



Research report

Ventral prefrontal cortex and serotonergic system activation during pedaling exercise induces negative mood improvement and increased alpha band in EEG

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ABSTRACT

This study evaluates a possible involvement of the prefrontal cortex (PFC) and serotonergic (5-HT) system in psychiatric and electroencephalography (EEG) changes during and after pedaling exercise (PE). The subjects performed PE for 15 min using a cycle ergometer. PE rate was kept at 60 rpm, and the work load (93 ± 5.4 W) was decided for each subject before the experiment based on a Rating of Perceived Exertion of 12–13 for self-selected exercise intensity. Cerebral oxygenation in the PFC was assessed by concentration changes in oxygenated hemoglobin (oxyHb) using 24-channel near-infrared spectroscopy. We found that PE evoked a significant increase in oxyHb levels in the ventral PFC during PE as compared with that in the dorsal PFC. Subjects had a feeling of reduced negative mood accompanied by a tendency of increased vigor-activity after PE, as assessed by the Profile of Mood States (POMS) questionnaire. Because the ventral PFC is associated with mood state, we hypothesized that the observed mood changes may have been induced by the activation of the ventral PFC. As for EEG changes during and after PE, we found a significant increase in the relative powers of high-frequency alpha bands (10–13 Hz) during and after PE. A significant increase in whole blood 5-HT level was obtained after PE. Because cortical attenuation would be caused by the 5-HT-induced inhibition of the basal forebrain, we hypothesized that the observed EEG changes are linked with the increased blood 5-HT level or an augmentation of the 5-HT system in the brainstem.

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1. Introduction

Pedaling exercise (PE) is known to induce changes in cerebral and psychiatric functions. Many reports demonstrate increased activity of the alpha band of electroencephalography (EEG), accompanied by a state of relaxation or decreased anxiety, during and after PE [6]. As for the effect of exercise on EEG, recent studies have emphasized changes in other frequency bands besides the alpha range. Schneider et al. [29] showed a decrease of beta power in EEG after higher intensity treadmill exercise. Therefore, we investigated beta and theta frequency bands, as well as the alpha frequency band. Despite the many studies evaluating the effect of exercise on EEG, the mechanisms underlying those EEG and psychiatric changes have not been fully understood. We conducted the present study to gain insight into this issue.

Clinical reports showing a negative correlation between depression severity and ventral prefrontal cortex (PFC) activity in patients [9,22] suggest that PFC may be associated with decreased anxiety during and after PE. The change in mood after exercise has also

been investigated in psychological studies based on a hypothesis suggesting that the left and right PFC are differentially related to negative and positive mood (i.e., frontal asymmetry) [7,8]. Thus, in this study, we evaluated activity of bilateral and ventral PFC during PE using 24-channel near-infrared spectroscopy (NIRS). Mood was assessed by the Profile of Mood States (POMS) test.

In addition, we measured EEG changes during PE. Our preliminary study demonstrated that PE induced the appearance of alpha band. We hypothesized that such EEG changes may be induced by activation of the serotonergic (5-HT) system based on experimental data by Jones and Mühlethaler [20], who revealed that microinjection of 5-HT into the basal forebrain, which projects to broad areas in the cortex, caused a shift to a lower-frequency band in EEG. This 5-HT-induced inhibition of the cholinergic pathway may be responsible for the appearance of lower-frequency band in EEG during PE. Therefore, we also evaluated the possibility that PE may cause such EEG changes through activation of the 5-HT system.

To assess activation of the 5-HT system in humans, we measured 5-HT levels in whole blood [21] based on the hypothesis that 5-HT system activation can be evaluated by an increase in 5-HT level in whole blood. This hypothesis has recently been proven by our in vivo experiment in rats demonstrating that augmented 5-HT within the brain can cross the blood–brain barrier (BBB) through the 5-

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HT transporter [27]. Because 5-HT released from the brain to the circulating blood moves quickly into the platelets, it is necessary to measure 5-HT in whole blood but not in the platelet-poor plasma. We compared 5-HT levels in whole blood before and after PE to estimate activation of the 5-HT system within the brain.

In the present study, we evaluated the hypothesis that activations of PFC and the 5-HT system may elicit psychiatric and EEG changes during and after PE.

2. Materials and methods

2.1. Subjects

Ten subjects (aged 32 ± 2.2 years; nine men and one woman) volunteered for this study. Oral and written informed consent was obtained from all subjects. All procedures were conducted in accordance with the ethical standards of the Committee on Human Experimentation at Toho University School of Medicine and with the Helsinki Declaration of 1964. The subjects were all healthy and screened to exclude those with psychiatric illness, systemic neuromuscular disease, a history of head injury, and intake of medications that may affect EEG recordings, regional cerebral blood flow, or 5-HT measurements. The daily activity level of subjects was not high, but they had been involved in light aerobic exercise for less than 3 weeks at the time of testing. It was made clear to the subjects that they were free to terminate the study at any time if they did not want to continue.

