Hemodynamic Response of the Frontal Cortex Elicited by Intravenous Thiamine Propyldisulphide Administration

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Abstract

The intravenous olfaction (IVO) test is a unique type of clinical olfactometry and is widely used in Japan. However, it is difficult to distinguish actual olfactory disturbance from feigned disturbance because the IVO test is a psychophysical test. To resolve this problem, we investigated the possibility of an objective IVO test assisted with near infrared spectroscopy (NIRS). IVO testing was performed according to the usual protocol with thiamine propyldisulphide (alinamin) administration. The relative oxy- and deoxyhemoglobin levels of the orbitofrontal area during olfactory stimulation by IVO test were measured by NIRS. Pairs of NIRS emitters and detectors were positioned on the bilateral frontal scalp. After administration of alinamin, oxyhemoglobin levels increased, though deoxyhemoglobin levels did not change. An increase in oxyhemoglobin levels was observed bilaterally. Administration of saline did not elicit any change in the oxy- or deoxyhemoglobin levels and concentration of the administered alinamin related increasing of the oxyhemoglobin level was observed. Oxyhemoglobin remained unchanged in anosmic subjects despite administration of alinamin. The latency of oxyhemoglobin increase on each side and smelling latency showed significant correlation. Latencies of oxyhemoglobin increases between the right and left sides also showed significant correlation. Oxyhemoglobin response appears to be linked to olfactory related response. NIRS is a useful technique for the development of an objective form of IVO testing.

Key words: alinamin, deoxyhemoglobin, intravenous olfaction test, olfaction, orbitofrontal cortex, oxyhemoglobin

Introduction

Many methods of clinical olfactory testing are used across the world and they are not globally standardized. Most clinical olfactometries are psychophysical tests, but objective olfactometry is very rare. There are two types of clinical olfaction tests performed in Japan (Takagi, 1989). The first method, T&T olfactometry, uses perfumer's strips and odorant bottles. The second method, the intravenous olfaction (IVO) test, is a unique clinical olfaction test with intravenous administration of thiamine propyldisulphide (alinamin; Takeda Pharmaceutical Company, Osaka, Japan). The main mechanism of intravenous olfaction is thought to be retronasal olfaction of the odorous breath gas produced by an intravenously administered odorous substance.

We conducted an electrophysiological study in an attempt to develop an objective IVO test. When alinamin was injected and the subject smelled its garlic-like odor, gamma band oscillation was recorded from the scalp (Ishimaru *et al.*, 2002). Hatanaka *et al.* (2004) investigated gamma band

oscillation elicited by IVO stimulation and discussed its potential as a clinical objective olfaction test. Near infrared spectroscopy (NIRS) of the olfactory cortex is also a promising method of objective olfactometry (Bartocci *et al.*, 2000, 2001; Ishimaru *et al.*, 2004). We investigated the potential of an NIRS-assisted, objective IVO test.

Materials and methods

Changes in levels of oxyhemoglobin and deoxyhemoglobin were recorded by two sets of light emitting diodes (LEDs) whose wavelengths were 750 and 830 nm and photo diodes placed on both sides of the frontal scalp near each orbitofrontal cortex (Figure 1). The distance between the LED and the photo diode was 3 cm. NIRS measurements of human olfactory response were recorded with a tissue oxygen meter (PSA500; Biomedical Science, Kanazawa, Japan) according to procedures detailed in our previous report (Ishimaru *et al.*, 2004). NIRS data were continuously sampled by every

Figure 1 Schema of NIRS measurement. Near infrared emitters with light emitting diodes (LED) were positioned on the frontal scalp's upper orbits; right LED (RL) and left LED (LL) were set. Detectors with photo diodes were positioned 3 cm lateral from the respective LEDs; right photo diode (RP) and left photo diode (LP) were set.

Figure 2 Typical NIRS response elicited by intravenous administration of alinamin. Saline (a) and alinamin (b) were administered intravenously. Oxyhemoglobin level (black line) increased when alinamin was administered. Administration of saline produced no response. Deoxyhemoglobin (dotted line) level was not changed by saline or alinamin. Horizontal bars indicate intravenous administration periods (10 s). The perpendicular bar indicates rate of change.

