NeuroImage 50 (2010) 1702–1710

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Acute moderate exercise elicits increased dorsolateral prefrontal activation and improves cognitive performance with Stroop test

Hiroki Yanagisawa [a], Ippeita Dan [b], Daisuke Tsuzuki [b], Morimasa Kato [a], Masako Okamoto [b], Yasushi Kyutoku [b], Hideaki Soya [a,*]

[a] Laboratory of Exercise Biochemistry, University of Tsukuba Graduate School of Comprehensive Human Sciences, 1-1-1 Tennodai, Tsukuba 305-8574, Japan
[b] National Food Research Institute, 2-1-12 Kannondai, Tsukuba 305-8642, Japan

ARTICLE INFO

Article history:
Received 10 July 2009
Revised 1 December 2009
Accepted 3 December 2009
Available online 16 December 2009

Keywords:
Optical topography
Cognitive performance
Acute moderate intensity exercise
Color-word matching Stroop task
Dorsolateral prefrontal cortex

ABSTRACT

A growing number of human studies have reported the beneficial influences of acute as well as chronic exercise on cognitive functions. However, neuroimaging investigations into the neural substrates of the effects of acute exercise have yet to be performed. Using multichannel functional near-infrared spectroscopy (fNIRS), we sought cortical activation related to changes in the Stroop interference test, elicited by an acute bout of moderate exercise, in healthy volunteers ($N=20$). The compactness and portability of fNIRS allowed on-site cortical examination in a laboratory with a cycle ergometer, enabling strict control of the exercise intensity of each subject by assessing their peak oxygen intake ($\dot{V}o_{2peak}$). We defined moderate exercise intensity as 50% of a subject's peak oxygen uptake (50%$\dot{V}o_{2peak}$). An acute bout of moderate exercise caused significant improvement of cognitive performance reflecting Stroop interference as measured by reaction time. Consistent with previous functional neuroimaging studies, we detected brain activation due to Stroop interference (incongruent minus neutral) in the lateral prefrontal cortices in both hemispheres. This Stroop-interference-related activation was significantly enhanced in the left dorsolateral prefrontal cortex due to the acute bout of moderate exercise. The enhanced activation significantly coincided with the improved cognitive performance. This suggests that the left dorsolateral prefrontal cortex is likely the neural substrate for the improved Stroop performance elicited by an acute bout of moderate exercise. fNIRS, which allows physiological monitoring and functional neuroimaging to be combined, proved to be an effective tool for examining the cognitive effects of exercise.

Introduction

A body of human and animal studies have reported the beneficial influence of exercise on cognitive and brain functions. Accordingly, exercise is drawing increasing research attention as a possible lifestyle factor for improving neurocognitive functions, and preventing or delaying dementia (Cotman et al., 2007; Hillman et al., 2008).

So far, the majority of studies have focused on the chronic effects of exercise, while studies on acute exercise effects on cognition have only started to draw growing attention (Tomporowski, 2003). Recent studies provide evidence that an acute bout of moderate aerobic exercise improves cognitive performance in a choice reaction task (Chmura et al., 1998), a simple reaction time task (Collardeau et al., 2001), as well as confliction tasks such as Eriksen flanker and Stroop tasks (Hogervorst et al., 1996; Kamijo et al., 2004, 2007).

Keeping pace with the examination of the cognitive effects of acute exercise, the search for their neural substrates has been accelerated mainly in the field of event-related potential (ERP) research. P300 (or P3) is a component believed to indicate the brain activity required to maintain working memory when the mental model of the stimulus environment is updated (Donchin & Coles, 1988), and is thus regarded as an appropriate neural substrate for improved cognitive performance. Several studies have generally demonstrated increased amplitude and shortened latency of P300 components in relation to the performance improvements caused by an acute bout of exercise (Hillman et al., 2003, 2008; Kamijo et al., 2004, 2007; Magnie et al., 2000; Nakamura et al., 1999; Polich and Lardon, 1997).

ERP provides high temporal information about brain activities, but it provides only rough information regarding where in the brain the effect was originated. In order to examine which brain regions change activation in response to exercise, the application of different neuroimaging techniques would be beneficial. As a promising neuroimaging technique for investigating the acute effects of exercise on cognition, we introduced functional near-infrared spectroscopy (fNIRS): an optical method that non-invasively monitors cerebral hemodynamics by measuring changes in the attenuation of near-infrared light passing through tissue (Koizumi et al., 2003; Obrig and Villringer, 2003; Villringer and Chance, 1997). In many studies, fNIRS has proven to be effective in assessing oxygenation changes in

* Corresponding author.
E-mail address: hsoya@taiiku.tsukuba.ac.jp (H. Soya).

1053-8119/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2009.12.023

response to cortical activities, utilizing the tight coupling between neuronal activity and regional cerebral blood flow. In contrast to other neuroimaging methods, fNIRS requires only compact experimental systems, is portable, and can be easily installed in a gym (Timinkul et al., 2008) (Fig. 1). This is advantageous for our study as exercise intensity can be strictly controlled using gym facilities, and on-site neuroimaging allows precise control of the interval between exercise and brain measurement. Moreover, since fNIRS allows for the least restrictive measuring environment among neuroimaging modalities, possible influences on cognitive tasks can be kept minimal.

An important factor that needed to be controlled for this study was the exercise intensity for each subject. Behavioral studies and recent ERP studies have shown that the effects of acute exercise on cognitive performance and brain response differ depending on the exercise intensity: The best improvements are generally achieved with a moderate intensity (Kamijo et al., 2004, 2007). Nevertheless, the same degree of difficulty of a physical task will have different impacts on each subject depending on an individual's fitness level. Therefore, we assessed the peak oxygen intake ($\dot{V}o_{2peak}$) for each subject and defined a moderate exercise intensity as 50% of a given subject's $\dot{V}o_{2peak}$ (50%$\dot{V}o_{2peak}$).

For the cognitive task, we chose the color–word matching Stroop task, a classical measure of prefrontal cortex (PFC) function (MacLeod, 1991), because it has been studied extensively using many neuroimaging techniques including fNIRS (Ehlis et al., 2005; Schroeter et al., 2002, 2003, 2004b), and the brain regions associated with the task are well known. In the color–word matching Stroop task, subjects observe the names of colors presented in various ink colors, and are instructed to name the presented ink color (Fig. 2A). Color names are presented in non-matching ink colors (e.g., the word green presented in red ink) in the incongruent condition, color names are presented in matching ink colors (e.g., the word green presented in green ink) in the congruent condition, and non-color letters are presented in any ink color (e.g., the letters XXXX presented in red ink) in the neutral condition. During the incongruent condition, the two conflicting sources of color information cause a competing effect known as Stroop interference, which is most typically observed as a prolonged reaction time compared to the neutral or congruent conditions (Laird et al., 2005).

