic nerves, excised from male Wistar rats according to the procedure detailed in [13], [15] and in agreement with the ETS 123 European Convention.

D. Ex vivo *Experimental Protocol*

After nerve excision, the sample was placed on the electrodes. To prevent nerve drying, it was wetted every 5 minutes with oxygenated (3 L/min) Krebs Ringer solution (Fisher Scientific, Merelbeke, Belgium). Electrical stimulations allowed to verify the ability of the *ex vivo* sample to produce a compound action potential.

For the optical stimulation, a pulse train, composed of pulses of 10 W peak power and 0.4 ms duration (4 mJ energy) at different stimulation rates was delivered to the nerve. Electrical stimulation was repeated between each optical stimulation to verify the integrity of the nerve and the absence of functional damage induced by the optical stimulation.

In our previous experiments, a sensitization effect was observed at a stimulation rate of 10 Hz [13], [15]. In this study, lower (5 Hz, 7.7 Hz) and higher (12.5 Hz, 20 Hz) stimulation rates were used to investigate the effect of repetition rate on sensitization and to determine how heat accumulation affects the CNAP elicitation. First, the 10 Hz stimulation was performed to verify the nerve responsiveness. Then a lower stimulation rate was tested, followed by a higher stimulation rate trial if the sample was still viable. The latter trial helps to confirm that the nerve remained responsive to optical stimulation even when the lower stimulation rate was not successful.

From previous experiments with this setup [15], the expected latency of the CNAP is 1 ms after the laser firing and it lasts about 3 ms. In that regard, the presence of a CNAP was based on the signal RMS value in the 1 ms to 4 ms window compared to the same measurement in a background window (taken from 22 ms to 25 ms). For each consecutive optical stimulation, the mean and the standard deviation of the two RMS signals were computed. A CNAP was considered

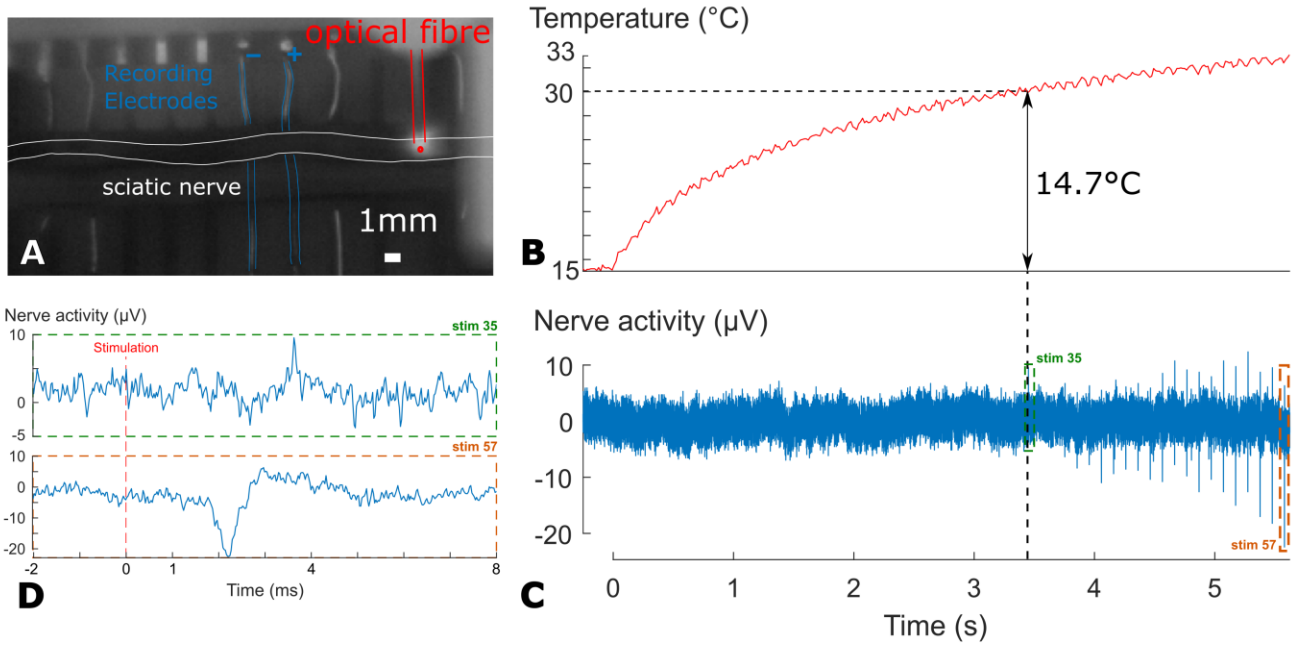

Figure 2. Infrared neural stimulation performed on an *ex vivo* sciatic nerve. Light pulse of 0.4 ms and 10 W peak power with a repetition rate of 10 Hz. (A) Grayscale image of the nerve obtained with the IR thermal camera. Brighter pixels are hotter. (B) Nerve temperature at the center of the laser spot highlighted in (A) along the time. (C) Electrophysiological recording of the nerve. (D) Zoom on the 35th and the 57th (last) compound neural action potentials evoked by the optical pulse train (see green and orange dashed boxes in C). The stimulation starts at t=0.

