

## Hemodynamic Responses of Rat Brain Measured by Near-infrared Spectroscopy During Various Whisker Stimulations

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NIRS (Near-infrared spectroscopy) is a relatively, new, non-invasive, and non-ionizing method of measuring hemodynamic responses in thick biological tissues such as the cerebral cortex. In this study, we measured the hemodynamic responses of the rat barrel cortex to whisker stimulation by using a frequency-domain NIRS system. We designed multiple optical probes comprising multi-mode optical fibers and manipulating arms, both of which can be easily applied to small animals. Various electrical stimulations were applied to rat whiskers at different voltage levels and stimulation frequencies. Our results show that the hemodynamic responses are highly dependent on the stimulation voltage level, and not so much on stimulation frequency. This paper suggests that NIRS technology is highly suitable for the study of small animal brains.

*Keywords* : Near-infrared spectroscopy (NIRS), Hemodynamic responses, Neuroimaging, Neurovascular coupling, Whisker stimulation

*OCIS codes* : (170.3880) Medical and biological imaging; (170.1470) Blood/tissue constituent monitoring; (170.3660) Light propagation in tissue

### I. INTRODUCTION

In neuroscience, small animals such as rats and knock-in and knock-out mice can be used as models of neurological disease, including stroke, epilepsy, and Alzheimer's disease. Various neuroimaging technologies are employed to approach this problem including positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and near-infrared spectroscopy (NIRS) [1].

PET images glucose metabolism using radioisotopes such as 2-deoxy-2- $^{18}\text{F}$ fluoro-d-glucose ( $^{18}\text{F}$ FDG) in the brain. However, these isotopes can be harmful to the human body. It is known that this device is very expen-

sive and has the drawback of requiring too much time to reconstruct images [2]. fMRI now is used as a universal imaging tool for neuroscience but it also has some disadvantages such as low signal-to-noise ratio of blood-oxygen level dependence (BOLD) signal level, low temporal resolution and high cost. It is especially problematic to use PET or fMRI in small animal studies due to the small brain sizes of small animals [3]. Even if our study is focused on hemodynamic signal detection and not on imaging, NIRS can be used for imaging purposes if a larger number of optical probes are used. Although NIRS has some disadvantages such as low spatial resolution and low penetration depth, it is an emerging tool for neuroscience since it costs less to build, shows high temporal resolution and uses harmless light.

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NIRS is used to measure concentrations of oxy-(HbO<sub>2</sub>) and deoxy-hemoglobin (Hbr) using absorption spectra variations in the near-infrared band. The first attempt to measure oxygenation in biological tissues was done by Franz Jobsis, in 1977 [4]. Since then, NIRS-based research has focused on various biological areas including neuroscience [5-8]. In recent years, interest has turned to using NIRS to study neurovascular coupling, which occurs between neuronal activities and hemodynamic responses when neurons are activated. Although many research groups have investigated these correlation phenomenon, it has not been clearly elucidated [8]. Depending on experiment conditions and stimulus parameters, neurovascular coupling has been found to be linear [9-10] or nonlinear [11-13]. In order to understand these linear or nonlinear phenomena, multiple imaging (or detection) modalities have been combined. For example, measurements using magnetoencephalogram (MEG) or electroencephalogram (EEG) have been studied simultaneously [14].

In this study, we measured electrical/hemodynamic responses of rat brain using a home-made probe to record EEG and NIRS signals simultaneously while stimulating whisker areas. Various stimulus parameters were used and corresponding hemodynamic responses including peak values of HbO<sub>2</sub> concentration were recorded.