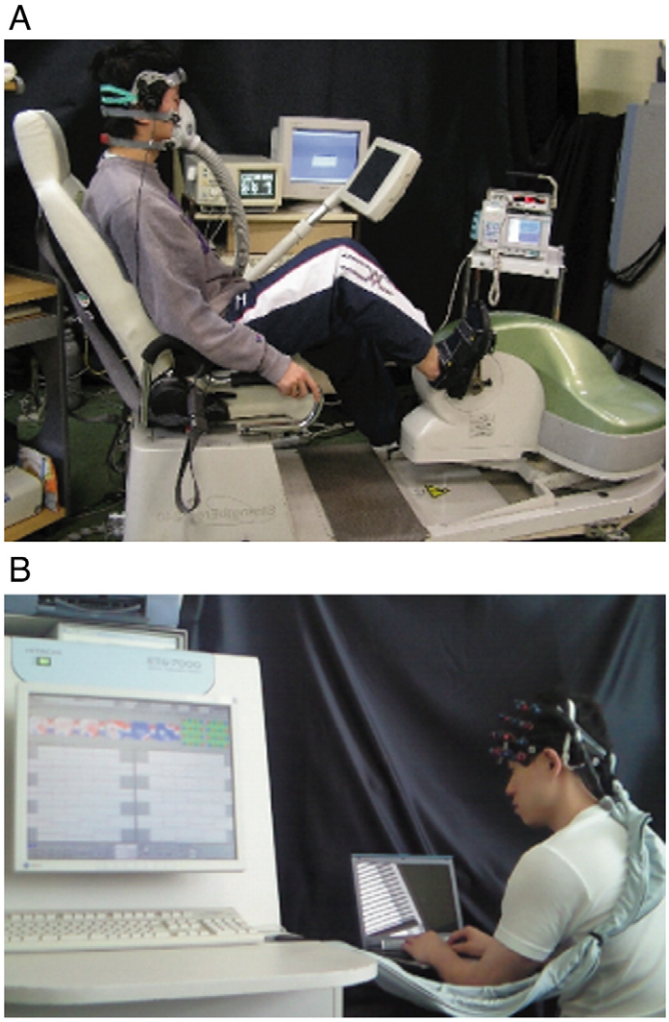

Fig. 1. Experimental settings for fNIRS experiments. (A) Pilot studies 1 and 2. Physiological parameters were measured while subjects performed exercise using a recumbent type cycle ergometer. (B) fNIRS measurements. Brain activity was measured while subjects performed the color-word matching Stroop task.

Stroop interference is consistently associated with the anterior cingulate cortex (ACC) and the lateral prefrontal cortex (LPFC), especially the dorsolateral prefrontal cortex (DLPFC), where the ACC is considered to be susceptible to conflict, and the DLPFC is purported to implement cognitive control (Carter et al., 2000; Leung et al., 2000). Although fNIRS cannot monitor the cortical activation in the ACC because its measurement is limited to lateral cortical surfaces, it has successfully monitored the activation of the LPFC associated with Stroop interference (Ehlis et al., 2005; Schroeter et al., 2002, 2003, 2004b), and some of these studies have also demonstrated LPFC activation related to Stroop task performance (Carter et al., 1995, 2000; Leung et al., 2000; Pardo et al., 1990; Taylor et al., 1997; Zysset et al., 2001). In addition, it has been observed that the color–word matching Stroop task does not always involve ACC activation (Zysset et al., 2001). Therefore we focused our analyses on the LPFC, and set fNIRS probes to cover the region.

In this way, we aimed to examine where in the LPFC activation related to Stroop interference changes due to an acute bout of moderate exercise. Using an event-related multichannel fNIRS targeting the LPFC, we compared the cortical activation pattern during the color–word matching Stroop task before and after the acute bout of moderate physical exercise. Together, we will provide the first experimental evidence that the improved cognitive performance after an acute bout of exercise has relevant neural substrates in specific regions of the LPFC.

Materials and methods

The overall procedure consisted of three major steps. First, in Pilot Study 1, we determined the $\dot{V}o_{2peak}$ to find the appropriate level of exercise for each subject. Second, in Pilot Study 2, we examined the effects of non-cortically derived physiological signals evoked by exercise on fNIRS measurements (skin blood flow etc.). Finally, we assessed the effects of an acute bout of exercise on cortical activation during a color–word matching Stroop task. Details of Pilot Studies 1 and 2 are presented in Supplement 1). This study was approved by the Institutional Ethics Committee of Tsukuba University, and was in accordance with the latest version of the Helsinki Declaration. Written informed consent was obtained from all subjects after giving them a complete description of the study.

Subjects

Twenty healthy, right-handed subjects (three females) participated in the study: mean age 21.5 ± 4.8 years [range 19–24 years], body mass 62.2 ± 7.8 kg, height 170.1 ± 5.1 cm. All subjects had normal or corrected-to-normal vision, normal color vision, and were Japanese speakers. No subject had a history of neurological, major medical, or psychiatric disorders, and none were taking medication at the time of measurement. The subjects underwent all the experimental sessions,

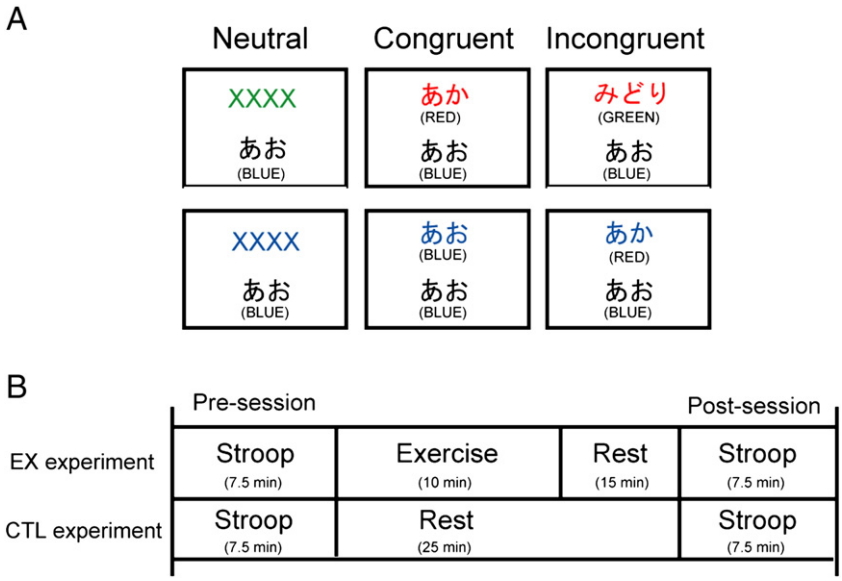

Fig. 2. Experimental design. (A) Instances of single trials for the neutral, congruent, and incongruent conditions of the color–word matching Stroop task are depicted. Stimuli were presented in Japanese. Their English translations are indicated in parentheses. The question given (in Japanese) was, "Does the color of the upper word match the meaning of the lower word?" For the top three examples, the correct answer is, "No" for the bottom three examples, the correct answer is, "Yes". (B) Flow of exercise (EX) and control (CTL) experiments.

comprising the two pilot studies and the fNIRS experiments, which were performed on different days.

fNIRS study: experimental design

We adopted the color–word matching Stroop task (Schroeter et al., 2002, 2003, 2004b; Stroop, 1935) in an event-related design. We presented two rows of letters on a computer screen, and instructed the subjects to decide whether the color of the letters in the top row corresponded to the color name printed in the bottom row (Fig. 2A), and to input their choice by pressing buttons to give "yes" or "no" responses with their middle fingers. The order of the two buttons was changed so that for half of the subjects the yes button was on the left and for the other half it was on the right. Correct answer rate and reaction time were also measured. For neutral trials, the top row contained groups of X's (XXXX) printed in red, green, blue, or yellow, and the bottom row contained the words 'RED', 'GREEN', 'BLUE,' and 'YELLOW' printed in black. For congruent trials, the top row contained the words 'RED', 'GREEN', 'BLUE,' and 'YELLOW' printed in a congruent color. For incongruent conditions, the color name word was printed in an incongruent color. All the word stimuli were presented in Japanese. The top row was presented 100 ms before the lower row to achieve sequential visual attention (Schroeter et al., 2002). The correct answer rate assigned to yes and no was 50% each. Each experimental session consisted of 30 trials including 10 neutral, 10 congruent, and 10 incongruent trials presented in random order with an inter-stimulus interval showing a blank screen for 12 s (Schroeter et al., 2002, 2004a). The stimulus remained on the screen until the response was given, or for 2 s. Prior to the experiment, a practice session consisting of seven trials was performed.

All subjects attended exercise (EX) and control (CTL) experiments with the order being counterbalanced across subjects. In the EX experiment, subjects performed a Stroop task before and 15 min after the exercise. In the CTL experiment, subjects rested instead of performing exercise. Brain activity was monitored with fNIRS while subjects performed Stroop tasks (Fig. 2B).