2.2. NIRS data acquisition

We employed a 24-channel NIRS system (OMM3000/8, Shimadzu Co., Kyoto, Japan) capable of detecting concentration changes in oxygenated hemoglobin (oxyHb), deoxygenated hemoglobin (deoxyHb), and their sum (totalHb) by using three types of near-infrared light (wavelengths, 780, 805, and 830 nm). Each parameter was calculated according to the following equations:

$$\text{oxyHb} = -1.49 \times \Delta A_{780} + 0.5970 \times \Delta A_{805} + 1.4847 \times \Delta A_{830}$$

$$\text{deoxyHb} = 1.845 \times \Delta A_{780} - 0.2394 \times \Delta A_{805} - 1.0947 \times \Delta A_{830}$$

$$\text{totalHb} = \text{oxyHb} + \text{deoxyHb}$$

A_{780} , A_{805} , and A_{830} represent the detected optical absorbances at 780, 805, and 830 nm, respectively. These were calculated every 130 ms, and we then employed 1300 ms (130 ms \times 10 points) of cumulative sampling data for analysis purposes.

A total of 16 optodes, namely eight emitters and eight detectors, were placed on the subject's frontal region using a holder. The subject's frontal region was covered with the holder to adjust and fixate the optode (Fig. 1). Distance between optodes was set to 3 cm. The optode of channel 2 was positioned at Fpz, according to the international 10/20 system used in EEG. Before the attachment of optodes, hairs under the optode were carefully brushed away to avoid problems with signal detection. We also checked whether the photomultiplier values were at an optimal level. The optimal level of photomultiplier value was obtained when an input level was 0.05–4 V. If the input level was over or under, caused by hair or inappropriate attachment of the probe, we reset the probe to the optimal input level. After completion of the procedure check, we began the experiment.

An anatomical 3-D T1-weighted 1.5-T magnetic resonance imaging (MRI) scan (Excelart, Toshiba Medical Systems Co., Tochigi, Japan) was performed by marking the optode location on the skull with vitamin D capsules. Voxel dimension, matrix, slice thickness, and FOV were 256×256 , 1 mm, and 220.01 mm, respectively. TR, TE, and flip angle were 3 s, 5 ms, and 30° degrees, respectively. Number of slices and slice orientation were 150 slices and transverse orientation, respectively. Based on the MRI measurements in the present study and other previous reports [16,28], these recorded areas correspond to the bilateral middle frontal gyrus and lower frontal gyrus (BA 8, 9, and 10).

2.3. EEG, EOG, ECG, and EMG recordings

EEG recordings were made with silver–silver chloride electrodes and fixed at two areas of the brain: central (Cz) and parietal (Pz) sites, according to the international 10/20 system. The linked electrodes of the left and right ear lobes were used as a reference. EEGs were recorded in an eyes-opened condition before, during, and after PE. Leads from these areas were amplified by a bioelectric amplifier (EEG-4217, Nihon Kohden Co., Tokyo, Japan) with a time constant of 0.3 s and a low-pass filter at 60 Hz (frequency range, 0.5–60 Hz). The signals were then continuously monitored on the recorder and digitized at a sampling rate of 200 Hz for a microcomputer-based analysis.

Spectral analyses of the EEG were performed for 1 min before, during, and after PE; the spectral analyses were performed at 1, 5, 10, and 15 min during PE. Each spectral analysis was made using the software ATAMAP II (Kissei Comtec Co., Nagano, Japan) as follows. First, a segment that did not include the artifacts originated by eye movements and head motion was visually selected piece by piece on the computer screen. Forty-seven segments were obtained from 1 min recordings. Because the segment that included artifacts was eliminated, data analysis of the power spectra was achieved by averaging 27 ± 1.0 segments/min ($58 \pm 2.1\%$ of maximum number of segments). Second, a Hanning window was applied to each segment without artifact, and thereafter fast Fourier transform was used to obtain the mean power areas in theta (4–8 Hz), low-frequency alpha (8–10 Hz), high-frequency alpha (10–13 Hz), and beta (13–30 Hz) bands. The ranges of low- and high-frequency alpha bands were

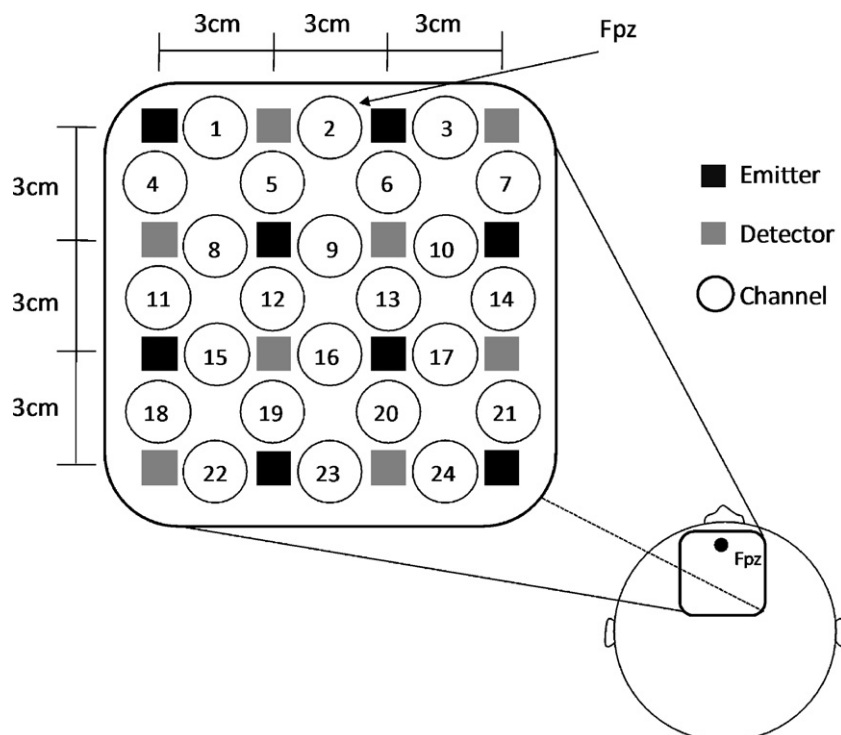


Fig. 1. Locations of optodes and channels. This figure illustrates an overhead view of an optode holder.