## II. MATERIALS AND METHODS

### II-1. Experimental setup

All animal experiments followed the experimental prototype of the Department of Physiology at Hallym University. We used three adult female Sprague-Dawley rats with body weight 300~350 g each. Each rat was anesthetized with urethane (1.25 g/kg) and the rat head was fixed to a stereotaxic frame to eliminate motion artifact. A heating pad (HB 101/2; LSI Letica) kept the rat's body temperature at a constant 37°C. After the scalp of each rat was resected, we placed the optical probe to the right barrel cortex which would be activated when the left whisker was stimulated (Fig. 1 (a)). Two optical fibers with 400  $\mu$ m core diameter carrying different wavelengths were carefully tied-up and co-localized on one point with the help of an optical manipulating arm (Fig. 1 (b)). Additionally, to leave no air gap between the fiber tip and skull, thus simplifying the boundary condition, dental cement was applied at the boundary.

The bio-stimulator electrodes (Stimulator 100, Palmer Bioscience) were inserted at B4 and E1 sites of the left whisker [15]. The location of the cerebral barrel cortex, which is known to respond to the whisker stimulation, extends 2 mm in posterior direction from the bregma point and 5 mm in lateral direction from the medial point.

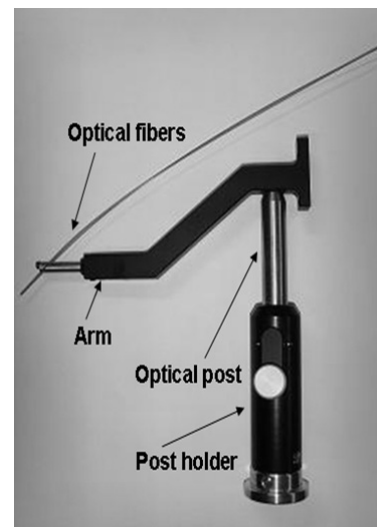
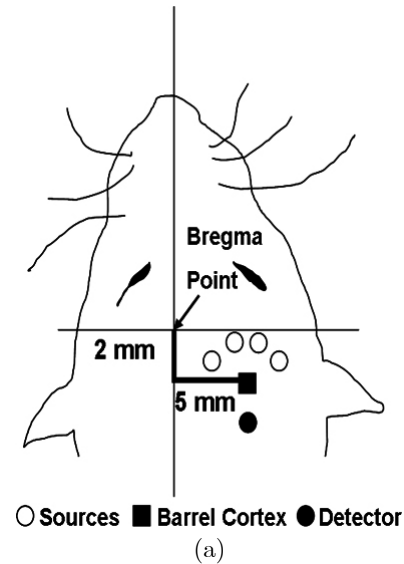


FIG. 1. (a) Arrangement of optical probes on the rat brain and (b) picture of the optical probe

We used the 8-channel frequency domain NIRS system (Imagent<sup>TM</sup>, ISS Corp.) which is composed of eight laser diode pairs (690 and 830 nm) modulated at 110 MHz as sources and two photomultiplier tubes as a detector, which gain is modulated at 110.005 MHz. We used heterodyne detection schematic and detected signal modulated at 5 kHz. The pulse width of the electrical stimulation was fixed at 20 msec and the total stimulation period was 6 sec. We varied both the voltages (i.e., 2, 5, and 10 V) and frequencies (i.e., 1, 3, and 5 Hz) in this study. Before stimulation, data recorded for 5 min were used as a baseline. After each set of 6 sec stimulation, we allowed 3 min for recovery and repeated each procedure in triplicate (Fig. 2). The data-acquisition frequency was 20 Hz for all cases.