100 ms. Alinamin (2 ml) was administered as an intravenous odorant, injected into the right median cubital vein over a period of 20 s, according to the standard protocols of the intravenous olfaction test (Takagi, 1989). The subjects were instructed to close their eyes and push a hand switch when they perceived intravenous olfaction of a garlic-like smell. This event was labeled the smelling response. The smelling response was captured via an analog to digital (A/D) converter along with the NIRS response and stored on a personal computer.

Seventeen people participated in this study. Seven male normosmia subjects (23.4 ± 1.1 years old) were volunteers who were compensated with a small amount of money. Two anosmic and eight hyposmic subjects (49.7 ± 19.0 years old, four males and six females) who were patients of Kanazawa University Hospital also participated in this study. All subjects understood the aim of the study and gave their informed consent. All the experimental procedures followed the World Medical Association Declaration of Helsinki Recommendations guiding physicians in biomedical research involving human subjects.

Time zero seconds of latency was defined as the onset of administration of alinamin.

Student's *t*-test and Pearson's product moment correlation coefficient were used for statistics.

Results

NIRS response from the normosmia subjects

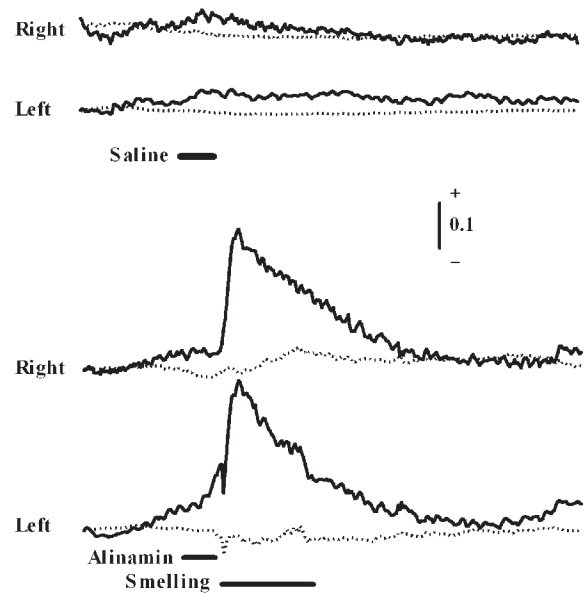Increases of oxyhemoglobin concentration produced by alinamin administration were observed in the seven subjects who declared normosmia. Four of the seven subjects were recorded with both alinamin and saline administrations as a control group. A typical case of a 23 years old male is shown in Figure 2. No changes in oxy- and deoxyhemoglobin levels within background were observed before administration. When saline was administered, no changes in oxy- and deoxyhemoglobin levels were observed. After administration of alinamin, a remarkable increase of oxyhemoglobin was observed, though the deoxyhemoglobin level did not change.

Oxyhemoglobin increased quickly when the subject experienced intravenous olfaction caused by alinamin. Most cases were similar to that shown in Figure 2. However, in two cases the increase in oxyhemoglobin occurred more gradually. Repetition smelling response was observed in one subject but only a long continuous smell response was observed in the other subject. A typical slow response case with repetition smell response is shown in Figure 3.

Oxyhemoglobin increase onset was 20.3 ± 5.3 s (right side) and 21.0 ± 5.4 s (left side) after onset of alinamin administration. There was no significant difference between the sides ($n = 7$, $P = 0.28$, paired *t*-test). Smelling response occurred 20.0 ± 5.4 s after onset of alinamin administration and there were no significant differences between the latency of oxyhemoglobin increase on each side ($n = 7$, $P = 0.76$ in right and $P = 0.33$ in left side, paired *t*-test).