defined according to previous studies by Cantero et al. [4] and Fumoto et al. [11]. Note that several studies reported that EEG samples of 20 s provide reliable measures of spontaneous EEG activity [13,23]. Therefore, 27 ± 1.0 segments (a 34.56 s total) in this study should be sufficient to estimate spontaneous EEG before, during, and after PE.

Electrooculography (EOG) was monitored with silver–silver chloride electrodes, which were placed above and below the eyes. EOG signal was amplified by a bioelectric amplifier with a time constant of 0.3 s and a low-pass filter at 60 Hz (frequency range, 0.5–60 Hz). The signals were then continuously monitored on the recorder.

Electrocardiography (ECG) was recorded to monitor heart rate (HR). The electrodes were placed on the right clavicle and left 8th thoracic rib. ECG signal was amplified by a bioelectric amplifier with a time constant of 2 s and a low-pass filter at 100 Hz (frequency range, 0.1–100 Hz). The signals were then continuously monitored on the recorder.

Electromyography of the quadriceps femoris muscle (EMG_{QF}) was recorded to monitor leg extension during PE. The paired electrodes of the EMG_{QF} were placed on the belly of the muscle; electrode spacing was 3 cm. The signals were amplified with a time constant of 0.03 s and a low-pass filter at 1 kHz (frequency range, 5–1 kHz). The signals were then continuously monitored on the recorder.

2.4. Experimental procedures

On the day of the study, all subjects accessed the laboratory at 1:00 p.m., after having a light meal, and rested for 60 min in a comfortable position. After the rest, the experimenter placed the electrodes for monitoring EEG, EOG, ECG, and EMG, as well as the optode holder for measurement of NIRS. PE was performed on a cycle ergometer (75XLII, Combi Wellness Co., Tokyo, Japan) with toe clips; the positions of the saddle and the handlebars were adjusted for each subject to secure a steady working posture. To avoid artifact signals for NIRS and EEG measurements, the subjects were asked to keep their head as stable as possible during cycling. We also asked the subjects to avoid shifting of their upper body weight onto the handlebars. Subjects cycled the ergometer for 15 min at 60 rpm. The subjects could confirm the speed of rotation by a sound that indicated the pitch. The mean power output of the work load was 93 ± 5.4 W. The work load for each subject was decided before the experiment. Each subject was asked to set the work load at a Rating of Perceived Exertion of 12–13 for self-selected exercise intensity [2]. As for the time course of changes in mean HR before and during PE, the mean HR increased from 70 ± 2.6 beats/min at rest to a plateau (119 ± 3.5 beats/min) at 5 min after the onset of PE. The mean HR at 15 min after the onset of PE was 128 ± 4.3 beats/min.

To assess psychological mood, the POMS questionnaire was submitted to each subject before and after PE. The POMS is widely used and standardized to assess mood profiles in the subclinical range after exercise [1,34]. In this study, we used POMS-B, which is a shortened version of the original 65-item POMS [24]. Participants rated a series of 30 mood-related adjectives on a five-point scale in response to the question. Scores (on a 5-point scale of 0–4) are grouped into six subscales: tension anxiety, depression, anger hostility, vigor, fatigue, and confusion. Reliability coefficients for internal consistency were reported as 0.90 in the manual accompanying the instrument [24], based on data from numerous validity studies and normative samples. To evaluate the 5-HT system, blood samples (5 ml) were obtained by venous puncture at the cubital fossa before, immediately after PE, and 30 min after PE.

For whole blood analysis, we applied the method described in detail by Kremer et al. [21] and Mohri et al. [25]. Half a milliliter of blood was suspended in 2.5 ml of water. Then, 30 μ l of the internal standard and 10 μ l of a 10% (weight/volume) solution of ascorbic acid in water were added to the suspended blood sample. The sample was then stored frozen at -20°C until the assay.

2.5. HPLC analysis

High-pressure liquid chromatography (HPLC) analysis was conducted within 2 weeks after the experiment. The whole blood sample was thawed, and 167 μ l of methanol was added to 1 ml of the whole blood sample to remove proteins. Then, the whole blood sample was centrifuged at $4670 \times g$ for 10 min at 4°C . The 500 μ l supernatant of the whole blood sample was suspended in 4.5 ml of mobile buffer. The mobile phase consisted of a phosphate buffer (Na^+ , 0.1 M) containing 50 mg/L EDTA-2Na and an ion pair (300 mg/L sodium-octyl-sulfate, Nacalai Tesque Co., Kyoto, Japan) and 20% methanol at pH 6.0. The whole blood sample was injected into the HPLC system.

