# Fast optical signals in the peripheral nervous system

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**Abstract.** We present a study of the near-infrared optical response to electrical stimulation of peripheral nerves. The sural nerve of six healthy subjects between the ages of 22 and 41 was stimulated with transcutaneous electrical pulses in a region located approximately 10 cm above the ankle. A two-wavelength (690 and 830 nm) tissue spectrometer was used to probe the same sural nerve below the ankle. We measured optical changes that peaked 60 to 160 ms after the electrical stimulus. On the basis of the strong wavelength dependence of these fast optical signals, we argue that their origin is mostly from absorption rather than scattering. From these absorption changes, we obtain oxy- and deoxy-hemoglobin concentration changes that describe a rapid hemodynamic response to electrical nerve activation. In five out of six subjects, this hemodynamic response is an increase in total (oxy+deoxy) hemoglobin concentration, consistent with a fast vasodilation. Our findings support the hypothesis that the peripheral nervous system undergoes neurovascular coupling, even though more data is needed to prove such hypothesis. *© 2006 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.2234319]

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## 1 Introduction

Biological information processing and communication are executed by the nervous system, a biocomputational system whose electrochemical operations are dependent on and tightly coupled to a supply of metabolic energy. Neural activity can be both stimulated and observed on a millisecond time scale utilizing electrical measurements and these activities can be correlated with coupled changes in the hemodynamic delivery of glucose and oxygen to local neural tissues through the blood vessels. This crucial coordinated and coupled neural and vascular relationship is called neurovascular coupling. If a patterned stimulus is presented to the central nervous system, two kinds of evoked responses are generated. The first appears within a millisecond time scale (5 to 500 ms) and is an electrical response that can be measured in the electroencephalogram. A second evoked response appears within a time frame of several seconds in which an increase in cerebral blood flow to the region of active neural tissue can be measured by several perfusion methods including direct observation of the delivery of fluorescent dyes,[1] measurement of the blood oxygen level-dependent (BOLD) signal in functional magnetic resonance imaging,[2] and measurement of hemoglobin signals in near-infrared spectrophotometry.[3] The application of optical techniques has attracted particular attention to the measurement of neurovascular coupling because of the potential for sensing changes in neural tissue on both time scales.

The interaction between light and biological tissue is characterized by the phenomena of scattering and absorption. Scattering occurs when photons encounter gradients of the refractive index that are present at a microscopic scale due to cellular and subcellular components. Absorption occurs when light-absorbing chromophores such as hemoglobin, cytochromes, and water are in the optical path of the photons. Optical methods are sensitive to interactions with biological tissues at varying temporal and spatial scales and thus can image both structural and physiological changes. Optical scattering has been correlated with membrane potential in invertebrate neuron cultures where changes in the optical signal are linearly proportional to both the resting membrane potential and to the action potential.[4,5] It has also been suggested that in the near-infrared spectral range (650 to 900 nm), optical signals measured from the exposed hippocampus during invasive animal experiments reflect both fast scattering and slower absorption changes.[6] Although there is an open debate about the biophysical mechanism of the fast signals detected in these experiments, the measured optical signals are usually divided into a fast signal representing events associated with the neural activation itself, and a slow signal representing the hemodynamic changes of neurovascular coupling. Consensus exists on the character of the slower or hemodynamic-associated optical signal from near-infrared spectroscopy (NIRS) experiments. However questions remain about the feasibility of measuring fast optical signals through the intact head of humans and animals (for a review see Steinbrink et al.[7]). There is not a general agreement about the robustness and repeatability of these experiments nor what mechanism might relate optical signals to electrophysiological activation. From a neurophysiological standpoint, a limitation of these studies is that virtually all studies using optical imaging of the nervous sys-

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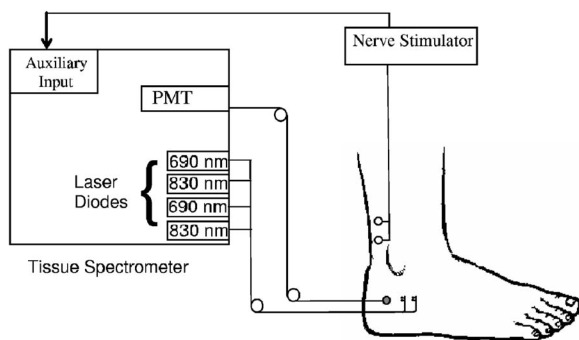


**Fig. 1** Experimental setup for the two-wavelength (690 and 830 nm) study of the optical response to electrical stimulation in the sural nerve. PMT: photomultiplier tube.