Since contrast between incongruent and neutral conditions yields the most appropriate measure for Stroop interference (Schroeter et al., 2002), we excluded the congruent condition from the analysis (data are shown in Supplement S2). Other than this, we used data from all the trials.

fNIRS instruments

We used the multichannel fNIRS optical topography system ETG-7000 (Hitachi Medical Corporation, Kashiwa, Japan), using two wavelengths of near-infrared light (785 and 830 nm) (Fig. 1B). We analyzed the optical data based on the modified Beer-Lambert Law (Cope et al., 1988) as previously described (Maki et al., 1995). This method allowed us to calculate signals reflecting the oxygenated hemoglobin (oxy-Hb), deoxygenated hemoglobin (deoxy-Hb), and total hemoglobin (total-Hb) concentration changes, calculated in arbitrary units (millimolar–millimeter) (Maki et al., 1995). The sampling rate was set at 100 ms.

fNIRS probe placement

We set the fNIRS probes to cover the LPFC activation foci as found in previous fMRI and PET studies (Derrfuss et al., 2005; Laird et al., 2005). Specifically, we used two sets of 4×4 multichannel probe holders, consisting of eight illuminating and eight detecting probes arranged alternately at an inter-probe distance of 3 cm, resulting in 24 channels (CH) per set (Figs. 3A, C).

As shown in Fig. 3C, the left probe holder was placed such that probe 5 (between CH 4 and CH 8) was placed over FT7, with the medial edge of the probe column parallel to the medial line. Likewise, the right probe holder was placed symmetrically. Among the 48 channels measured, we excluded those located outside the prefrontal cortex (CHs 1, 4, 25, and 28). Also, channels with poor signal conditions such as extraordinarily abundant high-frequency noise representing insufficient optical signals and abrupt simultaneous changes of oxy-Hb and deoxy-Hb signals suggesting body movements (CHs 5, 8, 11, 18, 21, 24, 29, 32, 35, 42, 45, and 48) were removed upon inter-rater agreement based on independent visual examination by two authors (H.Y. and M. O.). Cohen's Kappa index for inter-rater consistency was 0.979. This resulted in the exclusion of 16 channels. The remaining 32 channels were subjected to further analysis (Fig. 3C).

Virtual registration of fNIRS channels to MNI space

We employed virtual registration (Tsuzuki et al., 2007) to register fNIRS data to MNI standard brain space (Brett et al., 2002). Briefly, this method allows us to place a virtual probe holder on the scalp by

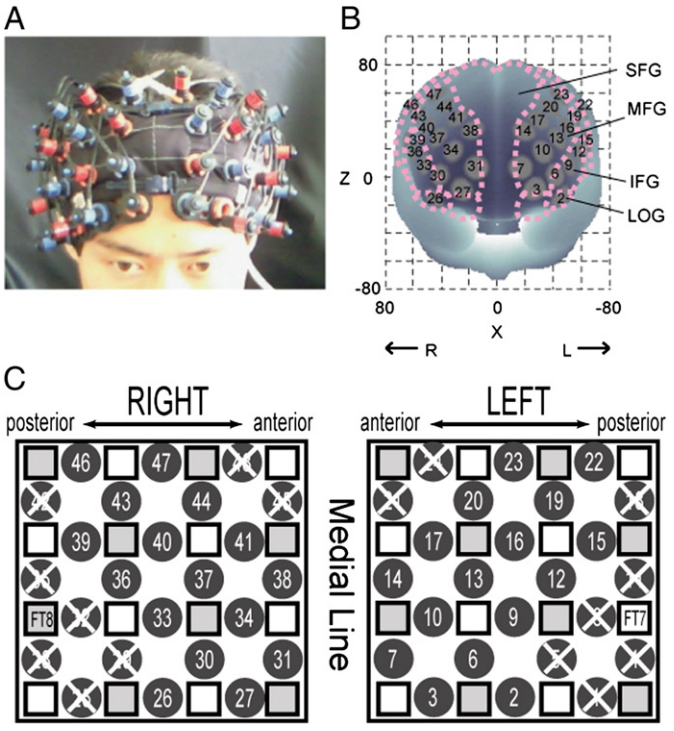

Fig. 3. Spatial profiles of fNIRS channels. (A) Front view of the probe arrangements. (B) Anatomical profiles. Estimated fNIRS channel locations are exhibited in MNI space. Corresponding channel numbers are indicated in black letters. Pink dotted lines indicate the border between anatomical regions. IFG: inferior frontal gyrus, MFG: middle frontal gyrus, SFG: superior frontal gyrus, LOG: lateral orbitofrontal gyrus. (C) fNIRS channel orientation. Detectors are shown as gray squares, illuminators as white squares, and channels as circles. The international 10-10 standard positions and other positional information is indicated. Channels that were not used in the analysis due to low signal-to-noise ratio are marked with an X.

simulating the holder's deformation and by registering probes and channels onto reference brains in our MRI database (Okamoto et al., 2004; Okamoto and Dan, 2005). We performed a statistical analysis of the MNI coordinate values for the fNIRS channels to obtain the most likely estimate of the location of given channels for the group of subjects, and the spatial variability associated with the estimation (Singh et al., 2005). Finally, we anatomically labeled the estimated locations using a Matlab function that reads anatomical labeling information coded in a macroanatomical brain atlas (Shattuck et al., 2008) (Fig. 3B, Supplement S3).

Analysis of NIRS data

When selecting which Hb signal to analyze, it is still a controversial issue whether oxy- or deoxy-Hb is more reliably related to brain activation (Schroeter et al., 2004b). The fNIRS apparatus (Hitachi Medical Co., ETG-7000) used in the current study utilized two wavelengths, 785 and 830 nm. This combination is suitable for detecting oxy-Hb signal, but not for deoxy-Hb signal. In addition, it is often observed that the oxy-Hb signal is characterized by a higher signal amplitude than the deoxy-Hb signal (Strangman et al., 2002). This was also the case in our experimental condition (Fig. 5A). Therefore, we used the oxy-Hb signal for statistical analyses. Individual timeline data for the oxy-Hb signal of each channel were preprocessed with a bandpass filter using cut-off frequencies of 0.04 Hz to remove baseline drift and 0.7 Hz to filter out heartbeat pulsations. From the preprocessed time series data, we obtained channel-wise and subject-wise contrast by calculating the inter-trial mean of differences between the oxy-Hb signals of peak (4–11 s after trial onset) and baseline (0–2 s before trial onset) periods. The contrasts obtained were subjected to second level, random effects group analysis.

Statistical analyses were performed using SPSS Statistical Packages (SPSS Inc., Chicago, USA). Specific flow of statistical analyses is described in the Results section. There are several notes on statistical procedures. We performed statistical analyses on channels and regions of interest (ROIs). We combined three or four neighboring channels based on a widely used anatomical label, LBPA40 (Shattuck et al., 2008), to form a ROI. This procedure is considered valid since optical properties of neighboring channels are known to be similar (Katagiri et al., in press). However, setting ROIs as a factor in ANOVA should be avoided because optical properties in different ROIs are known to vary systematically, causing systematic bias in the statistical analyses. Thus, we limited our statistical analyses to channel-wise or ROI-wise. For multiple comparisons whose number was below the degree of freedom, we used the Bonferroni method according to the criteria by Keppel and Wickens (2004). When the number of hypotheses was above the degree of freedom, as in multiple channel measurements, we used a false discovery rate (FDR) control (Singh and Dan, 2006).

Results

The subjects underwent two fNIRS experiments: exercise (EX) and control (CTL) experiments, each with two sessions (Fig. 2B). In EX experiments, subjects performed the Stroop task before (pre-session) and 15 min after (post-session) the acute exercise bout. In the CTL experiment, subjects rested during the interval between pre- and post-sessions instead of performing any exercise. Brain activity was monitored with fNIRS while subjects performed the Stroop task. The two experiments were performed using a crossover design, and the order was counterbalanced across subjects.

Stroop interference

We first examined whether a general tendency in the Stroop task could be reproduced in all the conditions used in this experiment. Reaction time (RT) and error rate were subjected to a repeated measures three-way ANOVA with the task-kind (incongruent-neutral), the exercise (EX/CTL), and the session (pre/post) being within-subject factors. Because the purpose of the ANOVA was to examine occurrences of the Stroop effect, we limited the range of our analysis to the main effect of task-kind. ANOVA on RT exhibited a significant main effect of the task-kind ($F_{(1,19)} = 249.59$, $p < 0.001$; Fig. 4A). ANOVA on error rate exhibited a significant main effect of the task-kind ($F_{(1,19)} = 60.18$, $p < 0.001$; Fig. 4B). These results verified that Stroop interference could be generally observed in all the conditions used in this study.

