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Identification of Three Factors Influencing Trail Making Test Performance Using Multichannel Near-Infrared Spectroscopy

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Recent advances in medical care have facilitated the survival of patients with stroke or traffic-related injuries. However, such patients may suffer from higher brain dysfunction; i.e., an impaired ability to plan and perform behaviors based on prior knowledge. The Trail Making Test (TMT) is a cognitive task that is used to evaluate higher brain dysfunction caused by frontal lobe injury. TMT consists of two tasks; TMT-A involves connecting consecutive numbers, and TMT-B involves connecting numbers and letters alternately. In this study, using near-infrared spectroscopy (NIRS) and the achievement value (TMT score), we investigated the effects of three factors on TMT performance: knowledge of the TMT, the order of TMT-A and TMT-B performance, and gender. The subjects were 48 healthy adults, consisting of college graduates and undergraduates (age: 22.8 ± 2.5 years, education: 16.0 ± 1.2 years, 24 males and 24 females). We measured the changes in oxygenated hemoglobin (oxy-Hb) levels using NIRS, showing that the increase in oxy-Hb was higher in subjects who had no knowledge of the TMT than those who had prior knowledge. In both TMT-A and TMT-B, the subjects who performed their first task displayed higher oxy-Hb levels. Moreover, the oxy-Hb level in males was higher than that in females. In contrast, only the order of TMT performance showed noticeable effect on the TMT score. In the present study, using NIRS we have shown that either knowledge of the TMT, the order of the TMT, or gender affects TMT performance, providing invaluable information for interpreting TMT results.

Keywords: near-infrared spectroscopy; trail making test; healthy adults; prefrontal cortex; higher brain function
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Higher brain function refers to the ability to plan and perform behaviors based on prior knowledge (Suzuki and Sakata 1988). Recent advances in medical care have facilitated the survival of patients who have suffered a stroke or traffic-related injuries from which they would previously not have survived. However, as a result, many such patients are diagnosed with higher brain dysfunction, making rehabilitation difficult. Furthermore, higher brain dysfunction persists in the chronic stage. This makes attending work difficult, resulting in the isolation of patients and their families from society and increasing stress related to domestic problems. Recently, government authorities and the mass media have emphasized these problems in order to encourage the development of solutions (Higher Brain Dysfunction Committee 2004).

Attention disorders and executive dysfunction are higher brain dysfunctions that are often caused by frontal lobe injury (Hecaen and Albert 1978; Stuss and Bensen 1986; McDonald et al. 2002). There are many standardized methods for evaluating frontal lobe dysfunction. The Trail Making Test (TMT) is a simple standardized neuropsychological test that has been widely used in clinical practice since its development as part of the U.S. Army Individual Test Battery in 1944 (Retzlaff et al. 1992). It is commonly used as a measure of frontal lobe function (Zakzanis et al. 2005). Injuries in the frontal lobe were associated with low performance (Stuss et al. 2001). Earlier studies suggested that this test was not specific enough to effectively localize brain injury, especially when differentiating between left and right hemispheric damage (Wedding 1979; Heilbronner et al. 1991; Lezak et al. 2004). Recently, there have been many studies of cerebral activity during the performance of cognitive tasks using brain imaging techniques such as near-infrared spectroscopy (NIRS), functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), and single photon emission computed tomography (SPECT). NIRS and fMRI are non-invasive methods for measuring brain activity. In studies using fMRI measurements, brain activity was mainly detected in the frontal lobe during TMT performance (Moll et al. 2002; Zakzanis et al.

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2005), and in NIRS measurement, frontal lobe activity was also noted during TMT performance (Shibuya-Tayoshi et al. 2007; Shoji et al. 2009). A difference in NIRS measurements during TMT performance was noted in a comparison of normal children and children with attention-deficit hyperactivity disorder (Weber et al. 2005). In addition, frontal lobe activity was confirmed using the Word Fluency Task, which evaluates the frontal lobe in a similar manner to TMT using NIRS (Kameyama et al. 2004).

Age, intelligence quotient (IQ), education, and gender may influence TMT performance (Davies 1968; Boll and Reitan 1973; Bornstein 1985; Wiederholt et al. 1993; Gaudino et al. 1995). However, the effects of gender on TMT performance are disputed (Heaton et al. 1986; Waldmann et al. 1992). Furthermore, posture may also contribute to oxy-Hb changes (Kurihara et al. 2003). In a previous study, we assessed TMT performance using NIRS in healthy adults in the twenties, consisting of college graduates and undergraduates (Shoji et al. 2009). The TMT is frequently used in clinical settings; therefore, it is important to understand the factors that affect TMT performance when interpreting the results. We often encounter patients who achieve better scores in cognitive tasks after they have acquired pattern-based skills or knowledge of the task. In this study, to aid the interpretation of TMT results, we studied the factors that affect TMT performance using NIRS.