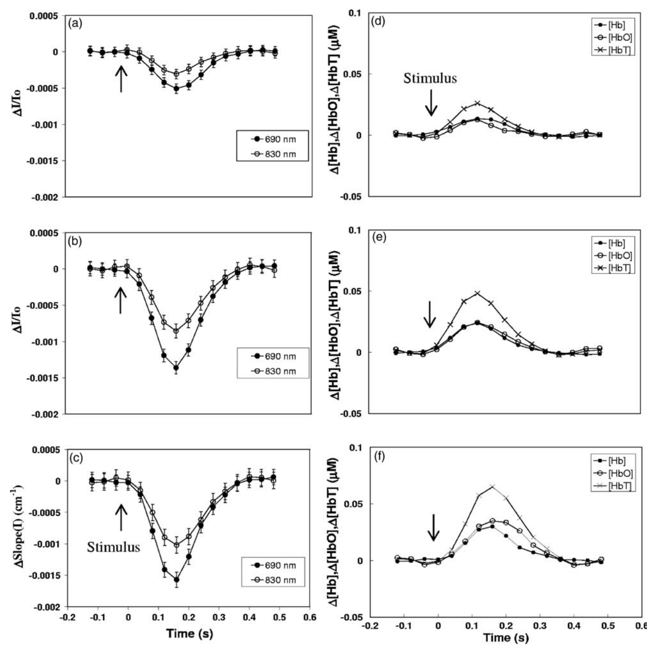


**Fig. 2** Time traces of the relative intensity change ($\Delta I/I_0$) at 690 and 830 nm for a source-detector distance of 1.0 cm (a) and 1.5 cm (b). (c) Time trace of the change in the intensity slope [$\Delta$Slope($I$)]. (d), (e), and (f) Changes in the concentration of oxy-hemoglobin, deoxy-hemoglobin, and total hemoglobin obtained from the intensity changes reported in (a), (b), and (c), respectively. The arrow in each panel indicates the time of the 1-ms electrical stimulation pulse.

tem have focused on the central nervous system. No reliable optical measurements of stimulus response with optical imaging in the peripheral nervous system (PNS) have been reported, though a single report[8] has suggested that fast scattering signals might be demonstrated in the PNS.

The PNS is an extensive neural network of motor and sensory nerves connecting the central nervous system to the body and the external environment. Diseases such as diabetes mellitus and normal aging affect the vascular integrity of the PNS causing significant morbidity and mortality especially when the contribution of aging of the PNS leading to falls and gait instability is taken into account. NIRS optical imaging of the PNS would be a novel method with significant clinical and research implications for exploring the stimulus-response behavior of the PNS to both direct neuronal activation and its hemodynamic response. We report a study on the PNS revealing the presence of an NIRS optical signal occurring on a millisecond time scale to which we assign the character of hemodynamic changes.

## 2 Experimental Setup and Protocol

The experimental protocol was approved by the Institutional Review Board of Tufts University. A Grass Model 10 ERS Evoked Response System (Grass Instrument Inc. Quincy, Mass.) was used to provide a 1-ms electrical stimulus at a frequency of 1.6 Hz with a range of intensity from about 10 to 40 mA. The skin over the sural nerve at the lateral ankle and its more proximal course were exposed in each subject (four males and two females, ages 22 to 41 years). The electrical stimulation electrodes were coupled to the skin with conducting gel over the proximal sural nerve, approximately 10 cm above the ankle. All subjects underwent neurological examination and a nerve conduction study, and they were all found to be within normal limits. The optical spectrometer (ISS, Inc., Champaign, Ill.) used for NIRS measurements featured two PMT-based detector channels and four fiber-coupled laser diodes, two emitting at 690 nm and two at 830 nm. The optical fibers were arranged over a flexible optical probe where the detector fiber and four pairs of source fibers were optically coupled to the skin via five prisms that deflected the light to/from a direction perpendicular to the optical fibers. Each source pair is made of two closely-spaced optical fibers guiding light at 690 and 830 nm, respectively. While the illumination optical fibers are 400 $\mu$m in diameter, after the internal reflection in the prism they result in a spot size of ~2 mm in diameter on the skin. Furthermore, the illumination spots at the two wavelengths are partially overlapped by about 50%. The distances between the detector fiber and the two source pairs were 1.0 and 1.5 cm, respectively. The source pair placed at 1.5 cm from the detector was used on all subjects to collect two-wavelength data at an acquisition rate of 50 Hz (i.e., 20 ms per data point). In addition to these single-distance measurements, a multidistance measurement, which minimizes sensitivity to possible probe-skin contact artifacts, was conducted on subject 1 using both source-detector distances of 1.0 and 1.5 cm. In the case of multidistance measurements, the acquisition rate was 25 Hz (i.e., 40 ms per data point). A schematic of the experimental setup is given in Fig. 1.