To clarify the effect of an acute bout of moderate exercise on a specifically defined cognitive process, we focused on the analyses of Stroop interference. We adopted RT as the measure of Stroop task performance: since the change of error rate was restricted to a small range of below 8%, it would be an unreliable measure of performance due to the floor effect (Nunnally and Berstein, 1994). The [incongruent–neutral] contrast that is assumed to represent Stroop interference was calculated and subjected to repeated measures of two-way ANOVA with the exercise (EX/CTL) and the session (pre/post) being within-subject factors. ANOVA exhibited a significant interaction between exercise and session factors ($F_{(1,19)} = 22.73$, $p < 0.001$). Next, to examine the interaction, we calculated the difference of the degree of Stroop interference between post- and pre-sessions: [[incongruent–neutral] of pre-session − [incongruent–neutral] of post-session] contrast for the EX and CTL groups, respectively, and compared the difference between both groups (Figs. 4C, D). RT difference was significantly more negative in the EX condition than in the CTL condition ($t_{(19)} = 4.70$, $p < 0.001$, paired t-test). This result demonstrates that the acute bout of moderate exercise caused significant

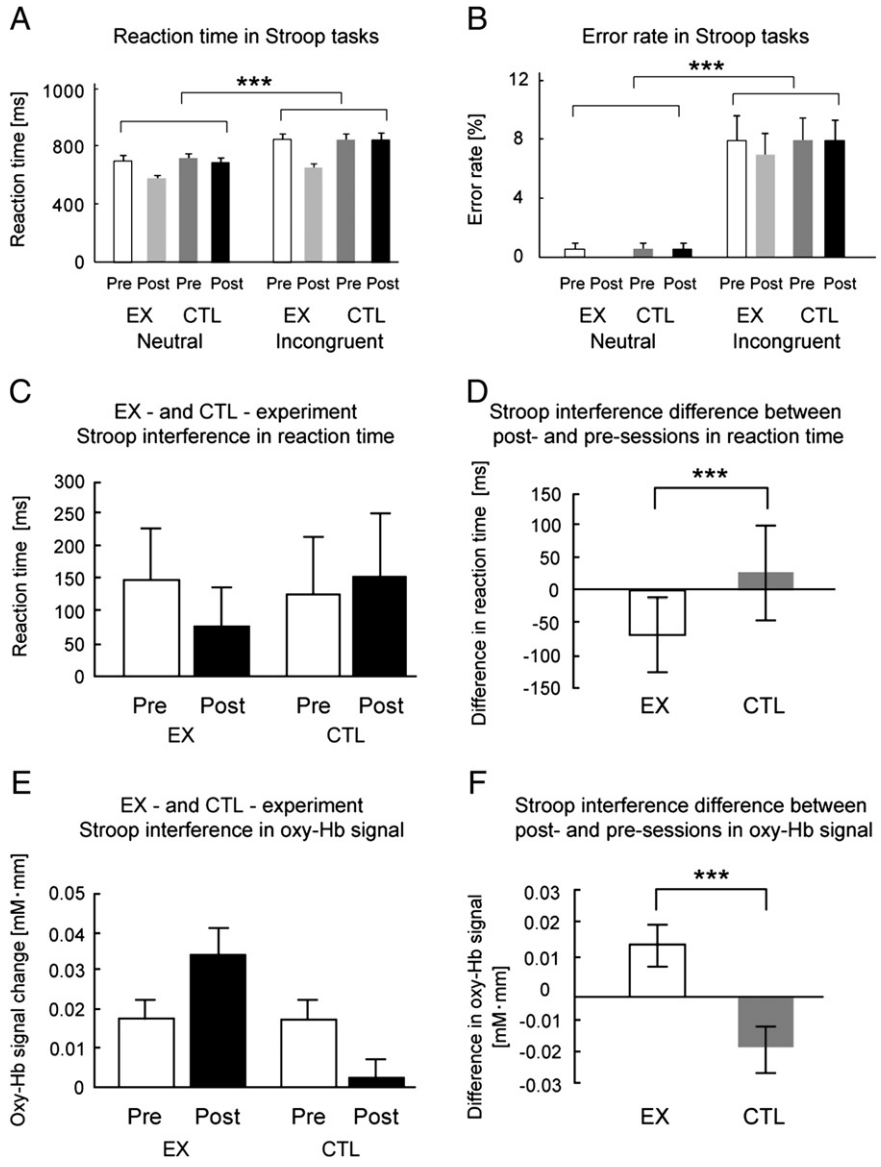

Fig. 4. Stroop task performance. Comparisons between incongruent and neutral conditions for reaction time (RT) (A) and for error rate (B). The mean difference of reaction times in incongruent and neutral conditions indicating the Stroop interference for exercise (EX) and control (CTL) conditions are shown in (C). Stroop interference difference between post- and pre-sessions in reaction time (i.e., [[incongruent–neutral] of pre-session − [incongruent–neutral] of post-session]) contrast is shown for the EX and CTL conditions in (D). The mean difference of oxy-Hb signals in the left DLPFC in incongruent and neutral conditions indicating the Stroop interference for EX and CTL conditions is shown in (E). Stroop interference difference between post- and pre-sessions in oxy-Hb signal (i.e., [[incongruent–neutral] of pre-session − [incongruent–neutral] of post-session]) contrast is shown for EX and CTL conditions in (F). Error bars indicate standard error. Statistically significant effects are indicated with asterisks (*** $p<0.001$).

improvement of cognitive performance reflecting Stroop interference as measured by RT.

fNIRS results

Fig. 5A illustrates patterns of cortical activation during Stroop tasks in the EX condition represented by [peak period–baseline period] contrasts for the oxy-Hb signal. Examples of one-channel timeline data for oxy-Hb and deoxy-Hb signals are also exhibited. First, we observed more stable event-related oxy-Hb signals than deoxy-Hb signals. Thus, oxy-Hb signals are more appropriate for our experimental conditions. Second, cortical activation was generally greater in incongruent conditions than in neutral conditions. This tendency suggests that the measurement of Stroop interference is feasible for our experimental conditions.

As aforementioned, the RT analysis exhibited an effect of exercise on Stroop interference. To explore its neural substrates, we focused on the hemodynamic response during the neutral and incongruent conditions, and used the [incongruent–neutral] contrast that is assumed to represent the hemodynamic response due to Stroop interference.

First, we sought the cortical regions associated with Stroop interference. Among the four conditions used in the current study, pre-EX and pre-CTL conditions are free from any effects of the exercise or prolonged rest periods. Therefore, we averaged the [incongruent–neutral] contrasts for the two conditions and performed a channel-wise analysis. Significant Stroop interference (i.e., incongruent>neutral) was found in ten channels on the left hemisphere (CHs 2, 3, 6, 7, 9, 10, 13, 14, 16, and 17) and CH 37 on the right (one-sample *t*-test, $p<0.05$, FDR-controlled). The activated channels were located over the left DLPFC, frontopolar area (FPA) and ventrolateral PFC (VLPFC), and the right DLPFC.