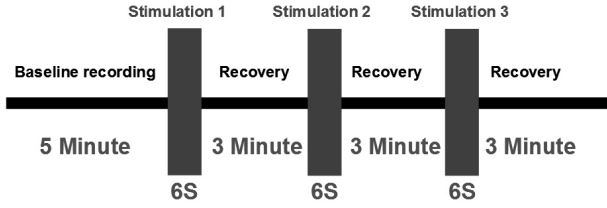


FIG. 2. Stimulation protocol

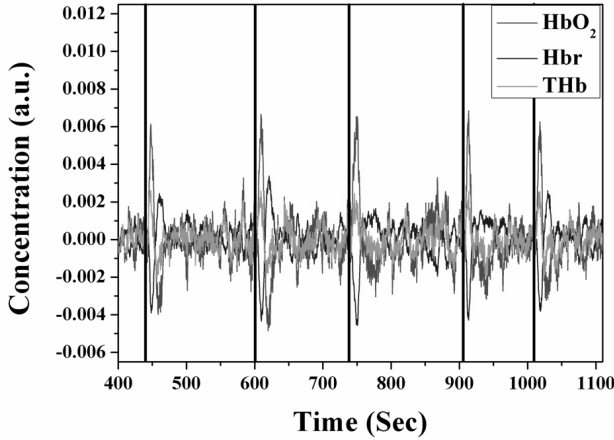
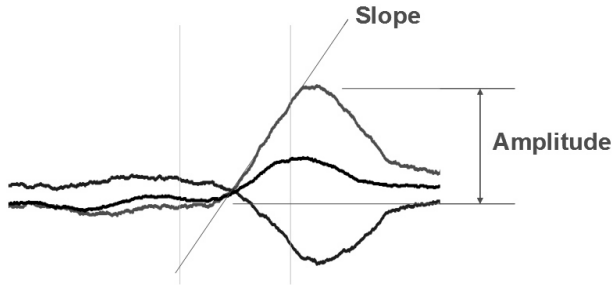
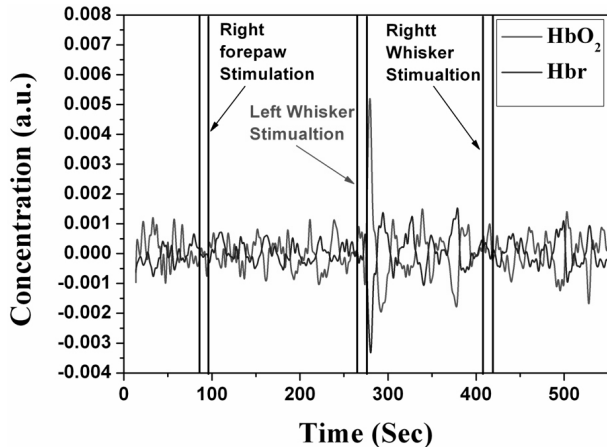
FIG. 3. Relative changes in HbO<sub>2</sub>, Hbr, and THb by induced whisker stimulation (red: HbO<sub>2</sub>, blue: Hbr, green: THb, black: stimulation on)FIG. 4. Slope and peak value of HbO<sub>2</sub> concentration

FIG. 5. Hemodynamic responses at various somatosensory cortices

## II-2. Calculation of hemoglobin concentration

We calculated the concentrations of HbO<sub>2</sub> and Hbr using the modified Beer-Lambert law [16] which requires intensity information in two different wavelengths:

$$\Delta OD^\lambda = -\ln \frac{I_{Final}}{I_{Initial}} = (\epsilon_{HbO_2}^\lambda \Delta[HbO_2] + \epsilon_{Hbr}^\lambda \Delta[Hbr]) B^\lambda L \quad (1)$$

Where  $I_{initial}$  is the source light intensity,  $I_{final}$  is the light intensity at the detector,  $\epsilon$  is the absorption coefficient,  $B$  is the differential pathlength factor,  $L$  is the source-detector distance, HbO<sub>2</sub> is the oxy-hemoglobin, Hbr is the deoxy-hemoglobin and  $\lambda_1$  and  $\lambda_2$  are 690 and 830 nm, respectively.