Materials and Methods

Subjects

The subjects were 48 healthy right-handed adults who ranged in age from 20 to 29 years (24 males and 24 females, mean age of 22.8 ± 2.5 years, mean education years of 16.0 ± 1.2 years). They were college graduates or undergraduates. The protocol for this study was approved by the Ethics Review Board of Medical Research Division, Kanazawa University (No. 252), and the signed informed consent was obtained from each subject after the procedures had been explained. Their visual acuity was normal, and none of the patients had any medical history that would have influenced the data collected in this study, such as neurological disorders.

NIRS measurements

We measured the relative changes in the concentrations of oxygenated (oxy-Hb) and deoxygenated hemoglobin (deoxy-Hb). Total hemoglobin values were calculated by combining the two parameters following collection of NIRS data (ETG-4000; Hitachi Medical Corporation, Tokyo, Japan) during performance of the Trail Making Test. The ETG-4000 uses two wavelengths of near-infrared light, 695 nm and 830 nm. The distance between pairs of emission and detection probes was set at 3.0 cm, which enabled cerebral blood volume measurements at a depth of 2-3 cm from the scalp; i.e., the surfaces of the cerebral cortices (Hock et al. 1997; Toronov et al. 2001).

To measure the area of the brain on either hemisphere, we used two quadrats of 9 × 15 cm^2 in size that came with the machine. We placed 15 probes (8 emission and 7 detection probes) in each quadrat. Fig. 1A and 1B show a representative quadrat. It was possible to obtain measurements from 22 channels in each quadrat; therefore, we obtained measurements from 44 channels on each hemisphere. Of these channels, we decided to use the data from the 24 gray channels indicated in Fig. 2, which were used to monitor the prefrontal cortex.

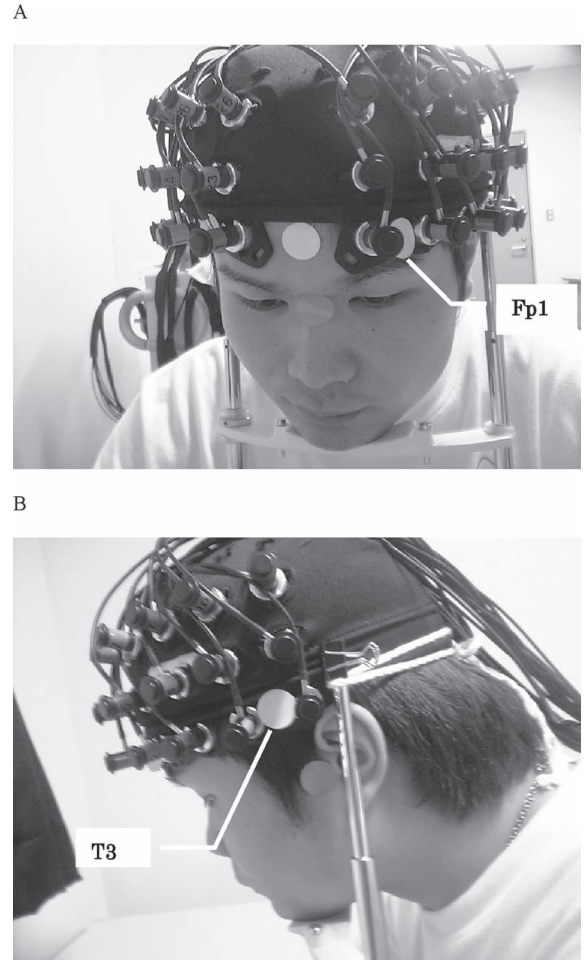

Fig. 1. Positions of the NIRS probes.
A, Front view; B, Side view; To unify the measurement sites, the lowest row of emission and detection probes was placed on a line connecting T3 with T4, which are described in the international 10-20 electrode system used for electroencephalography.

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Trail Making Test (TMT)

The TMT is commonly used in clinical settings to evaluate attention and executive functions. It consists of two forms, TMT-A and TMT-B (Reitan 1958; Lezak et al. 2004). In TMT-A, numbers from 1 to 25 are randomly scattered on a sheet, and the patients must a draw line through them in numerical order (Fig. 3A). This activity assesses attention, visual investigation, the velocity of synergic movement of the eyes and hands, and the velocity of information processing. In TMT-B, numbers from 1 to 13 and 12 Japanese characters are randomly scattered on a sheet, and the patients must draw a line that alternately passes through the figures and Japanese characters (Fig. 3B). In particular, TMT-B facilitates the assessment of an individual's ability to switch to the next task (executive function) and is useful