Next, we classified the activated channels into four ROIs according to the MNI-space-based anatomical labeling (Shattuck et al., 2008). In the left hemisphere, the channels located in the inferior frontal and lateral orbitofrontal gyri were classified into anterior VLPFC (aVLPFC;

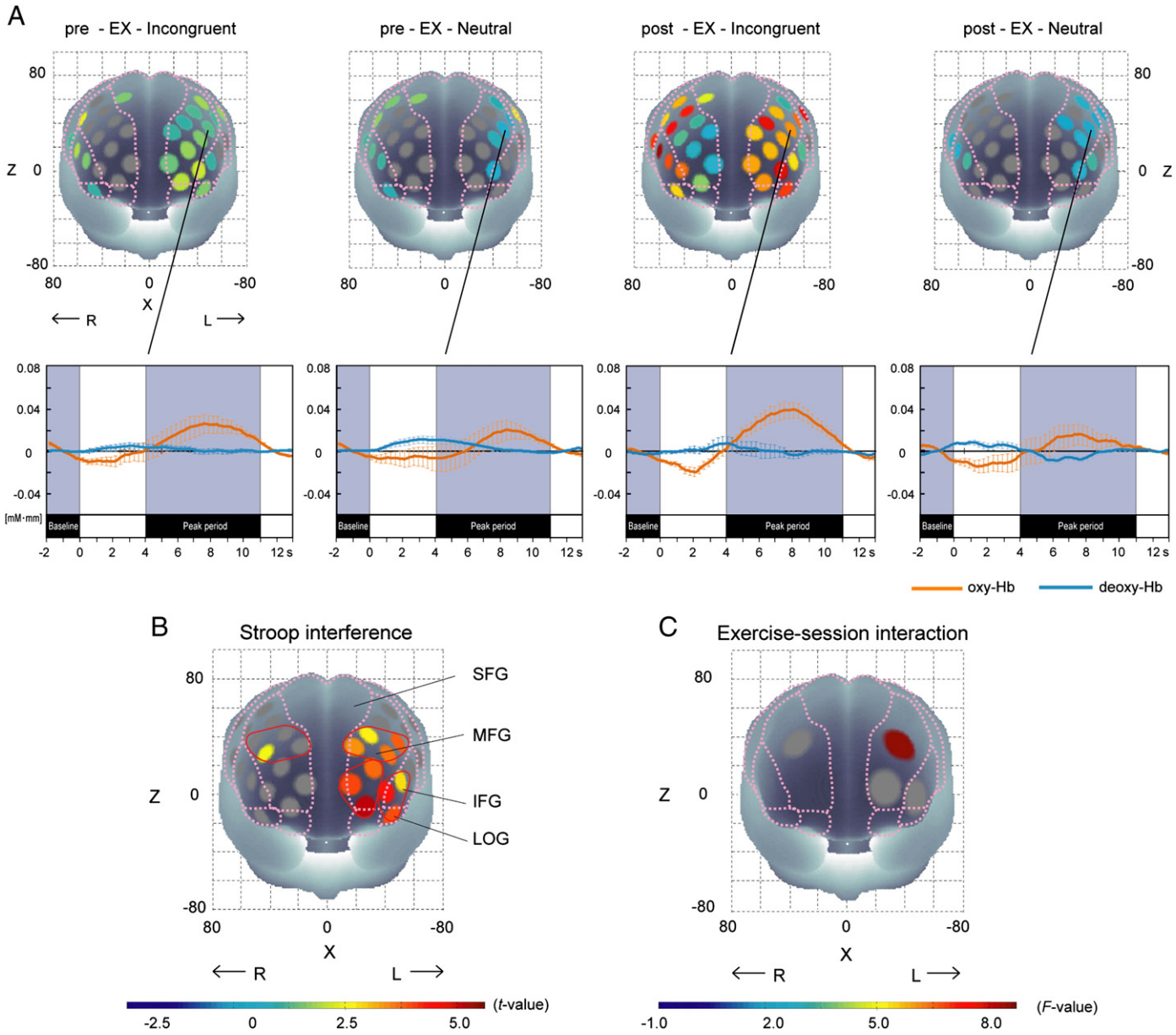

Fig. 5. Results of functional analysis. (A) Cortical activation pattern during Stroop task at pre-EX and post-EX sessions. T-maps of oxy-Hb signal change for [peak period–baseline] contrasts are shown in the upper row. *t*-values are shown according to the color bar. The channels that did not reach the significance level (p<FDR 0.05) are gray. The graphs on the lower row show the time lines of oxy-Hb and deoxy-Hb signals from a representative channel (CH16), which had the highest *t*-value for Stroop interference contrast in pre-Ex/pre-Ctl conditions in the left DLPFC ROI (refer to B). Error bars indicate standard error at given time points. Each time line is adjusted to the average value of the baseline period. Oxy-Hb and deoxy-Hb signals are shown in arbitrary units (mM·mm). (B) T-map of oxy-Hb showing Stroop interference effect (incongruent-neutral) at pre-EX and pre-CTL session average. Red solid lines indicate the ROIs based on the anatomical labeling. Other descriptions are as in (A). (C) F-map of oxy-Hb signal change showing the interaction between exersise (EX and CTL) and session (pre and post) conditions. Locations and activations of the four ROIs (left and right DLPFC, left FPA, and left aVLPFC) are shown. The center of a circle represents the centroid of the channels consisting the ROI. Other descriptions are as in (A).

CHs 2, 6, and 9). Regarding the other channels located in the middle frontal gyrus, those above $z=20$ were defined as left DLPFC (CHs 13, 14, 16, and 17), and those below $z=20$ as left FPA (CHs 3, 7, and 10). In the right hemisphere, the only activated channel located in the middle frontal gyrus was defined as right DLPFC (channel 37). To keep the balance of the ROI size between the right and left DLPFC, we modified the right DLPFC to make it symmetrical with the left DLCPF by incorporating CHs 38, 40, and 41 with CH 37.

As to the four ROIs thus determined, we assessed the effect of an acute bout of moderate exercise on Stroop interference. The [incongruent–neutral] contrasts were analyzed with a repeated measures two-way ANOVA including the exercise (EX/CTL) and the session (pre/post) as within-subject factors. In this design, the effect of an acute bout of moderate exercise on Stroop interference was expected to appear as an interaction between the two factors since pre-EX and pre-CTL were identical to each other. The ANOVA performed on each of the four ROIs revealed significant interaction between the exercise and session factors in the left DLPFC ($F_{(1,19)}=14.8$, $p<0.01$, Bonferroni-corrected) (Fig. 5C). To eliminate the possibility that the right DLPFC result arose because of the incorporation of channels without Stroop-interference-related activation (i.e., CHs 38, 40, and 41), we also performed the two-way ANOVA solely for channel 37. There was no significant interaction between exercise and session.

To clarify the exercise–session interaction in the left DLPFC ROI, we calculated the difference of the hemodynamic response due to Stroop interference between post- and pre-sessions: [[incongruent–neutral] of pre-session − [incongruent–neutral] of post-session] contrast of

oxy-Hb signal for the EX and CTL groups, respectively, and compared the difference between both groups (Figs. 4E, F). Oxy-Hb signal difference was significantly greater in the EX condition than in the CTL condition ($t_{(19)}=3.54$, $p<0.001$, paired t-test). This result demonstrates that the acute bout of moderate exercise led to an increased Stroop interference-related cortical activation in the left DLPFC.

Association between behavioral and fNIRS results

We examined the association between the Stroop-interference-related reaction time shortening and left DLPFC activation induced by exercise. Conventional correlation analyses were not suitable for the current data: both reaction time and oxy-Hb signals entail substantial individual differences, and a subtraction procedure to contrast out Stroop interference further jeopardizes the quantification of each parameter and narrows the range of parameters leading to a floor effect (Nunnally and Bernstein, 1994). Therefore, we performed a McNemar test: a robust nonparametric procedure, applicable to assess correspondence between two incidences (Siegel and Castellan, 1988). We examined whether the exercise-induced enhancement of Stroop interference-reflected in reaction time coincided with the exercise-induced Stroop-interference-related increase of oxy-Hb in a binominal manner. Specifically, the following contrast, [[[incongruent–neutral] of pre-session − [incongruent–neutral] of post-session] in EX condition − [[incongruent–neutral] of pre-session − [incongruent–neutral] of post-session] in CTL condition], was calculated for RT and oxy-Hb signal data, respectively, and they were subjected to the McNemar test. This revealed that the frequency of the coincidence as indicated in Table 1 was significant ($\chi^2_{mc(1,20)}=11.53$, $p<0.001$). Thus, we concluded that the improved cognitive performance demonstrated in RT reflecting Stroop interference and left DLPFC activation elicited by exercise significantly coincided.