$$\Delta[Hbr] = \frac{\epsilon_{HbO_2}^{\lambda_2} \frac{\Delta OD^{\lambda_1}}{B^{\lambda_1}} - \epsilon_{HbO_2}^{\lambda_1} \frac{\Delta OD^{\lambda_2}}{B^{\lambda_2}}}{(\epsilon_{Hbr}^{\lambda_1} \epsilon_{HbO_2}^{\lambda_2} - \epsilon_{Hbr}^{\lambda_2} \epsilon_{HbO_2}^{\lambda_1}) L} \quad (2)$$

and

$$\Delta[HbO_2] = \frac{\epsilon_{Hbr}^{\lambda_1} \frac{\Delta OD^{\lambda_2}}{B^{\lambda_2}} - \epsilon_{Hbr}^{\lambda_2} \frac{\Delta OD^{\lambda_1}}{B^{\lambda_1}}}{(\epsilon_{Hbr}^{\lambda_1} \epsilon_{HbO_2}^{\lambda_2} - \epsilon_{Hbr}^{\lambda_2} \epsilon_{HbO_2}^{\lambda_1}) L}$$

The total hemoglobin (THb) is the sum of HbO<sub>2</sub> and Hbr. Data collected were analyzed and the relationship between the stimulation parameters and the corresponding hemodynamic responses was studied.

## III. RESULTS

Fig. 3 illustrates the data collected while stimulating the whole left-whisker area using a piece of stick. Whisker stimulation lasted for about 10 sec and was repeated five times. Between each stimulation, 3 min of recovery time was allowed. Approximately within 1~1.5 sec after stimulation, the HbO<sub>2</sub> concentration started to increase. Also, we noticed that the concentration reached a peak value within 6~8 sec of stimulation. On the other hand, the peak value of Hbr concentration decreased in similar time scale. However, compared to the HbO<sub>2</sub> values, the peak value of Hbr change was approximately 60%.

Fig. 4 is part of the signal in Fig. 3 which shows typical hemodynamic responses to whisker stimulation. We extracted two different parameters from these data: peak and slope values of HbO<sub>2</sub> changes. A linear-fitting was conducted to calculate the slope value and all peak and slope values for HbO<sub>2</sub> concentration were averaged over the whole stimulation sequence.

To verify that our signal originated only from the left-side whisker, we stimulated the forepaw and the right side of the whisker. Fig. 5 shows that no significant hemodynamic responses are observed by stimulation

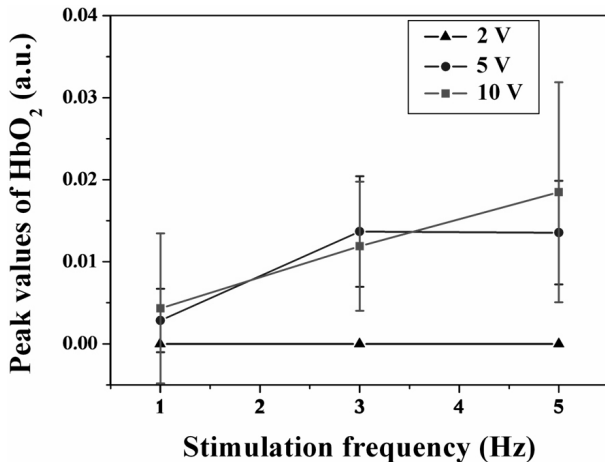


FIG. 6. Peak values of the HbO<sub>2</sub>, by various stimulation parameters

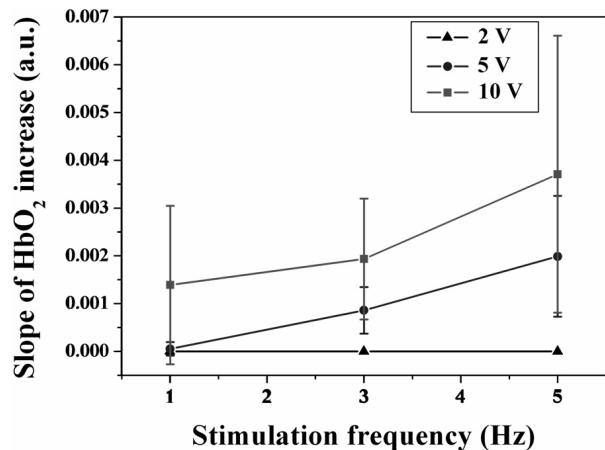


FIG. 7. Slope of the HbO<sub>2</sub>, by various stimulations

on any other areas but the left whisker.