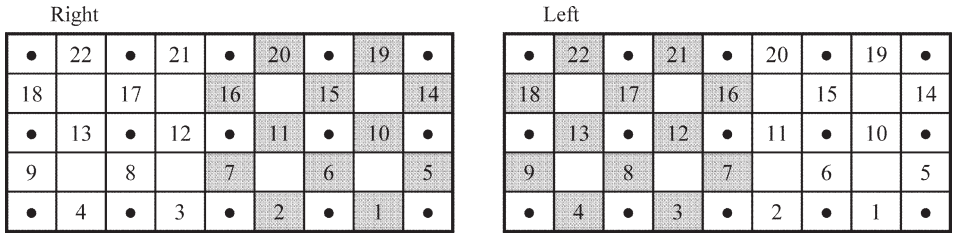


Fig. 2. The measurement channels.
In this research, we measured these gray parts as the frontal lobe.
●, emission and detection probes

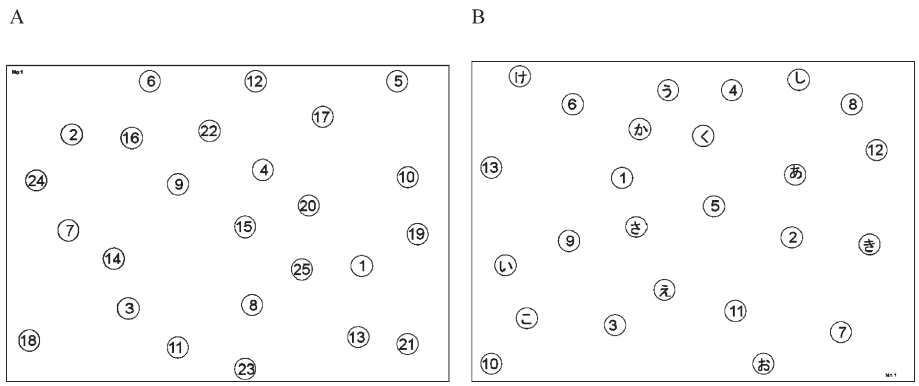


Fig. 3. Trail Making Test (TMT).
A, This test is TMT-A. In TMT-A, numbers from 1 to 25 are randomly scattered on a sheet, and the patients must a draw line through them in numerical order; B, This test is TMT-B. In TMT-B, numbers from 1 to 13 and 12 Japanese characters are randomly scattered on a sheet, and the patients must draw a line that alternately passes through the figures and Japanese characters.

for detecting frontal lobe dysfunction (Mitrushina et al. 1999).

In this study, we examined three factors during TMT performance. We named these three factors "knowledge of the TMT", "order of the TMT", and "gender". "Knowledge of the TMT" represents whether the subject had prior knowledge of the task (familiar or unknown). The "order of the TMT" represents the order in which TMT-A and TMT-B were performed and assesses the influence of pattern-based skills on TMT results. We confirmed whether the subjects were familiar with the TMT prior to administering the tests. The subjects were divided into 2 groups: those who were not familiar with the TMT (Unknown group) and those who were familiar with or had previously undergone the TMT (Known group). Both groups contained the same number of subjects. Concerning the order of the TMT, the subjects were assigned to initially perform the TMT-A or -B (Group A and Group B, respectively). Both groups contained the same number of subjects. The male-to-female ratio was 1:1 in all groups. The backgrounds of the subjects are shown in Table 1.

Tasks

The subjects were instructed to perform the TMT with a pencil at a desk while sitting in a quiet room, as is performed in clinical practice. Simultaneously, a probe for optical topography was used to measure brain activity. Screens were placed around each subject so that external visual and acoustic stimulation was blocked. As a previous study reported cephalic motion-related changes in cerebral blood flow (Kurihara et al. 2003), cephalic motion was restricted using a jaw rest.

Research design

The composition of the measurement session was as follows: first, we performed a 10-second pre-scan, which was followed by four 60-second rest periods and three 30-second performance periods in an alternating manner. We defined this as session 1. Session 1 lasted 340 seconds (Fig. 4). The subjects were instructed to gaze at a green point on the desk without moving their head during the rest period.

In the performance periods, we employed 3 tests: TMT-A, TMT-B, and a control. TMT-A and TMT-B were based on the Japanese version of the TMT, which was prepared by Kashima et al. (1986). Two random patterns were prepared for each test in accordance with the Japanese version of the TMT. A total of 3 patterns, including the Japanese version, were used, and a preliminary experiment demonstrated that there were no differences in their subjective difficulties or performance times.

In this study, we employed the random drawing of a line on a sheet of paper of the same size as the control in order to eliminate the influence of line-drawing movement. In the performance period, the subjects performed a similar task three times in 1 session. The subjects performed the tasks according to the randomly allocated order shown in Table 2. Sheets of paper were placed in front of the subjects immediately before the start of each task. The subjects initiated the TMT upon receiving the verbal signal "Please start". After 30 seconds, the subjects discontinued the TMT upon receiving the signal "Please stop". The examiner then collected the sheets. Each measurement session consisted of a total of 3 sessions (TMT-A, TMT-B, and control). Three-minute inter-session intervals were established,

Table 1. Detail of the subjects.