Levels of 5-HT were determined using an HPLC-ECD (Eicom 300, Eicom Co., Kyoto, Japan) system. The working electrode was a graphic carbon electrode set at a detector potential of +400 mV against an Ag/AgCl₂ reference electrode. The 5-HT was separated on a reversed-phase column (Eicompack CA-50DS). The flow rate was set at 0.22 ml/min, and the analysis temperature was 35°C .

2.6. Data analysis

Regarding the NIRS data, we analyzed changes in oxyHb as the best indicator of changes in blood flow in PFC [17,31] (see detailed description in Section 4). For analysis of oxyHb, the raw data from each channel were converted into a z-score. Detailed descriptions of the z-score are in Section 3.

The difference in mean z-score of oxyHb levels between the ventral PFC and the dorsal PFC regions was analyzed by paired *t*-test in this study. The mean z-score for the regional activation in the ventral PFC region was calculated by the data from 1 to 10 channels in Fig. 1. The corresponding data in the dorsal PFC region were analyzed by the data from 15 to 24 channels in Fig. 1. Note that the number 11, 12, 13, and 14 channels were excluded from further analysis in this study because of a boundary zone between ventral PFC and dorsal PFC.

For the purpose of analyzing frontal asymmetry, we used mean z-scores in the following six PFC regions. We named them LV (left ventral region; 1, 4, 8 channels in Fig. 1), MV (medial ventral region; 2, 5, 6, 9 channels in Fig. 1), RV (right ventral region; 3, 7, 10 channels in Fig. 1), LD (left dorsal region; 15, 18, 22 channels in Fig. 1), MD (medial dorsal region; 16, 19, 20, 23 channels in Fig. 1), and RD (right dorsal region; 17, 21, 24 channels in Fig. 1). We performed a one-way repeated measures analysis of variance (ANOVA) followed by a Scheffe's post hoc test for further comparison.

Comparison of EEG changes was made before and after PE, as well as among 1, 5, 10, and 15 min during PE. In each period, the raw EEG data were visually inspected, and segments that did not include the artifacts were visually selected piece by piece on the computer screen. The time course of changes in mean power areas of EEG and the effect of electrode positions of EEG were assessed by using two-way repeated measures ANOVA. In this study, the EEG recording condition was different for before/after PE versus during PE. Before and after PE were resting conditions. On the other hand, EEG was recorded in an active condition during PE. Therefore, we adopted two separate ANOVAs (i.e., before and after & during PE). Significance levels for the *F* values were obtained after the Greenhouse–Geisser correction when appropriate. If a significant main effect of the EEG changes was found, a paired *t*-test was used between before and after PE, and one-way repeated measure ANOVA was performed among 1, 5, 10, and 15 min during PE, followed by Dunnett's post hoc test.

The comparison of POMS questionnaire scores before and after PE was performed by paired *t*-test.

The mean whole blood 5-HT level was analyzed by one-way repeated measure ANOVA with the analysis periods, followed by Dunnett's post hoc test for further comparison.

Effects were considered to be statistically significant when the *p* values were less than 0.05. All data were expressed as the mean \pm S.E.

3. Results

3.1. Changes in the time course of hemoglobin levels in the PFC

Typical examples of changes in the time course of oxyHb, deoxyHb, and totalHb levels before, during, and after PE are shown in Fig. 2. There were two types of changes in the time course of oxyHb and totalHb levels during PE. One type of change was characterized by gradual increases in oxyHb and totalHb levels, which reached a steady state at the end of PE. Thereafter, gradual decreases in oxyHb and totalHb were observed after stopping PE. This type of change was mainly found in the ventral PFC regions (see #1–10 panels in Fig. 2). Based on MRI measurement in this study, the ventral PFC regions were considered to be BA 9–10.

By contrast, the other type of change showed no or little increase in oxyHb level or totalHb level during PE. This type of change was mainly observed in the dorsal PFC regions (including 15–24 panels in Fig. 2), corresponding to BA 8–9.

Regarding deoxyHb, there was only a small decrease or no change throughout PE in both the ventral and dorsal PFC regions.

3.2. Regional activation of oxyHb in PFC

For statistical analysis, we focused on oxyHb changes as the best indicator of changes in blood flow in PFC based on previous reports concerning NIRS experiments [17,31]. Additionally, the NIRS raw data were originally relative values, and such data could not be averaged directly across subjects or channels. We thus converted the raw data of oxyHb from each channel into a z-score because the z-score could be averaged regardless of unit. The z-score was calculated using the mean value and the standard deviation of oxyHb before PE. Therefore, the mean value and the standard deviation of oxyHb during the 5 min before PE were, respectively, changed into z-scores 0 and 1 for every channel. Thereafter, we calculated grand averages among the ventral PFC and dorsal PFC regions.

