# Chapter 39
# *Ginkobiloba* Extract Improves Working Memory Performance in Middle-Aged Women: Role of Asymmetry of Prefrontal Cortex Activity During a Working Memory Task

**Kaoru Sakatani, Masahiro Tanida, Naoyasu Hirao, and Naohiro Takemura**

**Abstract** In order to clarify the mechanism through which extract of *Ginkgo biloba* leaves (EGb) improves cognitive function, we examined the effects of EGb on cerebral blood oxygenation in the prefrontal cortex (PFC) and on performance during a working memory task, using near-infrared spectrometry (NIRS). First, we evaluated differences in behavioral performance of the Sternberg working memory test (ST) and in the activation pattern of the PFC during ST between 15 young and 19 middle-aged healthy women. Then, we examined the effect of EGb (120 mg/day for 6 weeks) on ST performance and PFC activation pattern in the middle-aged group. The middle-aged group exhibited a longer reaction time (RT) in ST than the young group and showed a different PFC activation pattern during ST, i.e., the middle-aged group showed bilateral activation while the young group showed right-dominant activation. In the middle-aged group, administration of EGb for 6 weeks shortened the RT of ST and changed the PFC activation pattern to right-dominant, like that in the young group. The results indicate the PFC plays a role in the physiological

K. Sakatani (✉)
Laboratory of Integrative Biomedical Engineering, Department of Electrical and Electronics Engineering, College of Engineering, Nihon University, 1 Nakagawara, Tokusada, Tamuramachi, Koriyama, Fukushima Prefecture 963-8642, Japan

Department of Neurological Surgery, Nihon University School of Medicine, Tokyo, Japan
e-mail: sakatani.kaoru@nihon-u.ac.jp

M. Tanida • N. Hirao
Shiseido Research Center, Yokohama, Japan
e-mail: masahiro.tanida@to.shiseido.co.jp

N. Takemura
Laboratory of Integrative Biomedical Engineering, Department of Electrical and Electronics Engineering, College of Engineering, Nihon University, Koriyama, Fukushima 963-8642, Japan
e-mail: naohiro.takemura@gmail.com

H.M. Swartz et al. (eds.), *Oxygen Transport to Tissue XXXVI*, Advances in Experimental Medicine and Biology 812, DOI 10.1007/978-1-4939-0620-8_39,

cognitive function-enhancing effect of EGb. EGb might improve working memory function in middle-aged individuals by counteracting the occurrence of aging-related hemispheric asymmetry reduction.

**Keywords** Ginkobiloba • Near-infrared spectroscopy • Prefrontal cortex • Cerebral blood oxygenation • Sternberg working memory test • Reaction time

## 1 Introduction

A standardized preparation of extract of the leaves of the *Ginkgo biloba* tree (EGb) is well known to alleviate symptoms of many cognitive disorders, including Alzheimer's disease or multi-infarct dementia [1]. EGb also improves cognitive functions in ageing healthy populations [2] and middle-aged subjects [3]. However, the mechanism of the effect of EGb on cognitive function, particularly the role of the prefrontal cortex, remains unclear.

Here, we examined the effect of EGb on bilateral prefrontal cortex (PFC) activity associated with working memory performance, using near-infrared spectroscopy (NIRS). We focused on the hemispheric asymmetry of PFC activity to explore the cognitive function-enhancing properties of EGb. Hemispheric asymmetry of PFC activity is well known to be reduced or eliminated during ageing [4]. Employing NIRS, we evaluated the PFC activity of young and middle-aged women while they performed a Sternberg-type working memory task [5]. Then, we evaluated the effect of EGb on the age-related change in the PFC activity pattern associated with a cognitive performance task.

## 2 Methods

We studied 15 young (aged 20–22 years, mean 21.8 ± 0.7 years) and 19 middle-aged (aged 44–50 years, mean 46.8 ± 1.6 years) healthy women. The subjects were all deemed right-handed according to the laterality quotient questionnaire of the Edinburgh Handedness Inventory. All subjects provided written informed consent as required by the Human Subjects Committee of the Shiseido Life Science Institute.

### *2.1 Experimental Protocol*

We employed the modified Sternberg test as a working memory task [5]. We used 2-channel time-resolved spectroscopy (TRS); the sampling rate was once per second, and the source-detector distance was 3 cm. Details of the TRS (TRS-20, Hamamatsu Photonics K.K., Japan) have been reported [6]. The concentrations of Hb were

expressed in μM. The NIRS probes were set symmetrically on the forehead; MRI confirmed that the emitter-detector was located over the dorsolateral and frontopolar areas of the PFC.