Discussion

In this study, we have for the first time applied fNIRS measurements to assess the neural substrates underlying the cognitive effect of an acute bout of moderate exercise. fNIRS measurements in a laboratory enabled strict control of the exercise intensity across subjects to be set at 50%$\dot{V}o_{2peak}$. Since most of the ERP studies performed thus far have not applied strict criteria related to physical fitness, the current study is valuable in that it couples physiological monitoring with functional neuroimaging to examine the cognitive effects of an acute exercise bout. The on-site measurements further allowed the monitoring of physiological parameters, including MCA V_{mean} (middle cerebral artery mean blood velocity), SBF (skin blood flow), CO_2, and HR (heart rate), and thus fNIRS measurements were devoid of possible contamination by exercise-induced physiological noise. These technical merits of fNIRS have led to the revelation of a neural substrate for improved cognitive performance after an acute bout of moderate exercise increased activation of the DLPFC (dorsolateral prefrontal cortex) in the left hemisphere to cope with Stroop interference.

From the behavioral measurements showing a shorter RT in the neutral compared to that in the incongruent condition, we verified that the Stroop effect could be stably observed in the conditions used in this experiment even after an acute bout of moderate exercise. Based on this observation, we assessed the effect of an acute bout of moderate exercise on Stroop interference, and confirmed that performance was improved. This is consistent with a former study that reports an acute-exercise-induced enhancement of Stroop interference (Hogervorst et al., 1996).

Given that the acute bout of moderate exercise caused improved cognitive performance to cope with the Stroop interference, the next step is to search for its neural substrates. In the current study, we observed significant oxy-Hb signal increases associated with the Stroop interference (incongruent–neutral contrast) in the LPFC covering the left FPA, aVLPFC, and bilateral DLPFC (Fig. 5B). This spatially specific activation pattern roughly matches the results of previous fNIRS studies on Stroop interference, reporting bilateral LPFC activation (Schroeter et al., 2002, 2003, 2004b), and left DLPFC activation around FC3 when the ROI was only the left hemisphere (Ehlis et al., 2005) (Fig. 6).

Former fMRI and PET studies have also consistently reported LPFC activation reflecting Stroop interference along with activation of ACC (Derrfuss et al., 2005; Laird et al., 2005) (Fig. 6). Since fNIRS cannot capture frontomedian activation due to the limited depth penetration of NIR light (Villringer and Chance, 1997), we limit our functional arguments to the LPFC.

Reviewing the Stroop-task-related LPFC activation consistently observed in former studies, Banich et al. (2000, 2001) postulated that the LPFC activation may reflect interference processing/response inhibition. Considering these previous studies, the LPFC activation may be regarded as a tight neural substrate for improved performance on Stroop interference. In the current study, we observed that the improved cognitive performance after an acute bout of moderate exercise coincided with increased activation of the left DLPFC due to Stroop interference, providing the first experimental evidence for neural substrate of the improved cognitive performance after an acute bout of moderate exercise.

Although this would be a phenomenologically interesting finding, the causal relation between an acute bout of exercise and improved cognitive performance may not at first appear coherent. However, the current study provides a crucial functional clue to revealing the effects of an acute exercise bout on cognitive performance. Interestingly, in the CTL experiment, where subjects performed the Stroop tasks after just sitting still for 25 min without performing any exercise, we observed slightly decreased cortical activation related to Stroop interference in the left DLPFC (Fig. 4F). Our first intention was to provide a control condition devoid of any exercise. However, this brought about decreased arousal levels among the subjects: most of them expressed sleepiness during the rest period before the session. This led us to postulate that the left DLPFC activation coinciding with the Stroop interference may be due to the increased level of wakefulness caused by an acute bout of moderate exercise. Intriguingly, recent fNIRS studies have reported decreased LPFC activation due to fatigue (Suda et al., 2009) and sleepiness (Suda et al., 2008) during a word-generation task, although such effects on Stroop interference have yet to be explored. Indeed, after reviewing P300 ERP experiments performed on natural and environmentally induced states, including exercise, Polich and Kok (1995) hypothesized that cognitive processing speed is influenced by changes in arousal state. The arousal hypothesis has provided an explanation for the inverted U-shaped function of task performance against physical strength (Yerkes and Dodson, 1908). As arousal states increase with physical exertion, cognitive performance improves to an optimal point, after which further increases in physical exertion result in decreased arousal levels and deteriorating performance (Tomporowski and Ellis, 1985). The physical exertion required of participants in the current study was

Table 1
Contingency table for McNemar Test on association between reaction time and oxy-Hb signal.

		oxy-Hb signal		Totals
		−	+	
RT	−	2	16	18
	+	1	1	2
	Totals	3	17	20

Frequency of exercise-induced Stroop interference-related shortening of reaction time and Stroop interference-related oxy-Hb increase in the left DLPFC is summarized. RT stands for reaction time.

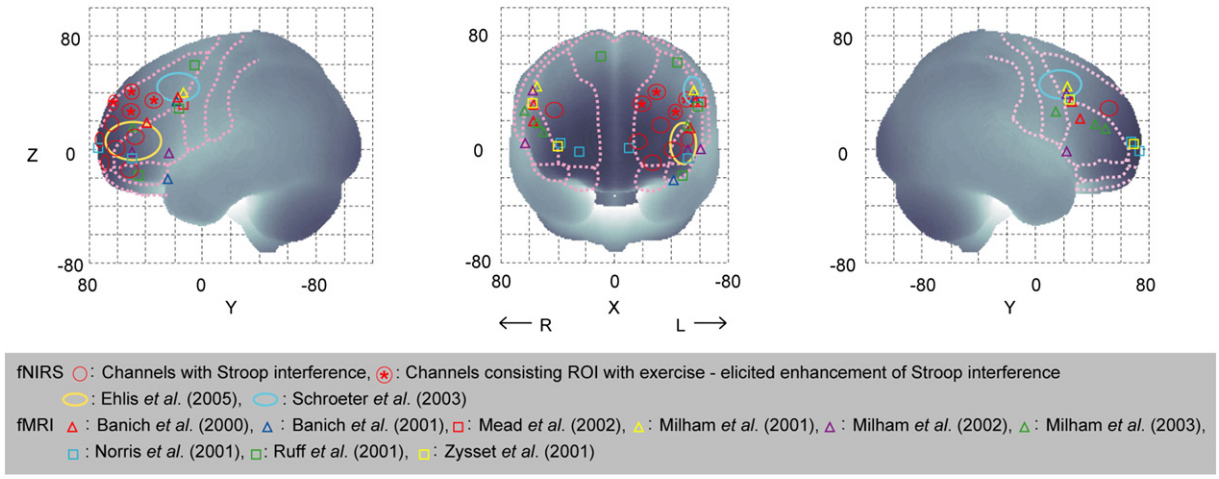

Fig. 6. Schematic illustrating the distribution of activation foci reported in the relevant fMRI and fNIRS studies. For fMRI studies, incongruent–neutral contrasts in color–word matching Stroop tasks with manual response were selected (Banich et al., 2000, 2001; Mead et al., 2002; Milham et al., 2001, 2002, 2003; Norris et al., 2002; Ruff et al., 2001; Zysset et al., 2001). Coordinates published in the Talairach system were converted to the MNI system using a transformation matrix called "tal2icbm_spm.m" developed by Lancaster et al. (2000). Activation foci reported in the relevant fMRI studies are plotted in assorted colors. Due to scarcity of fNIRS studies, incongruent–neutral contrasts in color–word matching Stroop task with manual response (Schroeter et al., 2002) and verbal response (Ehlis et al., 2005) were both included. Locations published in the 10–20 system were converted to the MNI coordinates according to Okamoto et al. (2004). For the current fNIRS study, the location of the fNIRS channels that exhibited significant Stroop interference-related activation is indicated with red circles. The channels consisting the left DLPFC with exersise-elicited enhancement are marked with asterisks. Left lateral, frontal, and right lateral views are shown.

set at 50%$\dot{V}o_{2peak}$, which corresponds to the increment phase of the inverted U-shaped function. Thus, it is plausible that an acute bout of moderate exercise gave rise to an increased arousal state and further led to improved cognitive performance. One may argue that the slightly decreased activation may be due to habituation, but this is unlikely because cortical activation for the Stroop task has been demonstrated to be robust with respect to repetition (Menz et al., 2006).