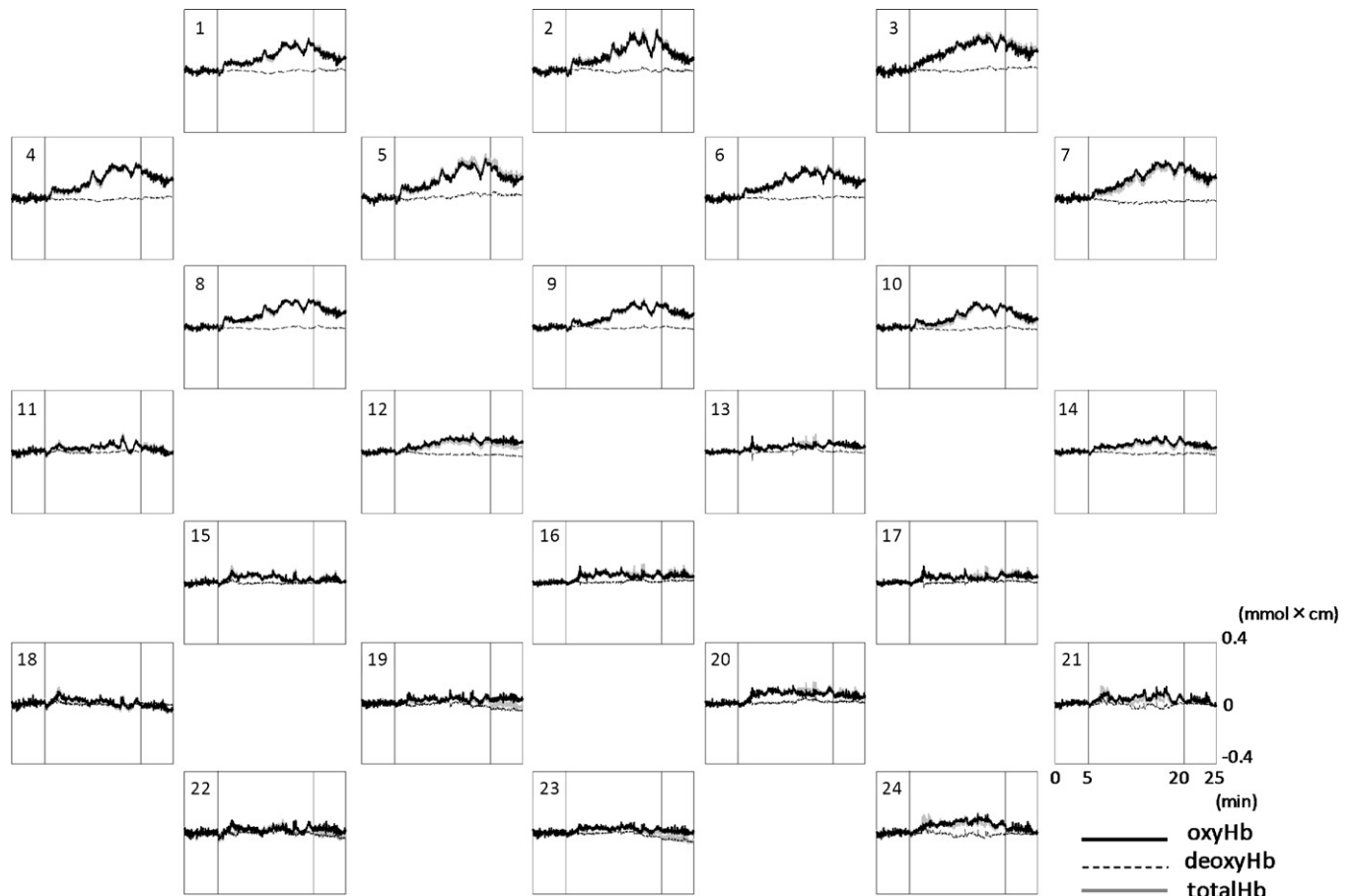


Fig. 2. Typical examples of changes in regional hemoglobin concentration in the prefrontal cortex (PFC) before, during, and after pedaling exercise (PE) from one subject. Solid lines indicate oxygenated hemoglobin (oxyHb) levels; thin dashed lines indicate deoxygenated hemoglobin (deoxyHb); and gray lines indicate the sum of oxyHb + deoxyHb (totalHb). The black vertical bars at 5 and 20 min represent the starting and end points of PE, respectively.