			The number of subjects	Age (years)	Education (years)
male	Known group	Group A	6	22.5 ± 1.0	16.5 ± 1.2
		Group B	6	22.8 ± 3.2	16.2 ± 1.5
	Unknown group	Group A	6	23.8 ± 3.0	15.7 ± 0.8
		Group B	6	24.2 ± 3.9	15.8 ± 1.3
female	Known group	Group A	6	23.0 ± 1.8	17.0 ± 1.7
		Group B	6	23.0 ± 2.2	16.2 ± 1.0
	Unknown group	Group A	6	21.3 ± 2.0	15.2 ± 0.4
		Group B	6	21.3 ± 1.4	15.3 ± 0.5
			48	22.8 ± 2.5	16.0 ± 1.2

The subjects were 48 healthy right-handed adults who ranged in age from 20 to 29 years. They were college graduates or undergraduates; Unknown group, those who were not familiar with the TMT; Known group, those who were familiar with or had previously undergone the TMT; Group A, the subjects were assigned to initially perform the TMT-A; Group B, the subjects were assigned to initially perform the TMT-B.

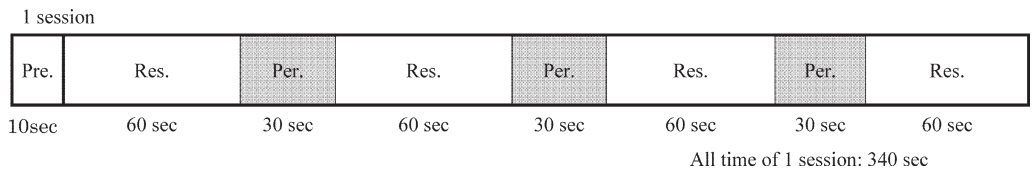


Fig. 4. The detail of one session about NIRS measurement.
The composition of the measurement session was as follows: first, we performed a 10 second pre scan (Pre.), which was followed by four 60-second rest periods (Res.) and three 30-second performance periods (Per.) in an alternating manner. We defined this as session 1. Session 1 lasted 340 seconds. In performance periods, subjects did a similar task three times in 1session.

Table 2. The combination with 6 patterns.

1	TMT-A — TMT-B — Control
2	TMT-B — Control — TMT-A
3	Control — TMT-A — TMT-B
4	TMT-B — TMT-A — Control
5	Control — TMT-B — TMT-A
6	TMT-A — Control — TMT-B

1, 3, and 6 are the pattern which does TMT-A earlier. 2, 4, and 5 are the pattern which does TMT-B earlier. Control is the tasks about the random drawing of a line on a sheet of paper in order to eliminate the influence of line-drawing movement.

during which the probes were worn.

Typically, when administering the TMT, the interval required to complete the tasks is usually recorded. However, in this study, the number of tasks that were accomplished within 30 seconds was recorded because NIRS was employed. We termed this the TMT score.

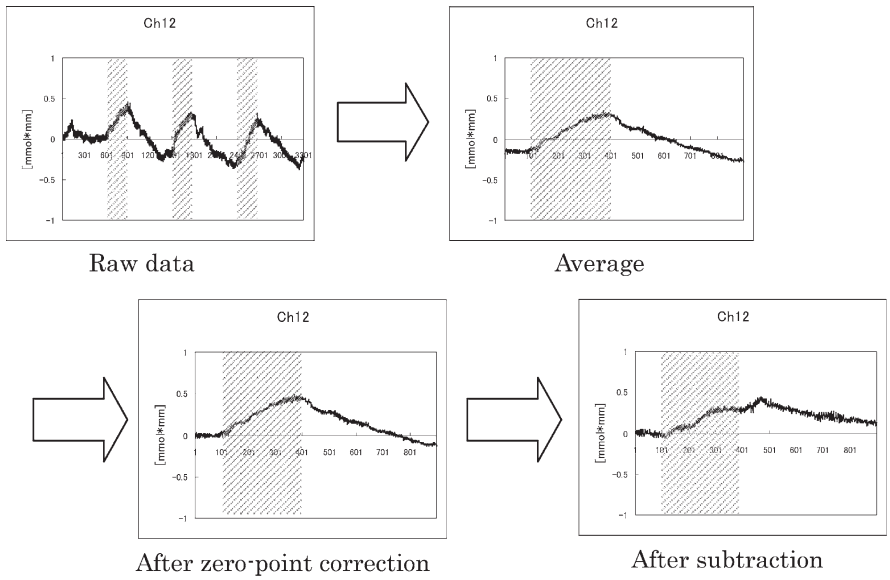
A previous study showed that the number of TMT-B tasks completed within 30 seconds was significantly smaller than that for TMT-A (Shoji et al. 2009). This was consistent with the finding that the TMT-B requires a longer performance time (Moll et al. 2002). Therefore, a similar method was employed in this study.