The oxyhemoglobin levels of the right and left sides reached a peak 74.2 ± 49.7 s and 72.5 ± 49.4 s after onset of
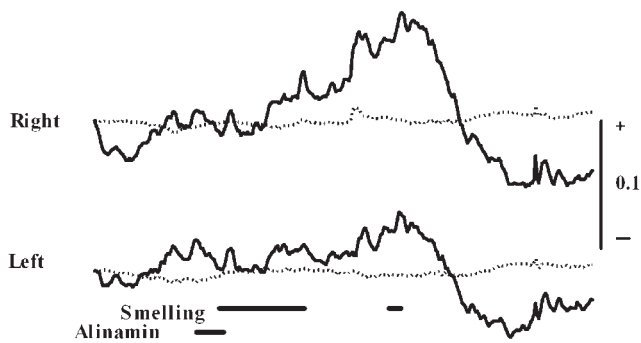
Figure 3 Slow response type of NIRS. Oxyhemoglobin (black line) peak was observed 100 s after onset of administration of alinamin. Deoxyhemoglobin (dotted line) did not change despite administration of alinamin. Horizontal bars indicate intravenous administration periods (10 s). The perpendicular bar indicates rate of change.

Figure 4 Correlation between NIRS amplitude and concentration of alinamin. The right **(a)** and left **(b)** peak amplitudes of oxyhemoglobin response of NIRS were well correlated to the logarithmic concentration of alinamin in the 27-year-old male. Solid and broken lines indicate a regression line and 95% confidence range, respectively.

alinamin administration, respectively ($n = 6$, mean ± SD). Data for a subject with noisy response on the right side and a peak latency that was greater than the measuring period were omitted. The right and left sided peak levels of oxyhemoglobin were 0.16 ± 0.08 (mean ± SD) and 0.16 ± 0.07 (mean ± SD), respectively (n = 6, same as above). There were no significant differences between right and left NIRS peak latencies ($n = 6$, $P = 0.19$, paired t-test) and levels ($n = 6$, $P = 1.00$, paired t-test).

Four of the above six subjects participated in the experiment with saline administration as a control group. The right and left sided oxyhemoglobin peak levels after administration of alinamin were 0.18 ± 0.07 and 0.17 ± 0.08, respectively (mean ± SD, $n = 4$). The homochronous right and left sided oxyhemoglobin levels after administration of saline were 0.00 ± 0.02 and 0.04 ± 0.01, respectively (mean ± SD, $n = 4$). Differences between each side's peak oxyhemoglobin level elicited by alinamin and homochronous oxyhemoglobin levels after saline administration were significant ($n = 4$, $P < 0.05$, paired t-test).

Concentration related response was recorded in the 27-year-old single male subject. Alinamin was diluted to 0.1, 1 and 10% with saline. The alinamin and the diluted alinamin were administered by the procedures described previously. Both side's peak amplitudes of oxyhemoglobin response were significantly correlated to logarithmic concentration of alinamin (right side $r = 0.98$, $P < 0.05$ and left side $r = 0.99$, $P < 0.01$; Figure 4a,b).