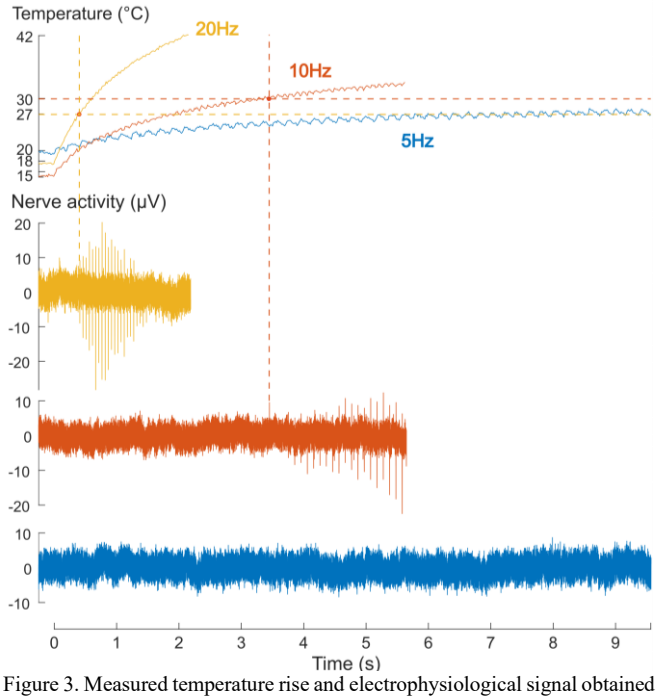

Figure 3. Measured temperature rise and electrophysiological signal obtained for a sequence of about 50 pulses with different stimulation rates (5, 10 and 20 Hz). Dashed lines highlight the onset of the CNAP elicitation associated to each successful stimulation rate.

present if its RMS value was higher than two times the standard deviation of the background window RMS.

III. Results

The recording of genuine electrophysiological activity induced by electrical and optical stimulations obtained with the described setup has been previously validated [13], [15]. In this work, experiments were performed in five animals.

A. Sensitization effect

During the 5th experiment, reported in Fig. 2 a pulse train of 57 light pulses at a stimulation rate of 10 Hz yielded a clear sensitization effect. The resulting heat accumulation is shown in Fig. 2.B. The rapid and large temperature spikes induced by each light pulse are smoothed out because of the limited temporal resolution of the thermal camera, yet the small modulations still allow to identify them. Fig. 2.C is a general view of the electrophysiological recording, where CNAPs are regularly triggered at the stimulation rate. Fig. 2.D provides a closer view of the 8 ms window following the 35th optical stimulation. This corresponds to the first CNAP detected and hence to the onset of the visible effects of the sensitization on the electrophysiological signal. The increase of the CNAP amplitude is clearly visible in Fig. 2.C and detailed in Fig. 2.D with CNAPs triggered by the 35th and by the 57th (last) optical stimulation pulse. As shown in Fig. 2, the amplitude of the nerve response increases with the stimulus order within the pulse train according to the sensitization effect. For this experiment, the onset of the responses is observed after reaching a local temperature build-up of 14.7°C, and so a temperature of 30°C at the center of the optical spot.

B. Impact of the Stimulation Rate

Fig. 3 shows the temperature at the center of the optical spot over time and the associated electrophysiological signal.

TABLE I. SUMMARY OF THE CNAP ONSETS OBSERVED IN THE EXPERIMENTS. DASHED CELLS INDICATE AN ABSENCE OF CNAP.

Exp.	Stim. rate (Hz)	Baseline temp. (°C)	Temp. CNAP onset (°C)	ΔT - build-up (°C)
	2	-	-	-
1	10	20.0	32.0	12.0
2	10	20.6	35.4	14.7
	7.7	-	-	-
3	10	19.0	32.0	13.0
	12.5	20.9	32.1	11.2
	5	-	-	-
4	10	20.6	32.8	12.2
	20	19.9	29.0	9.0
	5	-	-	-
5	10	15.2	30.0	14.7
	20	17.5	26.9	9.4

Optical pulse trains at three different stimulation rates are compared. Dashed lines highlight the onset of the CNAP triggering for both 10 Hz and 20 Hz and the corresponding temperatures. A difference can be seen between the absolute and the build-up temperatures at which the response becomes detectable. For the 10 Hz repetition rate, as mentioned above, the CNAP appears at the 35th stimulation, at a local temperature of 30.0°C and a build-up of 14.7°C compared to the baseline temperature. For the 20 Hz repetition rate, the CNAP appears at the 9th stimulation, at a temperature of 26.9°C and a build-up of 9.4°C. Despite reaching a temperature above 27°C, none of the stimulation from the 5 Hz pulse train elicits a CNAP.