At a more physiological level, the increased arousal state may be further attributed to an increased release of catecholamines (noradrenaline, etc.) in the central nervous system (CNS). An association between the concentration of central catecholamines and CNS activation in exercising rats has been suggested by Pagliari and Peyrin (1995). These authors claimed that mental improvement during exercise may be the consequence of central noradrenergic activation. In light of these observations, more research is necessary to elucidate the physiological mechanisms underlying cognitive performance increases induced by an acute exercise bout.

Given the results of the current study, it is tempting to postulate that the acute-exercise-induced increase in cognitive performance associated with increased left DLPFC activation may lead to chronic effects. Based on neuroimaging evidence, it has been reported that chronic exercise bouts lead to enhanced functioning of cortical regions, including the LPFC, during the Eriksen flanker paradigm, a cognitive interference task similar to the Stroop paradigm used in the current study (Colcombe et al., 2004). Longitudinal neuroimaging monitoring would be beneficial to fill in the missing link between the acute and chronic effects of moderate exercise on cognitive function. As another line of research, we are planning a neuroimaging examination of the cognitive effects of exercise bouts performed at different intensities. This would reveal the degree of intensity of brief exercise that most effectively improves cognitive performance. These research efforts will eventually lead to our final research goal: revealing whether exercise is effective in improving or maintaining cognitive functions in our daily lives, and if so, finding the neural basis for exercise-induced cognitive improvement.

Acknowledgments

This work was supported in part by a grant of the 21st Center of Excellence (COE) program from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, the Program for Promotion of Basic Research Activities for Innovative Bioscience, and Health and Labor Sciences Research Grants, Research on Psychiatric and Neurological Diseases and Mental Health.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2009.12.023.

References

Banich, M.T., Milham, M.P., Atchley, R., Cohen, N.J., Webb, A., et al., 2000. fMRI studies of Stroop tasks reveal unique roles of anterior and posterior brain systems in attentional selection. J. Cogn. Neurosci. 12 (6), 988–1000.
Banich, M.T., Milham, M.P., Jacobson, B.L., Webb, A., Wszalek, T., Cohen, N.J., Kramer, A.F., 2001. Attentional selection and the processing of task-irrelevant information: insights from fMRI examinations of the Stroop task. Prog. Brain Res. 134, 459–470.
Brett, M., Johnsrude, I.S., Owen, A.M., 2002. The problem of functional localization in the human brain. Nat. Rev. Neurosci. 3 (3), 243–249.
Carter, C.S., Macdonald, A.M., Botvinick, M., Ross, L.L., Stenger, V.A., Noll, D., Cohen, J.D., 2000. Parsing executive processes: strategic vs. evaluative functions of the anterior cingulate cortex. Proc. Natl. Acad. Sci. U S A 97 (4), 1944–1948.
Carter, C.S., Mintun, M., Cohen, J.D., 1995. Interference and facilitation effects during selective attention: an H215O PET study of Stroop task performance. Neuroimage 2 (4), 264–272.
Chmura, J., Krysztofiak, H., Ziemba, A.W., Nazar, K., Kaciuba-Uscilko, H., 1998. Psychomotor performance during prolonged exercise above and below the blood lactate threshold. Eur. J. Appl. Physiol. Occup. Physiol. 77 (1–2), 77–80.
Colcombe, S.J., Kramer, A.F., McAuley, E., Erickson, K.I., Scalf, P., 2004. Neurocognitive aging and cardiovascular fitness: recent findings and future directions. J. Mol. Neurosci. 24 (1), 9–14.
Collardeau, M., Brisswalter, J., Audiffren, M., 2001. Effects of a prolonged run on simple reaction time of well trained runners. Percept. Mot. Skills 93 (3), 679–689.
Cope, M., Delpy, D.T., Reynolds, E.O., Wray, S., Wyatt, J., van der Zee, P., 1988. Methods of quantitating cerebral near infrared spectroscopy data. Adv. Exp. Med. Biol. 222, 183–189.
Cotman, C.W., Berchtold, N.C., Christie, L.A., 2007. Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends Neurosci. 30 (9), 464–472.
Derrfuss, J., Brass, M., Neumann, J., von Cramon, D.Y., 2005. Involvement of the inferior frontal junction in cognitive control: meta-analyses of switching and Stroop studies. Hum. Brain Mapp 25 (1), 22–34.
Donchin, E., Coles, M.G.H., 1988. Is the P300 component a manifestation of context updating? Behav. Brain Sci 11, 357–374.
Ehlis, A.C., Herrmann, M.J., Wagener, A., Fallgatter, A.J., 2005. Multi-channel near-infrared spectroscopy detects specific inferior-frontal activation during incongruent Stroop trials. Biol. Psychol. 69 (3), 315–331.
Hillman, C.H., Erickson, K.I., Kramer, A.F., 2008. Be smart, exercise your heart: exercise effects on brain and cognition. Nat. Rev. Neurosci. 9 (1), 58–65.