All measurements reported here were taken below the threshold of muscle motion induced by the electrical stimulation. This threshold was established by placing a small mirror at the location on the foot where the optical probe was eventually placed during the experiment. A laser beam was then aimed at the mirror and reflected onto a wall 3 m away, to amplify the angular magnitude of any muscle movement. The stimulation current was increased until we observed a 1.6-Hz (the frequency of the electrical stimulation) oscillation of the laser beam on the wall. This current value was set as the threshold for detectable muscle motion.

The optical probe was placed so that the sural nerve would lie underneath the pair of source and detector fibers. The optical probe was secured to the foot by using double-sided tape

**Table 1** Summary of the results on the six subjects examined.

| Subject | $r$ (cm) | $I_{690}$ | $I_{830}$ | $t_{max}$ (ms) | $\Delta I_{830}/\Delta I_{690}$ | [Hb] | [HbO] | [HbT] | $\Delta$[HbO]/$\Delta$[HbT] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.5 | ↓ | ↓ | 100±20 | 0.73(3) | ↑ | ↑ | ↑ | 0.58(1) |
| 1(MD) | 1.0 | ↓ | ↓ | 160±40 | 0.6(1) | ↑ | ↑ | ↑ | 0.5(1) |
| | 1.5 | ↓ | ↓ | 160±40 | 0.63(8) | ↑ | ↑ | ↑ | 0.50(5) |
| | 1.0, 1.5 | ↓ | ↓ | 160±40 | 0.6(1) | ↑ | ↑ | ↑ | 0.54(7) |
| 2 | 1.5 | ↓ | ↓ | 120±20 | 0.9(6) | ↑ | ↑ | ↑ | 0.7(2) |
| 3 | 1.5 | ↓ | ↓ | 100±20 | 0.67(7) | ↑ | ↑ | ↑ | 0.53(3) |
| 4 | 1.5 | ↓ | ↓ | 60±20 | 1.5(6) | ↑ | ↑ | ↑ | 0.89(6) |
| 5 | 1.5 | ↓ | ↓ | 150±20 | 1.0(3) | ↑ | ↑ | ↑ | 0.77(5) |
| 6 | 1.5 | ↑ | ↑ | 120±40 | 1.4(4) | ↓ | ↓ | ↓ | 0.86(5) |

$r$: source-detector distance
$I_{690}$: Intensity at 690 nm
$I_{830}$: Intensity at 830 nm
$\Delta I_{830}/\Delta I_{690}$: ratio of maximal intensity changes at 830 and 690 nm
$\Delta$[HbO]/$\Delta$[HbT]: ratio of maximal oxy-hemoglobin to total hemoglobin concentration changes
MD: multidistance measurement
Numbers in parentheses indicate the standard error on the last digit

on the side of the probe in contact with the skin and by wrapping an elastic band around the probe and the foot. The duration of the measurement was approximately two minutes, during which about 200 electrical stimulation pulses were delivered. An auxiliary input channel of the NIRS instrument was used to record a synchronization signal from the electrical stimulation unit. A folding average was applied to the optical intensity data around each stimulus, starting 120 ms before and ending 480 ms after each stimulus.

## 3 Results

The optical data shown in Fig. 2 is obtained from subject 1, for whom we have collected multidistance data. Relative intensity changes at 690 and 830 nm measured at source-detector distances of 1.0 and 1.5 nm are shown in Figs. 2(a) and 2(b), respectively. The relative change in the intensity ($I$) is defined as $\Delta I/I_0$, where $\Delta I = I - I_0$ and $I_0$ is the average intensity during the 120 ms preceding the stimulation pulse. The changes in the intensity slope, which minimizes the sensitivity to potential contact artifacts associated with tissue motion, is reported in Fig. 2(c). The intensity slope is defined as $\ln(r_2^2 I_2 / r_1^2 I_1)/(r_2 - r_1)$, where $r_1$, $r_2$ are the two source detector distances, and $I_1$, $I_2$ are the corresponding intensities.[9] Under the assumption, justified in the discussion section, that the intensity changes of Figs. 2(a)–2(c) have an absorption origin, one can translate them into changes in the concentrations of oxy-hemoglobin ([HbO],deoxy-hemoglobin ([Hb]), and total hemoglobin ([HbT]=[HbO]+[Hb]). This can be done, for single-distance data, by using the modified Beer-Lambert law[10] (for which we have used differential pathlength factors of 6.51 at 690 nm, and 5.86 at 830 nm), and for multidistance data, by using the intensity slope algorithm[9] (for which we have used reduced scattering coefficients of 9.5 $cm^{-1}$ at 690 nm, and 7.2 $cm^{-1}$ at 830 nm). These hemoglobin concentration changes are shown in Figs. 2(d)–2(f), corresponding to the intensity data in Figs. 2(a)–2(c), respectively.

