Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model

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Hoshi, Yoko, Norio Kobayashi, and Mamoru Tamura. Interpretation of near-infrared spectroscopy signals: a study with a newly developed perfused rat brain model. J Appl Physiol 90: 1657–1662, 2001.—Using a newly developed perfused rat brain model, we examined direct effects of each change in cerebral blood flow (CBF) and oxygen metabolic rate on cerebral hemoglobin oxygenation to interpret near-infrared spectroscopy signals. Changes in CBF and total hemoglobin (tHb) were in parallel, although tHb showed no change when changes in CBF were small (<10%). Increasing CBF caused an increase in oxygenated hemoglobin (HbO2) and a decrease in deoxygenated hemoglobin (deoxy-Hb). Decreasing CBF was accompanied by a decrease in HbO2, whereas changes in direction of deoxy-Hb were various. Cerebral blood congestion caused increases in HbO2, deoxy-Hb, and tHb. Administration of pentylentetrazole without increasing the flow rate caused increases in HbO2 and tHb with a decrease in deoxy-Hb. There were no significant differences in venous oxygen saturation before vs. during seizure. These results suggest that, in activation studies with near-infrared spectroscopy, HbO2 is the most sensitive indicator of changes in CBF, and the direction of changes in deoxy-Hb is determined by the degree of changes in venous oxygenation and volume.

Cerebral blood flow; cerebral oxygen metabolic rate; oxygenated hemoglobin; deoxygenated hemoglobin; pentylentetrazole

NEAR-INFRARED SPECTROSCOPY (NIRS), a noninvasive optical technique, enables us to measure concentration changes in cerebral oxygenated (HbO2) and deoxygenated hemoglobin (deoxy-Hb) (8). Summation of these changes provides the concentration change in total hemoglobin (tHb), which reflects the change in cerebral blood volume within the illuminated area. This technique has been developed as a tool for noninvasive clinical monitoring. However, several recent studies have demonstrated that NIRS also has potential for neuroimaging of the human brain (7, 9, 10). Neuronal activation is coupled with increases in regional cerebral blood flow (CBF; rCBF), which is thought to be accompanied by increases in cerebral blood volume via volumetric expansion in vessels already perfused (vasodilatation) (12) or by increasing the portion of vessels actually perfused (recruitment) (3). It is widely accepted that the degree of increases in rCBF exceeds that of increases in the regional cerebral oxygen metabolic rate (CMRO2) (5), which results in a decrease in deoxy-Hb in venous blood. Thus increases in tHb and HbO2 with a decrease in deoxy-Hb are expected to be observed in activated areas in NIRS measurement. However, we and other groups found that deoxy-Hb and tHb did not necessarily show these changes: no change in tHb, with an increase in HbO2 and a reciprocal decrease in deoxy-Hb, and an increase or no change in deoxy-Hb accompanying increases in tHb and HbO2 were also observed (7, 9, 10). Thus HbO2 seems to be the most sensitive indicator of changes in CBF, whereas such unexpected changes in tHb and deoxy-Hb bring the accuracy of NIRS into question.

NIRS signals observed in activation studies reflect changes in oxygenation in venous blood and blood volume in both arterial and venous sides, which are attributed to changes in CBF and CMRO2 and are not distinguished from each other by NIRS. Thus, to interpret NIRS signals, one should understand the direct effect of each change in CBF and CMRO2 on NIRS parameters. However, it is very difficult to change either CBF or CMRO2 selectively, because procedures to change CBF and CMRO2 are often accompanied by alterations in the systemic circulation, which induces secondary changes in the cerebral circulation and metabolism. In addition, because of the coupling between CMRO2 and CBF, it is impossible to change only CMRO2. Recently, however, we developed a new rat model in which cerebral circulation is isolated from the systemic circulation while, unlike in the isolated perfused brain (1), the connection between the central and peripheral nervous systems is preserved (11). In this model, CBF and CMRO2 can be changed separately without direct influence on the systemic circulation, and it is possible to change CMRO2 under conditions in which CBF is constant. Using this model, we measured changes in cerebral HbO2, deoxy-Hb, and tHb while either CBF or CMRO2 was altered. The purposes of