NIRS data analysis

Initially, we collected data about the rates of change in the oxy-Hb level for the 10 seconds before the start of the test, during the 30 seconds of the test, and the 50 seconds after its completion three times and then averaged these data. Subsequently, zero-point correction was conducted so that the mean rate of change for the 10 seconds before the start of the test was set to zero. The rate of change in the oxy-Hb level observed during the control part of the test, which was calculated in the same manner, was subtracted from the corrected value. Subsequently, the mean rate of change in the oxy-Hb level for the 30 seconds of the test was calculated for each channel (Fig. 5).

Statistical analysis

We compared the mean number of TMT-A/-B tasks completed (TMT score) by all subjects and among the groups divided according to knowledge of the TMT, order of the TMT, and gender using the Student's *t*-test, with $p = 0.05$ set as the significance threshold. The mean changes in oxy-Hb level during TMT-A/-B performance for each channel were calculated for each subject (*t*-test for the null hypothesis $H_0 : \mu = 0$). Multiple regression analysis was performed to investigate oxy-Hb changes using knowledge of the TMT, order of the TMT, and gender as independent variables. Statistical analysis was performed with JMP6 software (SAS Institute, Cary, North Carolina).



→The mean rate of change in the oxy-Hb level for 30 seconds after starting was calculated at each channel.

Fig. 5. The processing method of the NIRS data.
Average = (the rates of change in the oxy-Hb level for the 10 seconds before the start of the test, the 30 seconds of the test, and the 50 seconds after its completion amount three times)/3; After zero-point correction = (Average) – (the mean rate of change in the oxy-Hb level for the 10 seconds before the start of the test); After subtraction = (After zero-point correction) – (the mean rate of change in the oxy-Hb level during the control part of the test). The gray hatching parts are performance periods.

Table 3. The mean of TMT score within 30 seconds in all subjects.

TMT score (mean ± S.D.)		*t* value	*p* value
TMT-A (*n* = 48)	TMT-B (*n* = 48)		
16.9 ± 2.9	13.6 ± 2.9	−8.93529	< 0.0001

Table 4. The mean TMT score of the knowledge of TMT.

task	TMT score (mean ± S.D.)		*t* value	*p* value
	Unknown group (*n* = 24)	Known group (*n* = 24)		
TMT-A	16.8 ± 2.7	17.1 ± 3.2	0.402165	0.6913
TMT-B	12.9 ± 2.6	14.3 ± 3.0	1.58528	0.1266

Results

TMT score

The mean scores ± standard deviation during the TMT-A and -B were 16.9 ± 2.9 and 13.6 ± 2.9, respectively, and were significantly different ($t = -8.93529$, $p < 0.0001$) (Table 3). Most subjects reported that the TMT-B was more difficult between the two tests. With respect to knowledge of the TMT, the mean TMT-A scores in the Known group and Unknown group were 17.1 ± 3.2 and 16.8 ± 2.7, respectively. There was no significant difference between the groups ($t = 0.402165$, $p = 0.6913$) (Table 4). Concerning the order of the TMT, the mean TMT-A scores were 17.0 ± 3.0 and 17.0 ± 2.9 in Group A and Group B, respectively. There was no significant difference between the groups ($t = -0.03361$, $p = 0.9735$) (Table 5). With respect to gender, the mean TMT-A scores were 16.7 ± 3.0 and 17.2 ± 2.9 in males and females, respectively, revealing no significant difference ($t = 0.435649$, $p = 0.6672$) (Table 6).

With respect to the knowledge of TMT, the mean TMT-B scores were 14.3 ± 3.0 and 12.9 ± 2.6 in the Known group and Unknown group, respectively. There was no significant difference between the groups ($t = 1.58528$, $p = 0.1266$) (Table 4). Concerning the order of the test, the mean TMT-B scores were 14.5 ± 2.8 and 12.6 ± 2.7 in Group-A and B, respectively, showing a significant difference ($t = -2.42374$, $p = 0.0236$) (Table 5). With respect to gender, the mean TMT-B scores were 13.2 ± 2.6 and 14.0 ±

Table 5. The mean TMT score of the order of TMT.

task	TMT score (mean ± S.D.)		*t* value	*p* value
	Group A (n = 24)	Group B (n = 24)		
TMT-A	17.0 ± 3.0	17.0 ± 2.9	−0.03361	0.9735
TMT-B	14.5 ± 2.8	12.6 ± 2.7	−2.42374	0.0236

Table 6. The mean TMT score of the gender.