Figures 2(a) and 2(b) shows that the intensity starts decreasing within 40 ms of the stimulus and reaches a minimum of 160 ms poststimulus, then gradually returns to baseline within 400 ms. Such intensity decrease in response to the electrical stimulus (and the associated increase in total hemoglobin concentration) was observed in all subjects except one (subject 6). Table 1 summarizes the results on all subjects in terms of increase (↑) or decrease (↓) of the intensity and hemoglobin concentrations, the ratio of the intensity changes at the two wavelengths (a measure of the wavelength dependence of the optical signal), and the ratio of the oxy-hemoglobin to total hemoglobin concentration (a measure of the oxygen saturation of the hemoglobin compartment involved with the measured optical response). Table 1 also reports the time of maximal optical response, which ranged between 60 and 160 ms poststimulus in the six subjects examined.

## 4 Discussion

With the only exception of subject 5, we have consistently recorded a significant difference (10 to 50%) between the intensity changes at 690 and 830 nm (see Table 1). This strong wavelength dependence of the optical response indicates that it is not dominated by contact artifacts. In fact, while we took care to collect data below the visible threshold, any volume change associated with nerve activation may affect the optical contact between the optical probe and the skin. However, such contact artifact should affect the data at the two wavelengths in a similar way, as we observed in separate measurements on

tissue-like phantoms (not shown). For control measurements, the optical probe was placed on the lateral malleolus and also in a position close to the sole of the foot to guarantee that it was off the nerve, but approximately at the same distance from the stimulating electrode as in the previous measurements. During these control measurements no optical signal was observed. Furthermore, because of the weak wavelength dependence of the scattering coefficient, it is unlikely that such strongly wavelength-dependent intensity changes have a scattering origin. We argue that the fast optical signals recorded in response to the electrical stimulation of the sural nerve have an absorption origin, which, in the near-infrared spectral region, points to hemodynamic changes. We also observe that a volume change of a weakly absorbing structure (such as the peripheral nerve) embedded in a strongly absorbing medium (such as blood-perfused tissue) may also result in an apparent absorption change. In fact, the partial volume of strongly absorbing medium within the optically probed region changes as a result of the expansion or contraction of a weakly absorbing compartment. However, if this was the case in our measurements, the observed intensity decrease on the order of 0.1% would require a contraction of the sural nerve axon-derived diameter on the order of 20 $\mu$m. The size of such a change in the nerve size is greater than expected during electrical activation, and, more importantly, the direction of such change (contraction) is opposite to the response, namely an expansion in the plasmalemma, during activation as has been demonstrated in the non-myelinated crayfish axon.[11] Additionally, the activation-induced nerve expansion occurs on a time scale much faster than that of the measured optical response. For these reasons, we favor the hemodynamic origin of the optical signal measured in response to nerve activation. Because of the reproducible findings (with the only exception of subject 6) that this hemodynamic change is an increase in hemoglobin concentration (see Table 1), we assign it to a fast vascular dilation. The ratio between the maximal changes in oxy-hemoglobin and total hemoglobin concentrations reported in Table 1 ranges between 50% and 89% across the six subjects. Because these values provide a measure of the oxygen saturation of the vascular compartment involved in the hypothesized vascular dilation, values lower than $\sim$70% (measured in subjects 1 and 3) are less than expected under rest conditions. These low saturation values may be the result of systematic errors associated with the approximated models use to translate intensity changes into hemoglobin concentration changes.

## 5 Conclusion

Previous experiments that observed an optical signal occurring in the 100-ms time scale using NIRS have studied evoked potential activation of the brain and attributed the most likely main source of this optical signal as originating from scattering.[12] In our experiments on the peripheral nervous system, scattering does not appear to be the prominent mechanism for the 100-ms optical signal. The pattern of optical response is best explained by localized absorption changes probably associated with oxy-hemoglobin and deoxy-hemoglobin changes. Based on this assumption, the calculated hemodynamic changes showed an increase in the concentrations of oxy- and deoxy-hemoglobin in response to electrical nerve stimulation. Further investigation is warranted to address the issue of scattering versus absorption through the collection of data with richer spectral information. We note that the possibility of an evoked vascular response occurring over a time scale of about 150 to 250 ms and correlated with event-related potentials in the central nervous system has already been suggested in the literature[13] though using a very different methodology. This study confirms and extends this phenomenon to the peripheral nervous system.

The observation that the stimulated peripheral nerve responds with a rapid optical signal consistent with a "neurovascular" coupling phenomenon is both novel and surprising because up until the results reported in this work the PNS has not generally been thought to undergo significant hemodynamic regulation nor neurovascular response.[14] While these initial data do not strictly demonstrate that neurovascular coupling is at the origin of the optical response associated with electrical stimulation of peripheral nerves, there are strong indications that this may actually be the case. The NIRS technology herein described will likely be vital in developing an understanding of this physiology, which may well be disrupted in the peripheral neuropathies associated with diabetes, inflammation, neurotoxicity, and aging.

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