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METHODS

Animal preparation. See Fig. 1. The details of animal preparation are reported elsewhere (11). Briefly, 31 male Wistar rats (10–12 wk, 200–260 g) were anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg body wt). Rats were tracheotomized and mechanically ventilated after they were paralyzed with an intravenous injection of pancuronium bromide (0.02 mg/100 g body wt). The tidal volume and respiratory rate were adjusted to give arterial partial pressure of carbon dioxide values of 37–42 Torr. Bilateral common carotid, external carotid, and vertebral arteries were ligated. Bilateral common carotid arteries were cannulated to infuse rinsed human type O red blood cells (expired blood obtained from the Japanese Red Cross Society in Sapporo) mixed with modified Ringer solution containing 2% albumin, and the pH, partial pressure of oxygen, and arterial partial pressure of carbon dioxide were adjusted to the normal range (see Table 3) and the temperature to 30°C. To drain cerebral venous blood, external jugular veins were cannulated. Constant flow non-recirculating perfusion was maintained by the use of two infusion pumps, and the infusion rate was adjusted to give normal CBF (1.1 ml·g⁻¹·min⁻¹ in the control state). For the measurement of electroencephalograms (EEGs), skin and muscle overlying the calvarium were reflected. Four needle electrodes were inserted into the skull symmetrically in the frontal and occipital regions, and a reference needle was inserted into the nasal bone. Perfusion pressure was monitored by a manometer 10 cm distal to the portion where the cannula was implanted in the left common carotid artery. This study was approved by the ethics committee of the Institute for Animal Experimentation of Hokkaido University.

NIRS. The basic principle of the NIRS apparatus used in this study has been previously published in detail (6). A portable apparatus was built whereby near-infrared light from a halogen lamp passed through a lens system with a rotating disk containing three interference filters (700-, 730-, and 750-nm wavelengths). The concentration changes in HbO₂ and deoxy-Hb were calculated by the following numeric formulas every 1 s

\[
K \Delta[HbO_2] = -0.868 \Delta \lambda_{700-750} - 1.74 \Delta \lambda_{730-750}
\]

\[
K \Delta[\text{deoxy-Hb}] = 0.868 \Delta \lambda_{700-750} - 2.26 \Delta \lambda_{730-750}
\]

\[
\Delta[tHb] = \Delta[HbO_2] + \Delta[\text{deoxy-Hb}]
\]

where K is the apparent difference absorption coefficient of either HbO₂ or deoxy-Hb of an arbitrary wavelength pair, \( \Delta \) is change, \( \Delta \lambda \) is an absorbance change at a two-wavelength pair (700–750 and 730–750 nm), and brackets denote concentration (6). Because the value of K cannot be determined experimentally, the results were expressed in relative amounts (arbitrary units) rather than in absolute values of concentration. The skull was illuminated with NIRS light 5 mm in front of an ear through use of a 2-mm-diameter light guide, and light transmitted through the cranial bone and cerebral tissue was collected at the hard palate by another light guide of the same type. NIRS measurement was started at the beginning of the blood perfusion.

Procedures. After the 20-min control state, we changed either CBF or CMRO₂. The flow rate was changed in a stepwise manner (every 10 ml/h). The highest and lowest flow rates were ~165 and 55–60% of the initial flow rate, respectively. In nine rats, the flow rate was increased, and in the other nine rats it was decreased. To induce cerebral blood congestion, the tube connected to the cannula implanted in the left external jugular vein was changed into another one with a narrower lumen in the above-mentioned 18 rats. Pentylenetetrazole (PTZ) was used to increase CMRO₂. PTZ was dissolved in the prepared blood (5 mg/ml) and infused at the same rate as in the control state in 13 rats.

Statistical analysis. To determine whether changes caused by increasing or decreasing the flow rate and PTZ administration were significant, the values for HbO₂, deoxy-Hb, and tHb during changes in the flow rate and during the appearance of spikes were compared with those in the control state using paired t-test for each rat. When directions of changes during seizures varied (see RESULTS), the values in each phase were compared with those in the control state. Other comparisons were also made by paired t-test. \( P < 0.01 \) was chosen as the level of significance.