NIRS response from patients with olfactory dysfunction

Alinamin was administered intravenously, but oxy- and deoxyhemoglobin levels did not change in the subjects with anosmia ($n = 2$). A typical anosmia case is shown in Figure 5a. Oxyhemoglobin increases were observed in 8 of 10 patients. Though the patient had respiratory hyposmia caused by chronic sinusitis, the patient could perceive the garlic odor after intravenous alinamin administration and oxyhemoglobin increased with a similar time course to
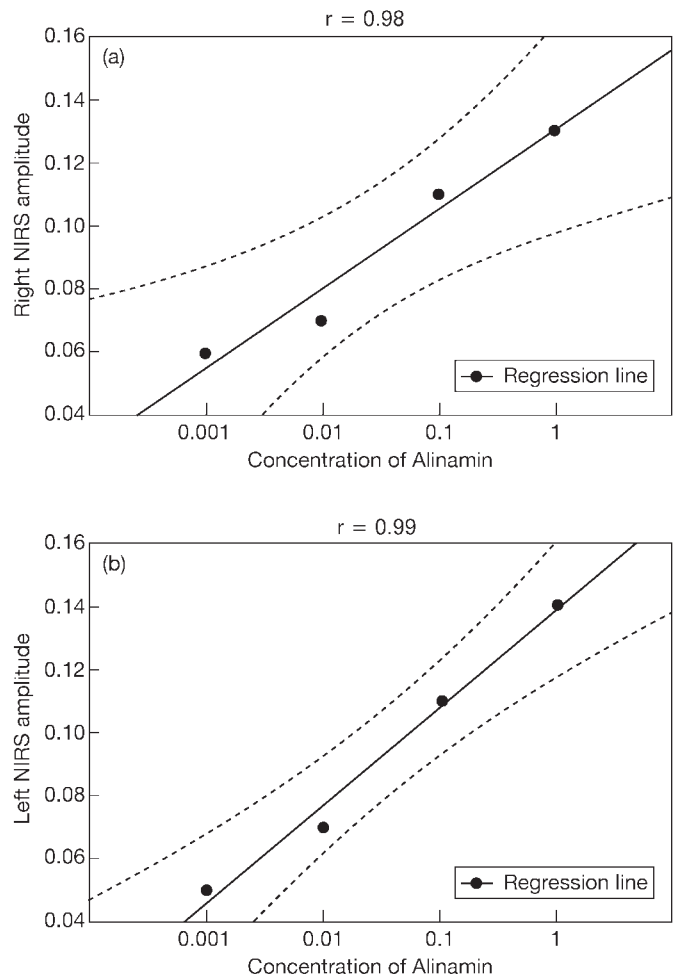
normal subjects ($n = 4$). A typical case is shown in Figure 5b. A substantially increased response of oxyhemoglobin was also observed in two patients recovering from hyposmia. A small increase of oxyhemoglobin was observed in one patient with central hyposmia. An extremely slow response onset of 128 s was recorded in a hyposmia patient who began to perceive smell 132 s after alinamin administration.

Data for three patients whose onset latency of oxyhemoglobin responses were unclear due to noise were omitted from the statistical analysis of latency. Differences in latency between oxyhemoglobin increases recorded from the right frontal scalp and smelling responses were not significant ($n = 5$, $P = 0.91$, paired t-test), but those from oxyhemoglobin increases recorded from the left frontal scalp and smelling responses were significant ($n = 5$, $P < 0.05$, paired t-test). Differences in oxyhemoglobin increase latencies of the right and left scalp were not significant ($n = 5$, $P = 0.24$, paired t-test). The peak of oxyhemoglobin
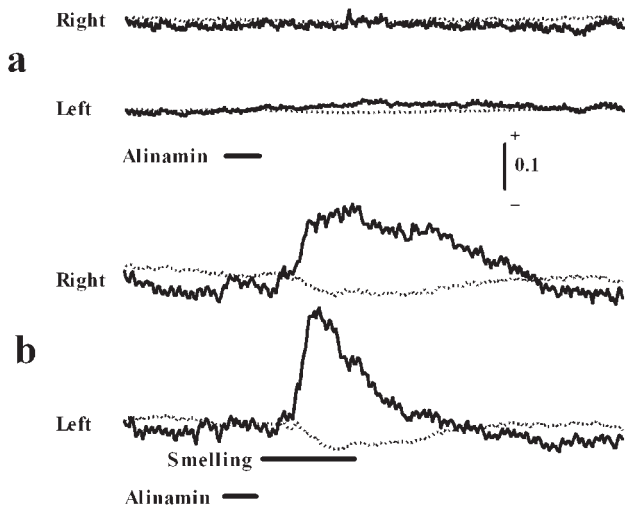
Figure 5 NIRS responses in anosmia and hyposmia. Both oxyhemoglobin (black line) and deoxyhemoglobin (dotted line) levels remained unchanged in subjects with anosmia **(a)**. Oxyhemoglobin level increased after administration of alinamin, but only a slight decrease in deoxyhemoglobin level was observed **(b)**.

increase was observed in four patients and their latencies were 33.8 ± 5.1 s (right) and 34.8 ± 5.7 s (left). No significant difference was observed in the peak latencies between right and left sides ($n = 4$, $P = 0.39$, paired t-test)

Correlation among smelling and NIRS latencies in all subjects

The relationships between smelling response (SR) and oxyhemoglobin increase latencies were analyzed in all cases in which oxyhemoglobin levels increased ($n = 12$). The oxyhemoglobin increase latency is termed 'NIRS latency' hereafter. The correlation between smelling and NIRS latencies was significant (right side $r = 0.92$, left side $r = 0.92$, $P < 0.001$; Figure 6a,b). The correlation of NIRS latencies between right and left sides was also significant ($r = 0.98$, $P < 0.001$; Figure 6c).