As described earlier, three animals were used for three sets of consecutive experiments, each of which was separated by 3 min recovery time. The voltage levels and stimulation frequencies were 1, 3, and 5 Hz and 2, 5, and 10 V, respectively. Therefore, the total number of data points was 81. Each set of HbO<sub>2</sub> data was averaged and results are shown in Figs. 6 and 7.

As shown in Fig. 6, the amplitude of HbO<sub>2</sub> change increases with stimulation frequencies. It seems that the amplitude change is less affected by the stimulation intensities. Also, it was observed that the cerebral hemodynamics changes were not changed when lower intensity stimulation (2 V) was applied regardless of stimulation frequencies. On the other hand, the HbO<sub>2</sub> rises faster proportionally to the stimulation frequencies and intensities as shown in Fig. 7.

#### IV. DISCUSSION AND CONCLUSION

Even if the diffuse optics has been used in human brain and breast studies for a long time, not many reports on small animal studies have been introduced. Our study shows that it is possible to measure hemodynamic responses in the barrel cortex of small animals using diffuse optical methods. Considering that the optical techniques are non-invasive and can be readily coupled with other imaging modalities, it will provide a unique tool for the neuroscience field.

Generally, after neurons are activated following appropriate stimulations, the blood volume that passes through the corresponding tissues increases. Then, due to metabolic demands, HbO<sub>2</sub> concentration increases while the Hbr concentration decreases, which phenomenon is called a “wash-out effect” [14, 17]. During the wash-out effect, the variations of HbO<sub>2</sub> and Hbr concentrations are out of phase with each other [18]. Sometimes, when the supply of HbO<sub>2</sub> is not sufficient to meet oxygen metabolism demands, there can be an initial dip in HbO<sub>2</sub> concentration for a short time, followed by an increase (biphasic response) [14, 17]. When neurons are activated, the increase in HbO<sub>2</sub> is larger than the decrease in Hbr. As a result, the concentration of total hemoglobin (HbO<sub>2</sub> + Hbr) increases.

We observed similar trends in our experiments. As expected, the relationship between HbO<sub>2</sub> and Hbr was out of phase, and the total hemoglobin concentration increased. However, we did not observe the aforementioned initial dip. Similar studies using a CCD camera that monitors the exposed brain cortex (skull is removed) and measures an optical intrinsic signal (OIS) have reported the biphasic phenomenon where HbO<sub>2</sub> increases after an initial dip [19-20]. It may be because the barrel cortex is exposed directly to light during OIS measurement, which offers better resolution than non-invasive NIRS. Meanwhile, Blood *et al.* found via OIS study that when forepaw stimulation occurs at a higher frequency and for a longer period of time, the HbO<sub>2</sub> concentration increases. The peak and slope values they reported are similar to our results [21].

Figs. 6 and 7 summarize our findings. It seems that the hemodynamic responses occur only when the stimulation exceeds certain threshold values. No NIRS signals were detected when 2 V electrical stimulation was applied. Electrical stimulations of 5 V and 10 V induced measurable hemodynamic responses. As shown in Fig. 7, the slope of HbO<sub>2</sub> changes is proportional both to the stimulation frequencies and magnitude. On the other hand, the amplitude (Fig. 6) does not seem to change proportionally to the stimulation magnitude, while it responds proportionally to the stimulation magnitude. Three subjects may not be enough to make the final conclusion. In the future, we plan to increase the number of subjects with more stimulation parameters. In conclusion, the hemody-

dynamic responses were successfully observed in the barrel cortex area using NIRS without the resection of the skull.

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