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Fig. 1. A: experimental setup. B: method of the brain perfusion. 1, Common carotid artery; 2, internal carotid artery; 3, external carotid artery; 4, vertebral artery; 5, external jugular vein; EEG, electroencephalogram; NIRS, near-infrared spectroscopy.
RESULTS

Effect of changes in CBF on NIRS parameters. Increasing the flow rate caused increases in HbO₂ and tHb with a decrease in deoxy-Hb (Fig. 2). When the flow rate was $111.3 \pm 6.5\%$ of the initial rate, however, tHb was not changed in eight of the nine examined rats (Fig. 2A). Venous oxygen saturation (SvO₂) increased as the flow rate increased: $\Delta$SvO₂/Δflow rate $= 4.08 \pm 1.2\% \cdot 10$ ml$^{-1} \cdot$ h$^{-1}$ when the flow change was less than $\pm 10\%$. The outflow rate from the bilateral external jugular veins almost matched the inflow rate while the flow rate increased. Table 1 summarizes the results.

Table 1. Changes in NIRS signals by increasing the flow rate

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Initial Flow Rate, ml/h</th>
<th>Highest Flow Rate, ml/h</th>
<th>HbO₂</th>
<th>deoxy-Hb</th>
<th>tHb</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>160</td>
<td>260</td>
<td>I</td>
<td>D</td>
<td>N → I</td>
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<td>160</td>
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<td>I</td>
<td>D</td>
<td>I</td>
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<tr>
<td>3</td>
<td>160</td>
<td>260</td>
<td>I</td>
<td>D</td>
<td>N → I</td>
</tr>
<tr>
<td>4</td>
<td>170</td>
<td>280</td>
<td>I</td>
<td>D</td>
<td>N → I</td>
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<tr>
<td>5</td>
<td>170</td>
<td>280</td>
<td>I</td>
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<td>N → I</td>
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<tr>
<td>6</td>
<td>170</td>
<td>280</td>
<td>I</td>
<td>D</td>
<td>N → I</td>
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<tr>
<td>7</td>
<td>180</td>
<td>300</td>
<td>I</td>
<td>D</td>
<td>N → I</td>
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<tr>
<td>8</td>
<td>180</td>
<td>300</td>
<td>I</td>
<td>D</td>
<td>N → I</td>
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<tr>
<td>9</td>
<td>180</td>
<td>300</td>
<td>I</td>
<td>D</td>
<td>N → I</td>
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</table>

NIRS, near-infrared spectroscopy; HbO₂, oxygenated Hb; deoxy-Hb, deoxygenated Hb; tHb, total Hb; I, increase; D, decrease; N, no change.

Decreasing the flow rate caused a decrease in HbO₂. When the degree of decreases in the flow rate was small ($\pm 90.2 \pm 5\%$ of the initial rate), however, tHb did not change in four of the nine examined rats (Fig. 3A). Changes in direction of deoxy-Hb were various: an increase, a decrease, and no change were observed

Table 2. Changes in NIRS signals by decreasing the flow rate

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Initial Flow Rate, ml/h</th>
<th>Lowest Flow Rate, ml/h</th>
<th>HbO₂</th>
<th>deoxy-Hb</th>
<th>tHb</th>
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<tbody>
<tr>
<td>1</td>
<td>160</td>
<td>100</td>
<td>D</td>
<td>N → I</td>
<td>D</td>
</tr>
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<td>2</td>
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<td>100</td>
<td>D</td>
<td>I</td>
<td>N → D</td>
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<tr>
<td>3</td>
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<td>N → D</td>
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<td>N → D</td>
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</table>
When the flow rate was decreased to less than 70% of the initial rate, deoxy-Hb started to increase in all nine rats, whereas HbO2 and tHb decreased. SvO2 decreased as the flow rate was decreased: 

$$\frac{\Delta \text{SvO}_2}{\Delta \text{flow rate}} = 4.26 \pm 1.23 \text{ ml}^{-1} \text{ h}^{-1}$$

when the flow change was less than 15%. The outflow rate almost matched the inflow rate while the flow rate decreased. Table 2 summarizes the results.