task	TMT score (mean ± S.D.)		*t* value	*p* value
	Male (n = 24)	Female (n = 24)		
TMT-A	16.7 ± 3.0	17.2 ± 2.9	0.435649	0.6672
TMT-B	13.2 ± 2.6	14.0 ± 3.2	1.020134	0.3183

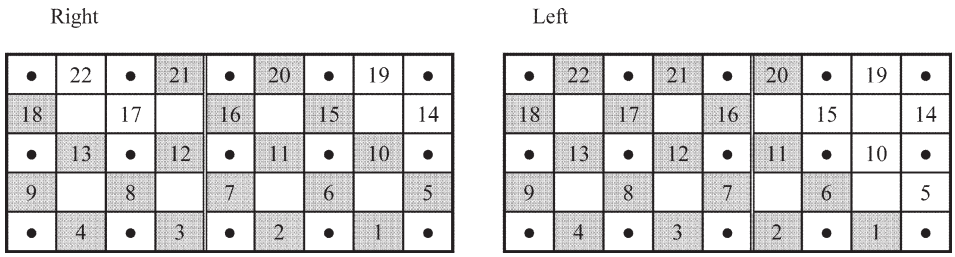


Fig. 6. Channels which are doing activation in TMT-A.
Gray parts are the Channels that oxy-Hb increase was confirmed ($p < 0.05$); ●, emission and detection probes.

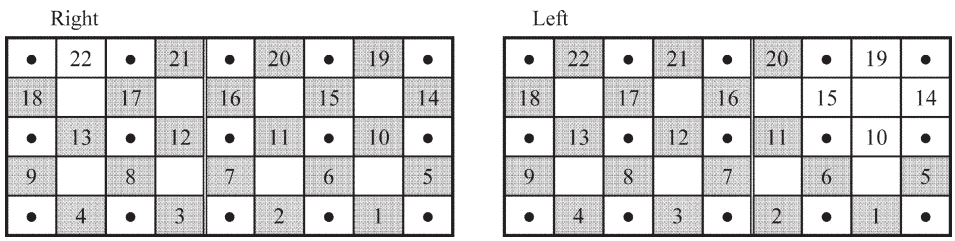


Fig. 7. Channels which are doing activation in TMT-B.
Gray parts are the Ch that oxy-Hb increase was confirmed ($p < 0.05$); ●, emission and detection probes.

3.2 in males and females, respectively, showing no significant difference (t = 1.020134, p = 0.3183) (Table 6).

Changes in the oxy-Hb level during TMT performance

During TMT-A performance, there were significant increases in the oxy-Hb level in comparison with that observed before the start of the test at all channels except channels (Ch) 5, 10, 14, 15, and 19 on the left side (t = 2.1758-5.3459, p = 0.035-0.0001). On the right side, the oxy-Hb level was also significantly increased at all channels except Ch 14, 17, 19, and 22 (t = 2.1479-4.2469, p = 0.0369-0.0001) (Fig. 6). Thus, during TMT-A performance, oxy-Hb increases were seen in many channels monitoring the bilateral frontal lobe.

During TMT-B performance, there were significant increases in the oxy-Hb level in comparison with that before the start of the test at all channels except Ch 10, 14, 15, and 19 on the left side (t = 2.2683-5.6327, p = 0.0279-0.0001). On the right side, the oxy-Hb level was also significantly increased at all channels except Ch 22 (t = 2.6230-4.8488, p = 0.0118-0.0001) (Fig. 7). Thus, in TMT-B performance, oxy-Hb increases were seen in many of the channels monitoring the bilateral frontal lobe.

Changes in the oxy-Hb level with respect to knowledge of the TMT, order of the TMT, and gender

For the TMT-A, multiple regression analysis was performed using 3 factors, knowledge of the TMT, order of the TMT, and gender, as independent variables. Significant changes were detected at Ch 2, 3, and 4 on the left side and

Table 7. The result according to the factors in TMT-A (knowledge of TMT).

The factor	Channel		β value	p value
Knowledge of TMT [Unknown group]	right	2	0.0321277	0.0427
		13	0.0361667	0.0239
	left	2	0.0499924	0.014
		3	0.0453533	0.0312
		4	0.0411926	0.0197

The table shows Channels that a significant difference was attended. Underlined Channels correspond to prefrontal cortex.

Table 8. The result according to the factors in TMT-A (order of TMT).

The factor	Channel		β value	p value
Order of TMT [Group A]	right	12	0.0432522	0.0293

The table shows Channels that a significant difference was attended.

Table 9. The result according to the factors in TMT-B (knowledge of TMT).