Discussion

Olfactory elicited NIRS response recorded from frontal scalp has been reported in newborns and adults (Bartocci *et al.*, 2000, 2001; Ishimaru *et al.*, 2004). Odor induces an increase of oxyhemoglobin levels of the orbitofrontal cortex. A similar effect was observed by functional MRI (Koizuka *et al.*, 1994, 2001; Sobel *et al.*, 1998). IVO testing also produced an increase in the frontal cortex tissue oxygen level (Koizuka *et al.*, 2001). In the present study, though administration of saline did not elicit an increase of oxyhemoglobin, administration of alinamin elicited increased oxyhemoglobin levels in the frontal cortex. When the smell of alinamin was perceived, NIRS response commenced, but this is not proof of pure olfactory response. In only a single case, the result that amplitude of NIRS was directly correlated to the concentration of administered alinamin
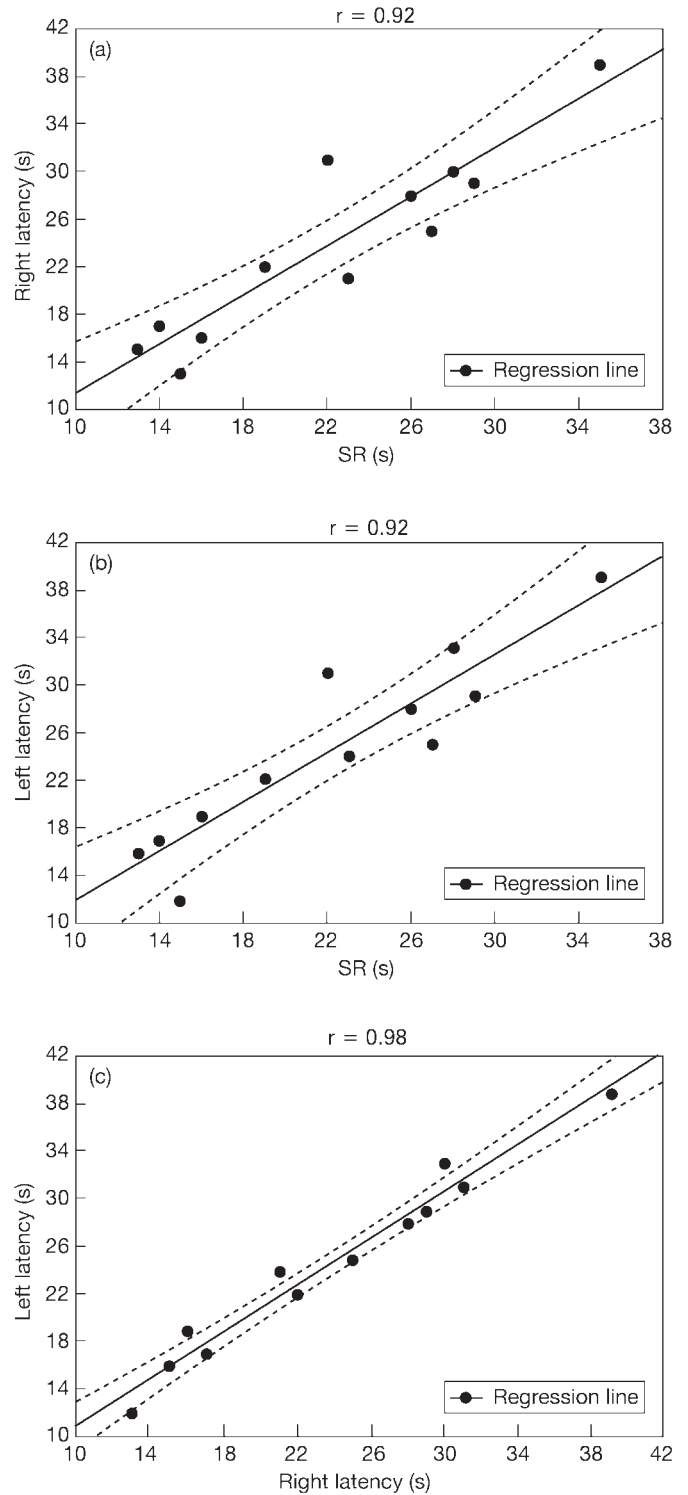