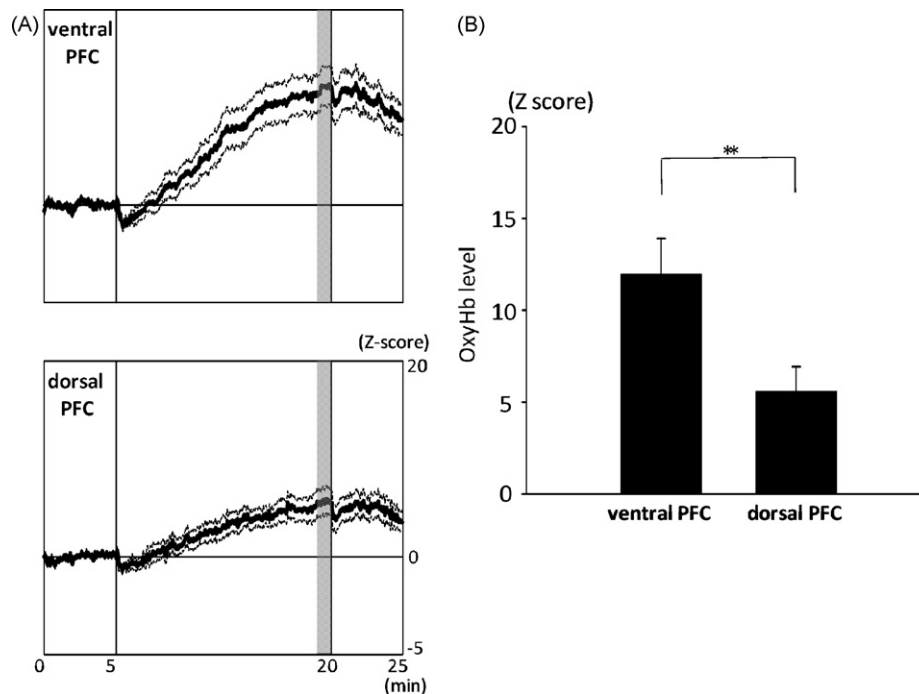


Fig. 3. (A) The grand averages for the time course of converted oxygenated hemoglobin (oxyHb) levels in ventral PFC (upper trace) and dorsal PFC (lower trace). The solid lines indicate the mean change in oxyHb, while the thin dashed lines indicate the standard error. The black vertical bars at 5 and 20 min represent the starting and end points of PE, respectively. The gray bars represent a period for statistical analysis. (B) Statistical changes in regional converted oxyHb levels in PFC during PE. Data are expressed as the mean \pm S.E. ($n = 10$). $**p < 0.01$. See text for details.

Fig. 3A shows the grand averages for converted data of oxyHb in the ventral and dorsal PFC regions. For statistical analysis, we calculated the mean converted level of oxyHb in each region for one minute immediately before the end of PE. The mean value was used as an index for regional activation within PFC in further analyses.

The mean indexes for the regional activation in the ventral and dorsal PFC are illustrated in Fig. 3B. The paired *t*-test for the changes in converted oxyHb levels revealed a significant increase in oxyHb in the ventral PFC region ($t=2.26$, $p<0.01$).

To evaluate frontal asymmetry, the mean indexes for the regional activation (LV, MV, RV, LD, MD, and RD regions) were statistically analyzed. The ANOVA for the changes in mean converted oxyHb levels revealed a significant main effect of PE for regions ($F=4.87$, $p<0.05$). Post hoc tests showed that the changes in the LV ($p<0.01$), MV ($p<0.05$), and RV ($p<0.05$) regions were significantly greater than that in LD. The change in the RV region was also significantly greater than that of the MD region ($p<0.01$). There was not any significant difference in the mean converted oxyHb levels among right, medial, or left regions within the ventral PFC as well as the dorsal PFC regions. Note that we did not show figures concerning the frontal asymmetry because we focused on the difference between ventral and dorsal PFC.

3.3. Changes in the mood state during PE

The investigator inquired about the mood of all subjects before and after PE using the POMS questionnaire. PE tended to improve vigor-activity (from 5.9 ± 1.8 to 7.3 ± 2.1 , $t=-1.35$, $p=0.21$) as assessed by the POMS subscale (Fig. 4). On the other hand, PE reduced negative mood in tension-anxiety ($t=2.64$, $p<0.05$) and confusion ($t=2.69$, $p<0.05$). Other subscales showed no significant

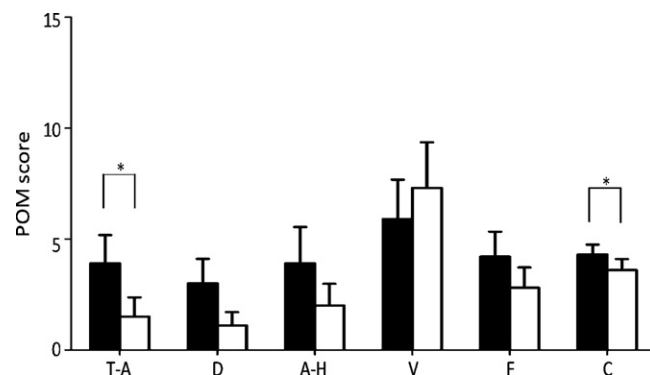


Fig. 4. Changes in mean POMS questionnaire scores before (closed bar) and after (open bar) PE. Data are expressed as the mean \pm S.E. ($n=10$). T-A, tension anxiety; D, depression; A-H, anger hostility; V, vigor; F, fatigue; C, confusion. * $p<0.05$.

change in depression ($t=2.08$, $p=0.06$), anger-hostility ($t=1.81$, $p=0.1$), or fatigue ($t=1.08$, $p=0.31$). In summary, subjects had a feeling of reduced negative mood accompanied by a tendency of increased vigor-activity after PE.

3.4. EEG during PE

Fig. 5 shows an example of the EEG (Cz, Pz), EOG, EMG_{QF}, and ECG before, during, and after PE. Because subjects performed PE with their eyes opened, EOG recordings exhibited vertical eye movement (due to eye blink) during PE. EMG_{QF} bursts occurred every 1 s during PE because subjects cycled the ergometer at 60 rpm, i.e., every 1 s. The ECG recording showed an increase in HR during PE.