The present study consisted of two phases. First, we evaluated the difference in working memory between the young and middle-aged groups. We compared the behavioral performance on the Sternberg test and the PFC activity evoked by the Sternberg test between the two groups. We evaluated reaction time (ms) as a measure of behavioral performance in the Sternberg test. In the second phase of the study, we evaluated the effect of a putatively cognitive function-enhancing supplement on the working memory of the middle-aged women. For this, we administered SuppleX (Shiseido, Japan), which contains EGb (120 mg), for 6 weeks. After 6 weeks, we compared the behavioral performance in the Sternberg test and the PFC activity evoked by the Sternberg test between the supplement ($n=9$) and placebo ($n=10$) groups. The present study was conducted as a single blind randomized controlled trial.

### *2.2 Data Analysis*

The evoked cerebral blood oxygenation (CBO) changes in the bilateral PFC were continuously monitored by TRS and were averaged every second during: (1) baseline conditions for 60 s; (2) the Sternberg test for 60–80 s (the period varied according to each subject's reaction time); and (3) recovery for 60 s. To analyze PFC activity in response to task performance, we calculated changes in oxy-Hb concentration during the Sternberg test. The mean baseline values (measured during 60 s) were subtracted from the mean activation values (measured during the first 60 s during task performance).

In order to determine left/right asymmetry of PFC activity during the working memory task, we calculated the right laterality score of Δoxy-Hb (right Δoxy-Hb − left Δoxy-Hb; positive values indicate greater activity of the right PFC, while negative values indicate greater activity of the left PFC.

For evaluation of the statistical significance of differences, two-way analysis of variance (ANOVA) was conducted with within-subject factors of treatment (control and Gingko supplement) and test period (pre and post).

## 3 Results

There was no significant difference in accuracy in the Sternberg test between the young and middle-aged groups ($p=0.47$). However, the reaction time of the middle-aged group was significantly longer than that of the young group ($p<0.0005$). TRS demonstrated increases of oxy-Hb and total Hb(t-Hb) associated with a decrease of deoxy-Hb in the bilateral PFC during performance of the Sternberg test in both the

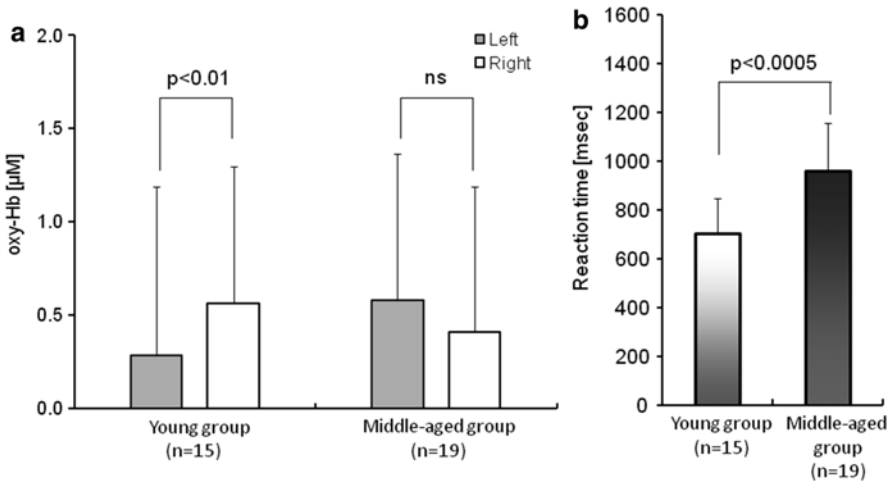


**Fig. 39.1** (**a**) Comparison of oxy-Hb changes between the right and left PFC during ST in the young and middle-aged groups. The right PFC exhibited a larger change of oxy-Hb than the left PFC in the young group, but there were no significant difference in the middle-aged group. (**b**) Comparison of reaction time of 6-digit ST between the young and middle-aged groups. The middle-aged group showed a significantly longer mean reaction timethan the young group ($p < 0.0005$)

young and middle-aged groups (Fig. 39.1). There was a significant difference in the right laterality score of Δoxy-Hb between the young and middle-aged groups ($p < 0.05$).

There are significant treatment × period interactions in both RT [($F(1, 17) = 5.79$, $p < 0.05$)] and laterality ratio [($F(1, 17) = 5.47$, $p < 0.05$)]. The main effects seen in the treatment and period comparisons were as follows: Administration of the supplement significantly decreased the reaction time in the Sternberg test ($F(1, 17) = 5.47$, $p < 0.05$), while there was no significant change of reaction time in the placebo group among the middle-aged subjects ($F(1, 17) = 1.14$, $p = 0.30$) (Fig. 39.2a). In addition, the right laterality score of Δoxy-Hb was significantly increased after administration of the supplement ($F(1, 17) = 11.14$, $p < 0.005$). In contrast, the placebo group exhibited no significant change in right laterality score (Fig. 39.2b).

Finally, we evaluated the baseline concentrations of oxy-Hb, deoxy-Hb, and t-Hb before and after administration of the supplement (Table 39.1). The baseline concentrations of Hb did not change significantly after administration of the supplement ($p > 0.05$).