The factor	Ch		β value	p value
Knowledge of TMT [Unknown group]	Right	4	0.0535119	0.0116
		8	0.0295528	0.0492
		9	0.0478512	0.0174
		13	0.0396766	0.0085
		18	0.0299352	0.0126
	Left	1	0.03092	0.0497

The table shows Channels that a significant difference was attended.

at Ch 2, 12, and 13 on the right side. Of these channels, the presence or absence of knowledge of the TMT influenced oxy-Hb changes at Ch 2, 3, and 4 on the left side (β = 0.0411926-0.0499924, p = 0.0312-0.014) and at Ch 2 and 13 on the right side (β = 0.0321277 and 0.0361667, p = 0.0427 and 0.0239, respectively) (Table 7). The oxy-Hb level was significantly higher in the Unknown group than in the Known group. Furthermore, the order of the TMT influenced the oxy-Hb changes at Ch 12 on the right side (β = 0.0432522, p = 0.0293) (Table 8). The oxy-Hb level was significantly higher in Group A than in Group B. Gender had no influence on oxy-Hb changes.

In TMT-B, multiple regression analysis was performed using 3 factors, knowledge of the TMT, order of the TMT, and gender, as independent variables. Significant changes were detected at Ch 1, 11, and 22 on the left side and at Ch 1, 2, 4, 5, 6, 8, 9, 11, 13, and 18 on the right side. Of these channels, knowledge of the TMT influenced oxy-Hb changes at Ch 1 on the left side (β = 0.03092, p = 0.0497) and at Ch 4, 8, 9, 13, and 18 on the right side (β = 0.0295528-0.0535119, p = 0.0492-0.0085) (Table 9). In the Unknown group, the oxy-Hb level was significantly higher than in the Known group. Furthermore, the order of the TMT influenced oxy-Hb changes at Ch 1, 2, 5, 6, 9, 11, and 18 on the right side (β = 0.0295236-0.0462721, p = 0.0394-0.0065) (Table 10). The oxy-Hb level was significantly higher in Group B than in Group A. At Ch 11 and 22 on the left side, gender influenced oxy-Hb changes (β = 0.0195985 and 0.0405865, p = 0.0473 and 0.0405, respectively) (Table 11). The oxy-Hb level was significantly higher in males than in females.

Discussion

In this study, we found that the mean number of TMT-B tasks completed within 30 seconds was significantly lower than that for TMT-A tasks. The subjective difficulty of the TMT-B was higher than that of the TMT-A. During TMT-A and -B performance, there was a significant increase in the oxy-Hb level in comparison with that observed before the start of the task at each channel. This is consistent with the results of previous studies (Shibuya-Tayoshi et al. 2007; Shoji et al. 2009). The results of this study also suggest that the frontal lobe is activated during TMT performance.

Moreover, we investigated whether knowledge of the TMT, order of the TMT, or gender affected TMT performance and/or brain activity. There were no TMT knowledge-related differences in the TMT-A or -B scores. However, multiple regression analysis showed that knowledge of the TMT influenced oxy-Hb changes during TMT-

Table 10. The result according to the factors in TMT-B (order of TMT).

The factor	Channel		β value	p value
Order of TMT [Group B]	Right	1	0.0404076	0.0065
		2	0.0358615	0.0282
		5	0.0378469	0.0182
		6	0.0308594	0.0394
		9	0.0462721	0.0212
		11	0.0295236	0.0356
		18	0.0308004	0.0104

The table shows Channels that a significant difference was attended. Underlined Channels correspond to prefrontal cortex.

Table 11. The result according to the factors in TMT-B (gender).

The factor	Channel		β value	p value
Gender [male]	Left	11	0.0405865	0.0473
		22	0.0195985	0.0405

The table shows Ch that a significant difference was attended. An underlined Channel corresponds to prefrontal cortex.

A/-B performance. The oxy-Hb level was not significantly higher in the Unknown group than in the Known group in the frontal lobe; however, in other areas of the brain, oxy-Hb level was significantly higher in the Unknown group than in the Known group. This suggests that knowledge of the TMT influences brain activity, but not the TMT-A or -B score.

With respect to knowledge of the TMT, subjects who had not experienced the test were not familiar with the TMT patterns. However, those who did have previous knowledge of the test were familiar with the patterns, and so the presence or absence of this knowledge may have contributed to differences in the patients' strategies for, and ability to perform the tests. As a result, a more extensive area of the brain may have to be examined. Executive function is regarded as a complex cognitive function involving volition, planning purposive action, and effective performance (Lezak et al. 2004). This function is necessary to establish a strategy for completing the task. Although there was no difference in TMT results, the brain activities of the patients in the Known group and Unknown group differed, possibly because there was a difference in executive function between these subjects. The results of this study suggest that brain activity monitoring during TMT performance facilitates a more accurate assessment of executive function.

Concerning the order of the TMT, in Group B, the TMT-B score was significantly lower than in Group A. Multiple regression analysis indicated that the order of the TMT was a factor influencing oxy-Hb changes. During TMT-A performance, the oxy-Hb level in Group A was significantly higher than that in Group B at some channels, whereas during TMT-B performance the oxy-Hb level in Group B was significantly higher than that in Group A at some channels. This suggests that the order of the test influences the TMT score and brain activity during TMT performance.