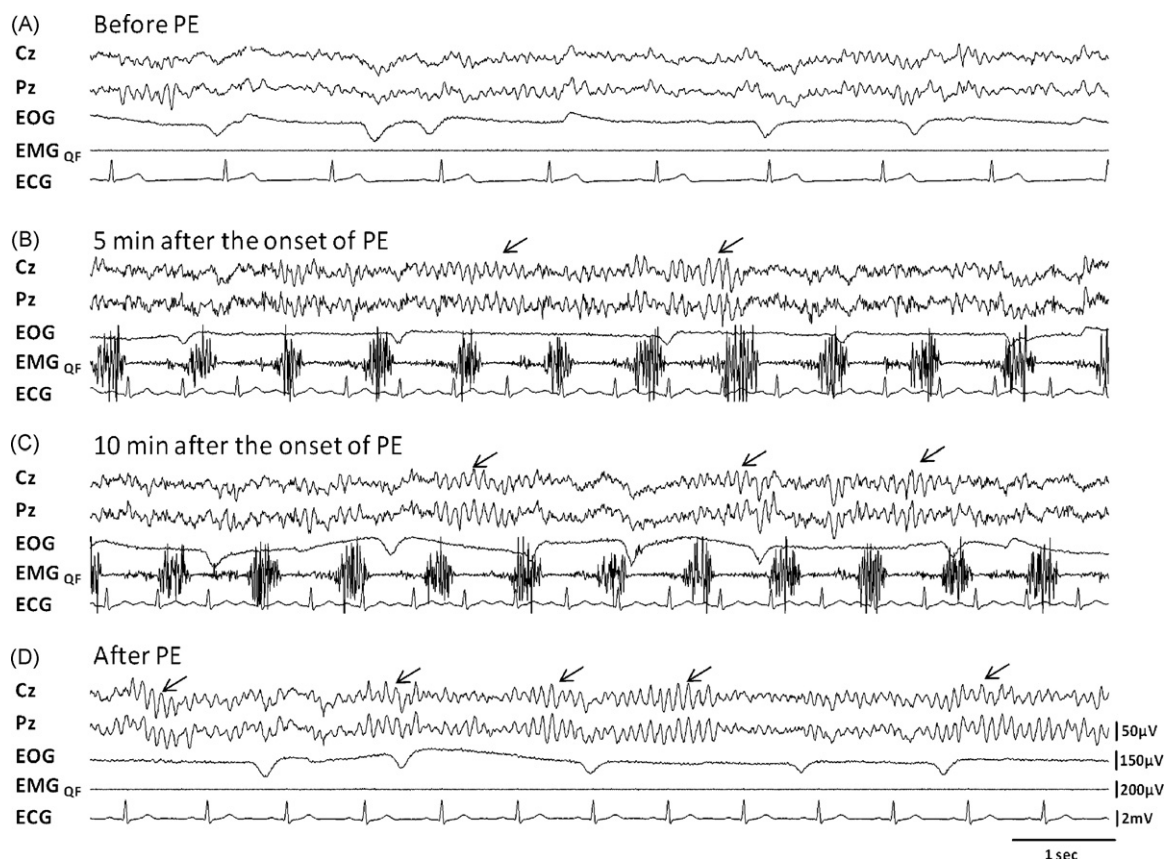


Fig. 5. An example of EEG (Cz, Pz), EOG, EMG_{QF}, and ECG from one subject. Data were obtained at before (A), during (B and C), and after (D) PE: during PE, data is shown at 5 and 10 min after the onset of PE. Arrows indicate the appearance of rhythmic waves with higher amplitude and slower frequency.