Hillman, C.H., Snook, E.M., Jerome, G.J., 2003. Acute cardiovascular exercise and executive control function. Int. J. Psychophysiol. 48 (3), 307–314.
Hogervorst, E., Riedel, W., Jeukendrup, A., Jolles, J., 1996. Cognitive performance after strenuous physical exercise. Percept. Mot. Skills 83 (2), 479–488.
Kamijo, K., Nishihira, Y., Hatta, A., Kaneda, T., Wasaka, T., Kida, T., Kuroiwa, K., 2004. Differential influences of exercise intensity on information processing in the central nervous system. Eur. J. Appl. Physiol. 92 (3), 305–311.
Kamijo, K., Nishihira, Y., Higashiura, T., Kuroiwa, K., 2007. The interactive effect of exercise intensity and task difficulty on human cognitive processing. Int. J. Psychophysiol. 65 (2), 114–121.
Katagiri, A., Dan, I., Tuzuki, D., Okamoto, M., Yokose, N., Igarashi, & K., H. T., Fujiwara, T., Katayama, Y., Yamaguchi, Y., Sakatani, K., in press. Mapping of optical pathlength of human adult head at multiwavelengths in near infrared. Advances in Experimental Medicine and Biology.
Keppel, G., Wickens, D.W., 2004. Design and Analysis: A Researcher's Handbook, 4th ed. Prentice Hall, Upper Saddle River.
Koizumi, H., Yamamoto, T., Maki, A., Yamashita, Y., Sato, H., Kawaguchi, H., Ichikawa, N., 2003. Optical topography: practical problems and new applications. Appl. Opt. 42 (16), 3054–3062.
Laird, A.R., McMillan, K.M., Lancaster, J.L., Kochunov, P., Turkeltaub, P.E., Pardo, J.V., Fox, P.T., 2005. A comparison of label-based review and ALE meta-analysis in the Stroop task. Hum Brain Mapp 25 (1), 6–21.
Lancaster, J.L., Woldorff, M.G., Parsons, L.M., Liotti, M., Freitas, C.S., et al., 2000. Automated Talairach atlas labels for functional brain mapping. Hum. Brain Mapp. 10 (3), 120–131.
Leung, H.C., Skudlarski, P., Gatenby, J.C., Peterson, B.S., Gore, J.C., 2000. An event-related functional MRI study of the Stroop color word interference task. Cereb. Cortex 10 (6), 552–560.
MacLeod, C.M., 1991. Half a century of research on the Stroop effect: an integrative review. Psychol. Bull. 109 (2), 163–203.
Magnie, M.N., Bermon, S., Martin, F., Madany-Lounis, M., Suisse, G., Muhammad, W., Dolisi, C., 2000. P300, N400, aerobic fitness, and maximal aerobic exercise. Psychophysiology 37 (3), 369–377.
Maki, A., Yamashita, Y., Ito, Y., Watanabe, E., Mayanagi, Y., Koizumi, H., 1995. Spatial and temporal analysis of human motor activity using noninvasive NIR topography. Med. Phys. 22 (12), 1997–2005.
Mead, L.A., Mayer, A.R., Bobholz, J.A., Woodley, S.J., Cunningham, J.M., Hammeke, T.A., Rao, S.M., 2002. Neural basis of the Stroop interference task: response competition or selective attention? J. Int. Neuropsychol. Soc. 8 (6), 735–742.
Menz, M.M., Neumann, J., Muller, K., Zysset, S., 2006. Variability of the BOLD response over time: an examination of within-session differences. Neuroimage 32 (3), 1185–1194.
Milham, M.P., Banich, M.T., Barad, V., 2003. Competition for priority in processing increases prefrontal cortex's involvement in top-down control: an event-related fMRI study of the Stroop task. Brain Res. Cogn. Brain Res. 17 (2), 212–222.
Milham, M.P., Banich, M.T., Webb, A., Barad, V., Cohen, N.J., Wszalek, T., Kramer, A.F., 2001. The relative involvement of anterior cingulate and prefrontal cortex in attentional control depends on nature of conflict. Brain Res. Cogn. Brain Res. 12 (3), 467–473.
Milham, M.P., Erickson, K.I., Banich, M.T., Kramer, A.F., Webb, A., Wszalek, T., Cohen, N.J., 2002. Attentional control in the aging brain: insights from an fMRI study of the Stroop task. Brain Cogn. 49 (3), 277–296.
Nakamura, Y., Nishimoto, K., Akamatu, M., Takahashi, M., Maruyama, A., 1999. The effect of jogging on P300 event related potentials. Electromyogr. Clin. Neurophysiol. 39 (2), 71–74.
Norris, D.G., Zysset, S., Mildner, T., Wiggins, C.J., 2002. An investigation of the value of spin-echo-based fMRI using a Stroop color–word matching task and EPI at 3 T. Neuroimage 15 (3), 719–726.
Nunnally, J., Berstein, I., 1994. Psychometric theory (3rd ed.). Applied Psychological Measurement 19, 303–305, McGraw-Hill, New York.
Obrig, H., Villringer, A., 2003. Beyond the visible—imaging the human brain with light. J. Cereb. Blood Flow Metab. 23 (1), 1–18.
Okamoto, M., Dan, H., Sakamoto, K., Takeo, K., Shimizu, K., et al., 2004. Three-dimensional probabilistic anatomical cranio-cerebral correlation via the international 10–20 system oriented for transcranial functional brain mapping. Neuroimage 21 (1), 99–111.
Okamoto, M., Dan, I., 2005. Automated cortical projection of head-surface locations for transcranial functional brain mapping. Neuroimage 26 (1), 18–28.
Pardo, J.V., Pardo, P.J., Janer, K.W., Raichle, M.E., 1990. The anterior cingulate cortex mediates processing selection in the Stroop attentional conflict paradigm. Proc. Natl. Acad. Sci. U S A 87 (1), 256–259.
Pagliari, R., Peyrin, L., 1995. Norepinephrine release in the rat frontal cortex under treadmill exercise: a study with microdialysis. J Appl Physiol 78 (6), 2121–2130.
Polich, J., Kok, A., 1995. Cognitive and biological determinants of P300: an integrative review. Biol. Psychol. 41 (2), 103–146.
Polich, J., Lardon, M.T., 1997. P300 and long-term physical exercise. Electroencephalogr. Clin. Neurophysiol. 103 (4), 493–498.
Ruff, C.C., Woodward, T.S., Laurens, K.R., Liddle, P.F., 2001. The role of the anterior cingulate cortex in conflict processing: evidence from reverse Stroop interference. Neuroimage 14 (5), 1150–1158.
Schroeter, M.L., Zysset, S., Kruggel, F., von Cramon, D.Y., 2003. Age dependency of the hemodynamic response as measured by functional near-infrared spectroscopy. Neuroimage 19 (3), 555–564.
Schroeter, M.L., Zysset, S., Kupka, T., Kruggel, F., Yves von Cramon, D., 2002. Near-infrared spectroscopy can detect brain activity during a color–word matching Stroop task in an event-related design. Hum. Brain Mapp. 17 (1), 61–71.
Schroeter, M.L., Zysset, S., von Cramon, D.Y., 2004a. Shortening intertrial intervals in event-related cognitive studies with near-infrared spectroscopy. Neuroimage 22 (1), 341–346.
Schroeter, M.L., Zysset, S., Wahl, M., von Cramon, D.Y., 2004b. Prefrontal activation due to Stroop interference increases during development—an event-related fNIRS study. Neuroimage 23 (4), 1317–1325.
Shattuck, D.W., Mirza, M., Adisetiyo, V., Hojatkashani, C., Salamon, G., Narr, K.L., Poldrack, R.A., Bilder, R.M., Toga, A.W., 2008. Construction of a 3D probabilistic atlas of human cortical structures. Neuroimage 39 (3), 1064–1080.
Siegel, S., Castellan, N., 1988. Nonparametric Statistics for the Behavioral Sciences, Second edition. McGraw-Hill, New York.
Singh, A.K., Dan, I., 2006. Exploring the false discovery rate in multichannel NIRS. Neuroimage 33 (2), 542–549.
Singh, A.K., Okamoto, M., Dan, H., Jurcak, V., Dan, I., 2005. Spatial registration of multichannel multi-subject fNIRS data to MNI space without MRI. Neuroimage 27 (4), 842–851.
Strangman, G., Boas, D.A., Sutton, J.P., 2002. Non-invasive neuroimaging using near-infrared light. Biol. Psychiatry 52 (7), 679–693.
Stroop, J., 1935. Studies of interference in serial verbal reactions. J. Exp. Psychol. 18, 643–662.
Suda, M., Fukuda, M., Sato, T., Iwata, S., Song, M., Kameyama, M., Mikuni, M., 2009. Subjective feeling of psychological fatigue is related to decreased reactivity in ventrolateral prefrontal cortex. Brain Res. 1252, 152–160.
Suda, M., Sato, T., Kameyama, M., Ito, M., Suto, T., Yamagishi, Y., Uehara, T., Fukuda, M., Mikuni, M., 2008. Decreased cortical reactivity underlies subjective daytime light sleepiness in healthy subjects: a multichannel near-infrared spectroscopy study. Neurosci. Res. 60 (3), 319–326.
Taylor, S.F., Kornblum, S., Lauber, E.J., Minoshima, S., Koeppe, R.A., 1997. Isolation of specific interference processing in the Stroop task: PET activation studies. Neuroimage 6 (2), 81–92.
Timinkul, A., Kato, M., Omori, T., Deocaris, C.C., Ito, A., Kizuka, T., Asada, T., Soya, H., 2008. Enhancing effect of cerebral blood volume by mild exercise in healthy young men: a near-infrared spectroscopy study. Neurosci. Res. 61 (3), 242–248.
Tomporowski, P.D., 2003. Effects of acute bouts of exercise on cognition. Acta Psychol. (Amst) 112 (3), 297–324.
Tomporowski, P.D., Ellis, N.R., 1985. The effects of exercise on the health, intelligence, and adaptive behavior of institutionalized severely and profoundly mentally retarded adults: a systematic replication. Appl. Res. Ment. Retard. 6 (4), 465–473.
Tsuzuki, D., Jurcak, V., Singh, A.K., Okamoto, M., Watanabe, E., Dan, I., 2007. Virtual spatial registration of stand-alone fNIRS data to MNI space. Neuroimage 34 (4), 1506–1518.
Villringer, A., Chance, B., 1997. Non-invasive optical spectroscopy and imaging of human brain function. Trends Neurosci. 20 (10), 435–442.
Yerkes, R.M., Dodson, J.D., 1908. The relation of strength of stimulus to rapidity of habit-formation. J. Comp. Neurol. Psychol. 18, 459–482.
Zysset, S., Muller, K., Lohmann, G., von Cramon, D.Y., 2001. Color–word matching Stroop task: separating interference and response conflict. Neuroimage 13 (1), 29–36.