Figure 6 Correlation among smelling (SR) and NIRS latencies. Relationship of smelling latencies versus right **(a)** and versus left **(b)**. Oxyhemoglobin increase of NIRS and the right versus left **(c)**. Solid and broken lines indicate a regression line and 95% confidence range, respectively.

indicates the possibility that the NIRS response was not an emotional response. The NIRS response was only thought to be a response elicited by alinamin administration. The

fact that significant correlation was observed in the latencies of smelling response and NIRS and that NIRS response was not observed in the anosmia cases supports the hypothesis that the increase in oxyhemoglobin was elicited by intravenous olfaction rather than by a direct effect of alinamin.

Oxyhemoglobin levels of both sides of the frontal scalp were remarkably increased after administration of alinamin. In a typical case, when the subject recognized the smell of alinamin a rapid increase of oxyhemoglobin was observed. In some other cases the increase of oxyhemoglobin levels was more gradual. The peak latencies of oxyhemoglobin levels also showed a large deviation. Intravenous olfaction was thought to be retronasal olfaction caused by odorous breath. When the odorous density in breath was measured by an artificial nose, the latency of odorous breath had an onset of 8–15 s and the peak of the density–time curve was observed 30–40 s after administration of alinamin (Nakashima *et al.*, 2002). Threshold levels of odorous density in breath to oxyhemoglobin increase seemed to be different for each subject. Therefore, it seemed that the increase in oxyhemoglobin levels began close to smelling response latency in some subjects, but in others the increase began at the peak latency of odorous breath gas concentration. Nakashima *et al.* (2002) also reported that repetitive peaks of alinamin smell per 1 min were detected in some subjects. Two smelling periods at an interval of 61 s were observed in the subject with slow response in Figure 3. Therefore, extremely slow response of NIRS might be an expression of mean repetition peaks occurring every 1 min.

Lateralization of the olfactory cortex was also an interesting problem. Zatorre *et al.* (1992) reported that the right side was superior to the left in a PET study.

The distance between the emitter and the detector produces the optical pathlength in a scattering medium such as tissue (Delpy *et al.*, 1988). Because the difference of scattering characteristics between right and left frontal cortices seemed to be slight, the optical pathlengths between the LED and the photo diode seemed to be the same for both sides. Therefore, NIRS data obtained from both cortices are directly comparable.

An NIRS study with stimulation by T&T olfactometry also indicated right sided superiority (Ishimaru *et al.*, 2004). The intensity of stimulation by perfumer's strip with odorant, T&T olfactometry, is weaker than in IVO testing. Because the intensity of odorous stimulation by IVO testing seems extremely strong, differences of oxyhemoglobin peak latency and levels between right and left sides seem to be unclear.

Conclusion

Increases in oxyhemoglobin produced by intravenous administration of alinamin were observed on the bilateral frontal scalp. Intravenous olfaction testing with NIRS was performed on patients with olfactory disturbances to determine whether oxyhemoglobin response detected by NIRS depended on intravenous olfaction or the direct alinamin effect. There was no NIRS response in subjects with anosmia and significant correlation between latencies of NIRS and subjective consciousness. Therefore, NIRS measurement of oxyhemoglobin levels is assumed to be a measurement of intravenous olfactory response. Application of NIRS to intravenous olfaction testing is a promising method for the clinical field.

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