Figure 4 shows changes in hemoglobin oxygenation caused by interference with venous drainage. Increases in HbO2, deoxy-Hb, and tHb were observed in all 18 examined rats.

**Effect of PTZ-induced seizures on NIRS parameters under conditions in which CBF was constant.** Infusion of PTZ induced epileptic discharges on the EEG (Fig. 5) and decreased perfusion pressure from 223.4 ± 19.4 mmHg during the preseizure state to 151.3 ± 17.9 mmHg during the seizure. All 13 examined rats showed increases in HbO2 and tHb during seizure. Deoxy-Hb decreased in 10 rats (Fig. 6A), whereas the remaining three rats showed various changes in the direction of deoxy-Hb (Table 3). Rat 1 first showed a decrease, then an increase, and again a decrease in deoxy-Hb (Fig. 6B). Rat 2 showed first a decrease and then no change in deoxy-Hb (Fig. 6C). An increase in deoxy-Hb was observed only in one rat (rat 3, Fig. 6D), which did not show a decrease in SvO2. There were no significant differences in cerebral mixed-venous blood-gas data before vs. during seizure in all of the rats (Table 3). Venous blood sampling was performed in the control state and while bursts of spikes appeared (number 4 in Fig. 6). Although it was not statistically significant, 10 rats showed higher SvO2 during seizure than in the preseizure state. Increasing flow rate while bursts of spikes and/or rhythmic spike and wave complexes still appeared on the EEG caused an increase in SvO2 and a decrease in deoxy-Hb.

**DISCUSSION**

Because, in our experimental conditions, active dilatation of arterioles did not occur while the flow rate was being increased, increases in tHb were attributed to passive vasodilatation or recruitment. Such increases in tHb were not observed until the flow rate was increased to a certain degree. This means that a small degree of increases in the flow rate did not cause either vasodilatation or recruitment but rather increases in the flow velocity. When oxygen delivery increases via an augmentation in CBF with no change in oxygen demand, HbO2 increases and deoxy-Hb decreases in venous blood. Thus no change in tHb with an increase in HbO2 and a reciprocal decrease in deoxy-Hb (Fig. 2A) means that the flow velocity increased without accompanying either vasodilatation or recruitment, which resulted in increases in venous oxygenation. The same pattern of changes in hemoglobin oxygenation as that in Fig. 2A has also been observed in the activated area in NIRS studies (7, 10), although the dilative response of pial arterioles to neuronal activation has been determined videomicroscopically in several studies (4, 12). Kleinschmidt et al. (10) proposed two explanations for this absence of the tHb response during activation: changes in local cerebral hematocrit associated with flow velocity changes, or a short interval between task performance, which does not allow for recovery of vasomotor tone. However, the
mechanisms of dilatation of pial arterioles are still controversial. There is no valid evidence to deny the possibility that, when increases in CBF are very small, the degree of dilatation of arterioles is too small to detect. This possibility is supported by the observation in microspectroscopic measurements through a cranial window and a thinned skull in rats that pial arterioles did not show detectable dilatation, whereas optical and electrocortical signal changes were observed in capillary areas (personal communication). In general, activity-dependent changes in rCBF for subtle cognitive tasks are small (<10%) (13). Thus an increase in flow velocity without detectable vasodilatation might account for the absence of an increase in tHb during activation.