Typically, the TMT-A is performed first, followed by the TMT-B. However, in this study, a reverse order was introduced because we speculated that pattern-based skills might influence TMT performance. For example, when performing TMT-B after TMT-A, it might be easier to complete the TMT-B task using the same method as employed for TMT-A. Kameyama et al. (2004) first indicated that oxy-Hb changes were more marked in patients who had completed fewer patterns in the Word Fluency Task. They assumed that such patients were unable to complete the tests efficiently, leading to cerebral activation due to an excess level of stress. Ehlis et al. (2005) reported that finding the test difficult increased blood flow in the cerebral cortex. Based on these studies, the order of the TMT might have influenced the level of difficulty of the TMT-A/-B, resulting in differences in the number of patterns accomplished and oxy-Hb variations.

When reviewing oxy-Hb changes, we found that in Group B the oxy-Hb level at many channels in the frontal lobe during TMT-B performance was significantly higher than that in Group A. This reflects the characteristic function of the frontal lobe, especially that of the prefrontal cortex.

The prefrontal cortex is only activated when learning new activities. In the presence of an effective learning capacity, the level of activation in this area does not differ from that at rest. The lateral region of the anterior motor area is activated during the learning of new activities. In contrast, the supplementary motor area is more markedly

activated during the performance of previously learned skills (Hashimoto 2007). Thus, the prefrontal cortex does not respond to skill use; therefore, in Group A, in which the TMT-A was performed first, TMT-B was conducted after the subjects had become accustomed to the TMT, whereas in Group B, the TMT-B, which was considered to be the harder task, was performed first, which may have led to significant differences in the oxy-Hb level due to varying levels of stress.

There were no marked differences in the TMT-A score or the rate of change in oxy-Hb levels among the groups. This was possibly due to the TMT-A consisting of tasks that are simple to complete for healthy adults and so produced no difference related to the presence or absence of skill. These parameters may show differences in elderly individuals, in whom TMT-A performance time is significantly longer than in young individuals, as well as those with frontal lobe injuries.

With respect to gender, there were no marked differences in the TMT-A score or the rate of change in oxy-Hb levels. However, multiple regression analysis of alterations in oxy-Hb revealed that gender influenced oxy-Hb changes during TMT-B performance. In males, the oxy-Hb level was significantly higher than in females at some channels. Previous studies of TMT indicated that gender influenced the TMT score (Wiederholt et al. 1993; Gaudino et al. 1995). Furthermore, another study examined brain activity during performance of the Word Fluency Task, which can be used to evaluate frontal lobe function, using NIRS and confirmed that oxy-Hb levels in the frontal and temporal lobes were significantly higher in males than in females (Kameyama et al. 2004). According to another study, brain activity during the test differed between males and females (Jausovec and Jausovec 2009). In this study, there was no gender difference in the number of tasks completed. During TMT-B performance, oxy-Hb levels in males were significantly higher than that in females, as reported previously. However, this finding was only noted at 2 channels (frontal lobe: only 1 channel) on the left side. This result is consistent with the results of a study using the Word Fluency Task (Kameyama et al. 2004). However, marked gender differences were only observed at a few channels, suggesting that the influence of gender on brain activity during TMT performance is less marked than that of other factors.

In this study, during TMT-B performance, factor-related differences in the right frontal lobe were more marked than those in the left frontal lobe. Concerning frontal lobe laterality, the right frontal lobe is involved in negative emotions, and the left frontal lobe is associated with positive emotions (Harmon-Jones 2004). In patients who have suffered left frontal lobe injury, depression is significantly more severe than in those with injuries at other sites (Robinson et al. 1984). The results of this study showed that brain activity was significantly enhanced in the Unknown group and Group-B, in which TMT-B was performed first, in comparison with the other groups. This was possibly because performing the more difficult TMT-B was stressful for subjects who were not familiar with the TMT. This may have caused the subjects to experience negative emotions, resulting in significant activity in the right frontal lobe. However, all subjects reported that the TMT-B was more difficult than the TMT-A. In this study, we did not examine emotions during TMT-B performance. In future, the emotions induced by TMT performance should be examined.

The results of this study suggest that knowledge of the TMT influences alterations in oxy-Hb levels during TMT-A and -B performance, and that the order of the TMT and gender also affect these changes. In addition, the order of the test may influence TMT-B score. Therefore, these 3 factors, in addition to age and educational background, may influence TMT score and brain activity during TMT performance. We are convinced that the discovery of these three factors provides meaningful information that will aid the interpretation of TMT results.

Furthermore, a review of brain activity during TMT performance would be useful for clarifying brain function, which cannot be clarified based on the results of desk-based tests alone.

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