The key numbers collected for each experiment (animal) are reported in Table I. The main observation is the decrease of the temperature onset with the increase of the stimulation rate. In the experiments where a 20 Hz stimulation rate successfully elicits CNAPs, the temperature at the CNAP detection onset and the temperature build-up required for nerve activation are lower.

Considering only the 10 Hz stimulation trials, the average temperature of the CNAP onset is 32.4 ± 2.0°C and the average build-up temperature is 13.3 ± 1.3°C. The consistency of the temperature build-up required before CNAP elicitation can be noticed. Indeed, the first three experiments with a higher baseline temperature have a higher temperature CNAP onset. Still, the ΔT is similar to the build-up of experiment 5, where the baseline temperature was lower.

Note that because of the varying lifetime of the *ex vivo* sample, every stimulation rates could not be tested in each experiment.

IV. Discussion

CNAPs with varying amplitude were recorded at different stimulation rates. This confirms the effect of repetition rate on the amplitude of the response to INS, despite the lack of consensus in the literature [7], [14]. In our experiments, increasing the stimulation rate lowers the stimulation threshold. A repetition rate as high as 10 Hz was required to elicit CNAP with 4 mJ light pulses. The heat accumulation induced by repeated optical stimulations is likely the cause of the amplitude variation of CNAPs. However, it is difficult to determine whether a temperature accumulation or a

temperature threshold is responsible for this sensitization effect. Both values for temperature and temperature build-up found at the onset of CNAPs appear to be roughly reproducible, and the variations between experiments are small compared to the temperature change that affects biological processes. For example, the Q_{10} factor, which represents the effect of a 10°C increase on biological processes, is in the range of 2.4-4 for ion channels opening rate [18]. Based on these observations, the cause of the sensitization effect remains unclear, as does its possible mechanism of action. An increase in the nerve temperature could sensitize the thermosensitive ion channel. Alternatively, a locally deeper penetration of heat could activate more fibers, resulting in a larger CNAP amplitude. In our view, the sensitization effect is an important parameter that needs to be better understood for future INS applications.

The main disadvantage of a high stimulation rate is the potential damage caused by heat accumulation. At 20 Hz repetition rate, a decrease in CNAP amplitude was observed after 17 light pulses. This may be due to critical damage induced in nerve fibers responding to INS. However, the CNAP evoked by electrical stimulation after the optical stimulation had the same amplitude as prior to the INS. If the same fibers are involved, this would exclude functional damage. Whether the nerves recover after some time or not, should still be investigated. Nevertheless, a previous study recommends avoiding heat accumulation during INS to prevent nerve damage [9].
If the formation of nanopores is considered as the mechanism of INS [6], then specific damages leading to ion leaks might be present. In the case of a stimulus triggered by classical ion channels, there is also an entry of Na^+ ions and an exit of K^+ ions. However, a whole membrane machinery is immediately activated to restore the ions balance. In the case of microlesion of the membrane, even reversible ones, this machinery could take longer to return to a pre-stimulus state and the rapid repetition of stimuli could lead to a decrease in CNAP amplitude.

Although our study is conducted at the macro level and the results are preliminary, some fundamental questions can be raised. A temporal temperature gradient is often considered necessary to induce neural activity [5]. However, the results presented here showed the effect of a slower temperature gradient caused by a repetition rate higher than about 8 Hz. We cannot exclude that an absolute temperature of the nerve contributes to the CNAP, while a fast and localized temperature gradient may be required to trigger it. Hypothetically, this would involve thermosensitive ion channels, which have been shown to be more sensitive to temperature increases [19]. Further experiments will be performed to verify if the sensitization effect and the resulting lowered stimulation threshold can be reproduced by increasing the baseline temperature of the whole nerve. If not, the importance of local heat accumulation would argue in favor of mechanisms based on spatial and temporal temperature gradients, such as the optocapacitance effect [5] or membrane nanoporation [6].

ACKNOWLEDGMENT

We are grateful for the help provided by Geoffrey Vanbienne in the realization of our hardware setup.

REFERENCES

[1] E. S. Krames, P. Hunter Peckham, A. R. Rezai, and F. Aboelsaad, *What Is Neuromodulation?*, Volume 1., vol. 1. Elsevier Ltd, 2009.

[2] J. Wells *et al.*, "Frontiers in optical stimulation of neural tissues: past, present, and future," *Opt. Interact. with Tissue Cells XIX*, vol. 6854, no. 2008, p. 68540B, 2008, doi: 10.1117/12.776988.