Table 3. Cerebral mixed-venous blood-gas analysis before and during seizure and changes in deoxy-Hb during seizure

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Before Seizure</th>
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<th>During Seizure</th>
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<tbody>
<tr>
<td></td>
<td>pH</td>
<td>PO₂</td>
<td>PCO₂</td>
<td>SvO₂%</td>
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<tr>
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<td>38.5</td>
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<td>40.9</td>
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<td>39.1</td>
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Means ± SD 7.28 ± 0.03 40.8 ± 5.5 45.7 ± 3.9 67.5 ± 8.3 7.29 ± 0.03 43.3 ± 5.1 42.5 ± 5.1 72.9 ± 7.2

SvO₂, venous oxygen saturation. Infused blood without pentylentetrazole: pH, 7.33 ± 0.03; PO₂, 174.5 ± 26.5 Torr; PCO₂, 39.2 ± 1.3 Torr; O₂ saturation, 99.1 ± 0.5%. Infused blood with pentylentetrazole: pH, 7.35 ± 0.03; PO₂, 177.0 ± 23.1 Torr; PCO₂, 34.5 ± 1.3 Torr; O₂ saturation, 99.2 ± 0.3%.
As seen in Fig. 3 and Table 2, decreasing the flow rate caused a decrease in $SvO_2$, whereas changes in direction of deoxy-Hb were various. Thus the direction of change in deoxy-Hb measured by NIRS does not necessarily only reflect venous oxygenation. In fact, cerebral congestion, which caused venous dilatation, was accompanied by an increase in deoxy-Hb. These results indicated that the direction of changes in deoxy-Hb was determined by changes in both venous oxygenation and venous blood volume. It is likely that the increase in deoxy-Hb in Fig. 3A mainly reflects venous hypoxia, whereas the decrease in deoxy-Hb in Fig. 3C mainly reflects a decrease in venous blood volume. When the degree of changes in deoxy-Hb attributed to venous hypoxia is the same as that attributed to decreases in venous blood volume, no change in deoxy-Hb is expected to be observed, such as in Fig. 3B. This can also explain the various changes in direction of deoxy-Hb observed in activation studies. That is, when the degree of decreases in deoxy-Hb attributed to venous hyperoxygenation due to overcompensation of the flow is the same as that of increases in deoxy-Hb attributed to venous dilatation, no change in deoxy-Hb is observed. However, the contribution of venous dilatation to the change in deoxy-Hb is larger than that of venous hypoxia, resulting in an increase in deoxy-Hb. In contrast to $tHb$ and deoxy-Hb, the direction of changes in $HbO_2$ was always the same as that of the change in rCBF. It is, therefore, proposed that $HbO_2$ is the best indicator of changes in rCBF in cognitive studies with NIRS.

It is widely accepted that epileptic seizures induce increases in CBF and CMRO$_2$. To examine whether the direction of changes in deoxy-Hb in activated areas is actually determined by changes in both venous oxygenation and venous blood volume, we had planned to first administer PTZ without increasing the flow rate, which had been expected to cause venous hypoxia, and then increase the flow rate. However, PTZ caused a decrease in deoxy-Hb in 10 of the 13 examined rats, whereas all of the rats showed increases in $tHb$ and $HbO_2$. Furthermore, decreases in $SvO_2$ were not observed during seizures in these rats, including three rats that showed an increase or no change in deoxy-Hb, which might have been due to venous dilatation. Rather, 10 rats showed increases in $SvO_2$, even though they were not statistically significant. These results indicate that there is no global change in CMRO$_2$ during PTZ-induced seizures in the present model. This may be explained by the heterogeneity of activated areas. Ben-Ari et al. (2) reported that PTZ increased glucose metabolism in the neocortex, substantia nigra, red nucleus, nucleus of the oculomotor nerve, the cerebellum, and vestibular nuclei, whereas a decrease in glucose consumption was observed in the hippocampal formation and amygdala. It is conceivable that CMRO$_2$ and CBF increase in the former areas, whereas they decrease in the latter areas. The decrease in flow pressure caused by PTZ administration means that pial arterioles were dilated while responding to neuronal activation. It is thus speculated that required blood was supplied to activated areas from deactivated areas through the pressure difference. This also explains why $tHb$ and $HbO_2$ increased, whereas the flow rate was not increased. However, further studies are required to give a valid explanation.

In summary, the present study demonstrated that $HbO_2$ is the most sensitive indicator of changes in rCBF in NIRS measurements. The direction of the change in deoxy-Hb is determined by changes in both venous oxygenation and blood volume.

REFERENCES