## 4 Discussion

The present findings indicate that the PFC plays a role in the physiological cognitive function-enhancing effect of EGb in humans. TRS demonstrated differences in PFC activation pattern during the Sternberg test between the middle-aged and young

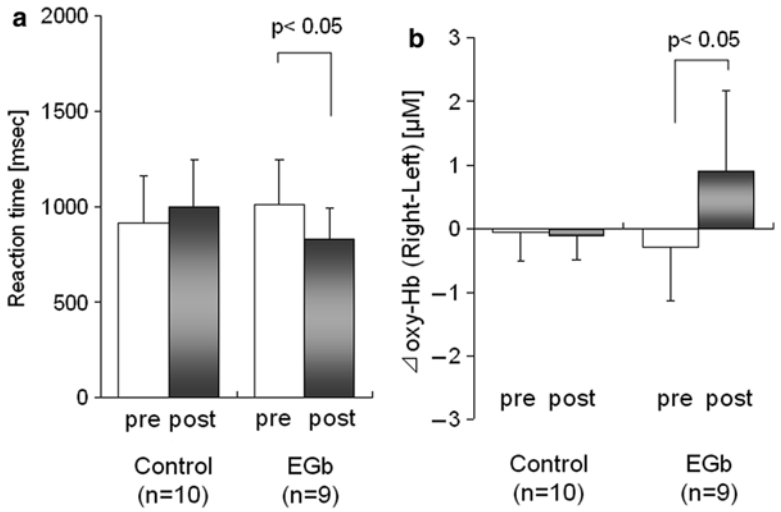


**Fig. 39.2** Effects of EGb for 6 weeks on the reaction time of the Sternberg test (**a**) and the right laterality score of Δoxy-Hb in the middle-aged group (**b**)

**Table 39.1** Differences in Hb concentrations under a resting condition before and after administration of the supplement

| Hb concentration (μM) | Before | After |
|---|---|---|
| Oxy-Hb (L) | 39.3 ± 4.9 | 39.9 ± 4.2 |
| Oxy-Hb (R) | 38.7 ± 4.8 | 37.8 ± 5.1 |
| Deoxy-Hb (L) | 19.8 ± 1.8 | 20.1 ± 2.0 |
| Deoxy-Hb (R) | 19.2 ± 1.9 | 19.1 ± 1.3 |
| t-Hb (L) | 59.1 ± 5.0 | 59.9 ± 4.4 |
| t-Hb (R) | 57.8 ± 4.5 | 56.9 ± 5.3 |

groups; the middle-aged group showed bilateral activation, while the young group exhibited right-dominant activation. It has been reported that working memory tasks cause asymmetrical PFC activation in younger adults, while older adults tended to exhibit a reduction in hemispheric lateralization (i.e., reduced asymmetry) [4]. It has also been reported that right-lateralized PFC activity is associated with more successful cognitive performance [7].

The reduction of asymmetry in PFC activity is known as HAROLD (hemispheric asymmetry reduction in older adults) [4]. Interestingly, we found that administration of the supplement changed the activation pattern of PFC in the middle-aged group during the Sternberg test to right-dominant, resembling the pattern observed in the young group. These observations suggest that EGb might improve working memory function in middle-aged subjects by counteracting the occurrence of HAROLD. However, it should be noted that such reduction of hemispheric asymmetry in older adults can be also explained by the CRUNCH (compensation-related utilization of neural circuits hypothesis) model [8].

TRS allows measurement of baseline concentrations of Hb under a resting condition. In the present study, TRS demonstrated that the baseline concentration of Hb in the PFC did not change after administration of the supplement for 6 weeks, suggesting that vascular effects such as cerebral vasorelaxation and reduction of blood viscosity [9] might not be involved in the effect of the supplement on PFC activity during the Sternberg test. Hadjiivanova and Petkov demonstrated that administration of EGb induced a significant decrease in the density of beta-adrenoreceptors in the frontal cortex and hippocampus in the rat [10]. Silberstein et al. investigated the chronic effects of EGb on steady state visually evoked potential (SSVEP) topography in healthy middle-aged subjects whilst completing an object working memory task, and found that EGb increased the SSVEP amplitude at frontal sites during the hold component of the task, suggesting that EGb may induce more efficient processing during the task performance [11]. Such neurotransmitter system effects might be involved in the cognitive enhancement in normal adults.

Finally, the limitations of the present study need to be considered. *First, we measured neuronal activity only in the PFC in the present study*. Therefore, we could not evaluate the effect of EGb on other brain regions. Second, we did not evaluate the effect of the supplement containing EGb on the young group. It is necessary to evaluate the neural correlates of EGb in normal subjects of various ages, as well as in patients with cognitive dysfunction. Therefore, it should be emphasized that further work is needed to characterize the influence of EGb on brain function.

**Acknowledgements** This research was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Sciences and Technology of Japan (B23300247), and grants from Alpha Electron Co., Ltd. (Fukushima, Japan) and Iing Co., Ltd. (Tokyo, Japan).

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