[3] 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*, vol. 163, no. 2, pp. 326–337, 2007, doi: 10.1016/j.jneumeth.2007.03.016.

[4] R. T. Richardson, M. R. Ibbotson, A. C. Thompson, A. K. Wise, and J. B. Fallon, "Optical stimulation of neural tissue," *Healthc. Technol. Lett.*, vol. 7, no. 3, pp. 58–65, 2020, doi: 10.1049/htl.2019.0114.

[5] M. Plaksin, E. Shapira, E. Kimmel, and S. Shoham, "Thermal Transients Excite Neurons through Universal Intramembrane Mechanoelectrical Effects," *Phys. Rev. X*, vol. 8, no. 1, p. 11043, 2018, doi: 10.1103/PhysRevX.8.011043.

[6] H. T. Beier, G. P. Tolstykh, J. D. Musick, R. J. Thomas, and B. L. Ibey, "Plasma membrane nanoporation as a possible mechanism behind infrared excitation of cells," *J. Neural Eng.*, vol. 11, no. 6, 2014, doi: 10.1088/1741-2560/11/6/066006.

[7] V. Goyal, S. M. Rajguru, A. I. Matic, S. R. Stock, and C. P. Richter, "Acute Damage Threshold for Infrared Neural Stimulation of the Cochlea: Functional and Histological Evaluation," *Anat. Rec.*, vol. 295, no. 11, pp. 1987–1999, 2012, doi: 10.1002/ar.22583.

[8] O. Yetis, O. Guner, I. Akkaya, E. Guneli, A. Bagriyanik, and S. Tozburun, "Vagus nerve bundle stimulation using 1505-nm laser irradiation in an in-vivo rat model," *J. Biophotonics*, 2021, doi: 10.1002/jbio.202100197.

[9] J. Wells *et al.*, "Biophysical mechanisms of transient optical stimulation of peripheral nerve," *Biophys. J.*, vol. 93, no. 7, pp. 2567–2580, 2007, doi: 10.1529/biophysj.107.104786.

[10] A. D. Izzo *et al.*, "Laser stimulation of auditory neurons: Effect of shorter pulse duration and penetration depth," *Biophys. J.*, vol. 94, no. 8, pp. 3159–3166, 2008, doi: 10.1529/biophysj.107.117150.

[11] R. G. McCaughey, C. Chlebicki, and B. J. F. Wong, "Novel wavelengths for laser nerve stimulation," *Lasers Surg. Med.*, vol. 42, no. 1, pp. 69–75, 2010, doi: 10.1002/lsm.20856.

[12] M. J. Alemzadeh-Ansari, M. A. Ansari, M. Zakeri, and M. Haghjoo, "Influence of radiant exposure and repetition rate in infrared neural stimulation with near-infrared lasers," *Lasers Med. Sci.*, vol. 34, no. 8, pp. 1555–1566, 2019, doi: 10.1007/s10103-019-02741-4.

[13] J. Cury *et al.*, "Infrared neurostimulation in ex-vivo rat sciatic nerve using 1470 nm wavelength," *J. Neural Eng.*, vol. 18, no. 5, p. 056018, 2021, doi: 10.1088/1741-2552/abf28f.

[14] A. D. Izzo *et al.*, "Laser stimulation of auditory neurons at high repetition rate," *Opt. Interact. with Tissue Cells XVIII*, vol. 6435, p. 64350R, 2007, doi: 10.1117/12.700846.

[15] L. Vande Perre *et al.*, "A Setup for Conduction Velocities and Temperature Gradients Measurements during Infrared Neurostimulation," in *2022 IEEE Biomedical Circuits and Systems Conference (BioCAS)*, 2022, pp. 453–457, doi: 10.1109/BioCAS54905.2022.9948671.

[16] H. Smets *et al.*, "Analysing vagus nerve spontaneous activity using finite element modelling," *J. Neural Eng.*, vol. 18, no. 5, p. 56008, 2021, doi: 10.1088/1741-2552/abe68f.

[17] J. Cury *et al.*, "Optical Birefringence changes in myelinated and unmyelinated nerves: a comparative study," *J. Biophotonics*, vol. 15, p. e202200028, 2022, doi: 10.1002/jbio.202200028.

[18] D. C. Sterratt, "Q10: The Effect of Temperature on Ion Channel Kinetics," *Encyclopedia of Computational Neuroscience*. 2014, doi: 10.1007/978-1-4614-7320-6_236-1.

[19] J. A. Lamas, L. Rueda-Ruzafa, and S. Herrera-Pérez, "Ion Channels and Thermosensitivity: TRP, TREK, or Both?," *Int. J. Mol. Sci.*, vol. 20, no. 10, 2019, doi: 10.3390/ijms20102371.