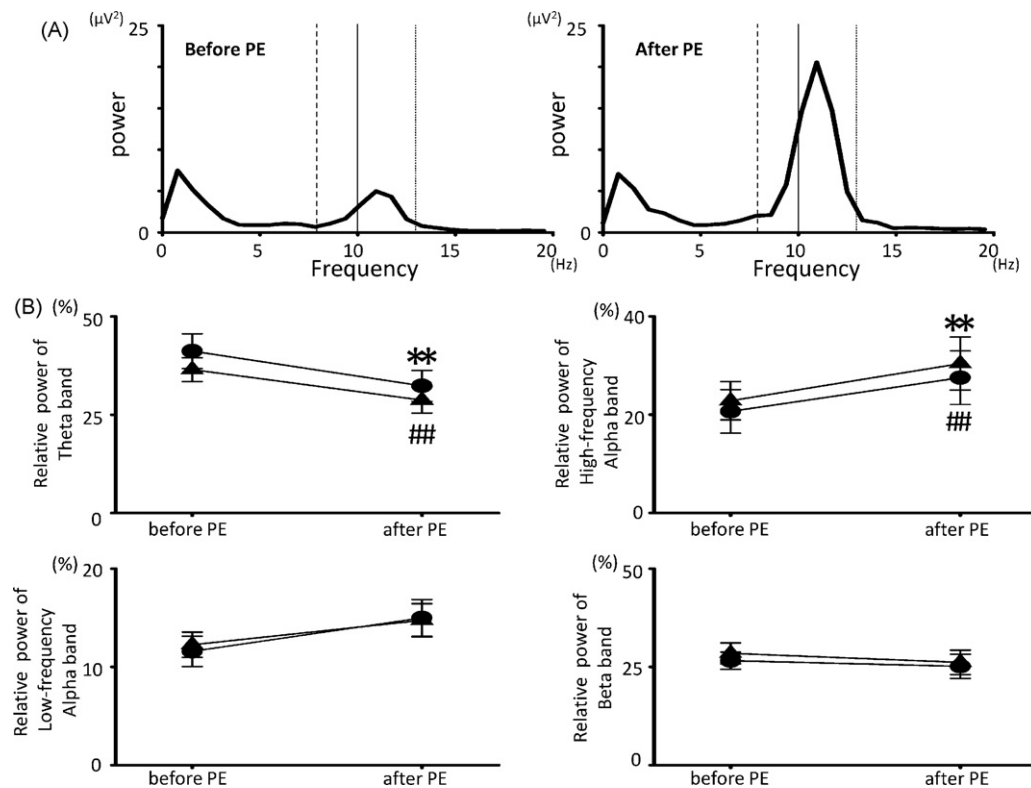


Fig. 6. (A) An example of spectral analysis of EEG in the parietal area before and after PE. Spectral analyses for 1 min of the EEG were performed before and after PE. Dashed, solid and dotted vertical lines indicate 8, 10, and 13 Hz, respectively. (B) The changes in mean relative power of theta (4–8 Hz), low-frequency alpha (8–10 Hz), high-frequency alpha (10–13 Hz), and beta (13–30 Hz) bands before and after PE. Black circle and triangle indicate the relative powers of EEG in Cz and Pz position, respectively. Data are expressed as the mean \pm S.E. ($n = 10$). ** $p < 0.01$ (for Cz position). ## $p < 0.01$ (for Pz position).

Before PE, a low-amplitude wave with a fast frequency was predominant as shown in Fig. 5A. On the other hand, a rhythmical wave with higher amplitude and slower frequency appeared at 5 min after the onset of PE (see arrows in Fig. 5B). This kind of rhythmical wave also occurred in the later stage of PE (Fig. 5C) and after PE (Fig. 5D).

We performed spectral analysis of the EEG in the Cz and Pz regions and obtained the mean power areas in theta (4–8 Hz), low-frequency alpha (8–10 Hz), high-frequency alpha (10–13 Hz), and beta (13–30 Hz) bands. The changes in the mean relative powers of theta, low- and high-frequency alpha, and beta bands were illustrated before and after PE in Fig. 6, as well as among 1, 5, 10, and 15 min during PE in Fig. 7.

As for the changes in theta power before PE and after PE (Fig. 6B), two-way repeated measures ANOVA showed a significant main effect ($F = 18.70$, $p < 0.01$). The interaction was not significant with the EEG changes before and after PE \times the electrode positions ($F = 0.34$, $p = 0.57$). There was a significant post hoc difference between before PE and after PE (Cz, $t = 3.94$, $p < 0.01$; Pz, $t = 3.88$, $p < 0.01$).

Concerning the changes in low-frequency alpha power before and after PE (Fig. 6B), two-way repeated measures ANOVA showed no significant main effect ($F = 4.19$, $p = 0.71$) or interaction (the EEG changes before and after PE \times the electrode positions, $F = 2.91$, $p = 0.12$).

Regarding the changes in high-frequency alpha power before PE and after PE (Fig. 6B), two-way repeated measures ANOVA showed a significant main effect ($F = 16.59$, $p < 0.01$). The interaction was not significant for the EEG changes before and after PE \times the electrode positions ($F = 0.26$, $p = 0.62$). There was a significant post hoc difference between before PE and after PE (Cz, $t = -4.18$, $p < 0.01$; Pz, $t = -3.54$, $p < 0.01$).

As for the changes in beta power before PE and after PE (Fig. 6B), two-way repeated measures ANOVA showed no significant main effect ($F = 1.45$, $p = 0.26$) or interaction (the EEG changes before and after PE \times the electrode positions, $F = 0.57$, $p = 0.47$).

Concerning the time course of theta power during PE (Fig. 7B), two-way repeated measures ANOVA showed a significant main effect ($F = 10.63$, $p < 0.01$). The interaction was not significant for the time course during PE \times the electrode positions ($F = 0.13$, $p = 0.79$). One-way repeated measure ANOVA showed no significant main effect (Cz, $F = 1.27$, $p = 0.31$; Pz, $F = 2.74$, $p = 0.63$).

Regarding the time course of low-frequency alpha power during PE (Fig. 7B), two-way repeated measures ANOVA showed a significant main effect ($F = 3.67$, $p < 0.05$). The interaction was not significant for the time course during PE \times the electrode positions ($F = 1.77$, $p = 0.21$). Regarding Cz position, one-way repeated measure ANOVA showed a significant main effect ($F = 3.14$, $p < 0.05$). There was a significant post hoc difference between 1 min of PE and 10 min of PE ($p < 0.05$). For the Pz position, the ANOVA showed no significant main effect ($F = 1.90$, $p = 0.15$).

As for the time course of high-frequency alpha power during PE (Fig. 7B), two-way repeated measures ANOVA showed a significant main effect ($F = 4.66$, $p < 0.05$). The interaction was not significant for the time course during PE \times the electrode positions ($F = 0.61$, $p = 0.49$). One-way repeated measure ANOVA showed a significant main effect (Cz, $F = 4.92$, $p < 0.01$; Pz, $F = 10.18$, $p < 0.01$). There was a significant post hoc difference between 1 min of PE and 15 min of PE (Cz, $p < 0.01$; Pz, $p < 0.01$).

Concerning the time course of beta power during PE (Fig. 7B), two-way repeated measures ANOVA showed a significant main effect ($F = 6.28$, $p < 0.05$). The interaction was not significant for the time course during PE \times the electrode positions ($F = 0.42$, $p = 0.74$). Regarding Cz position, one-way repeated measure ANOVA showed

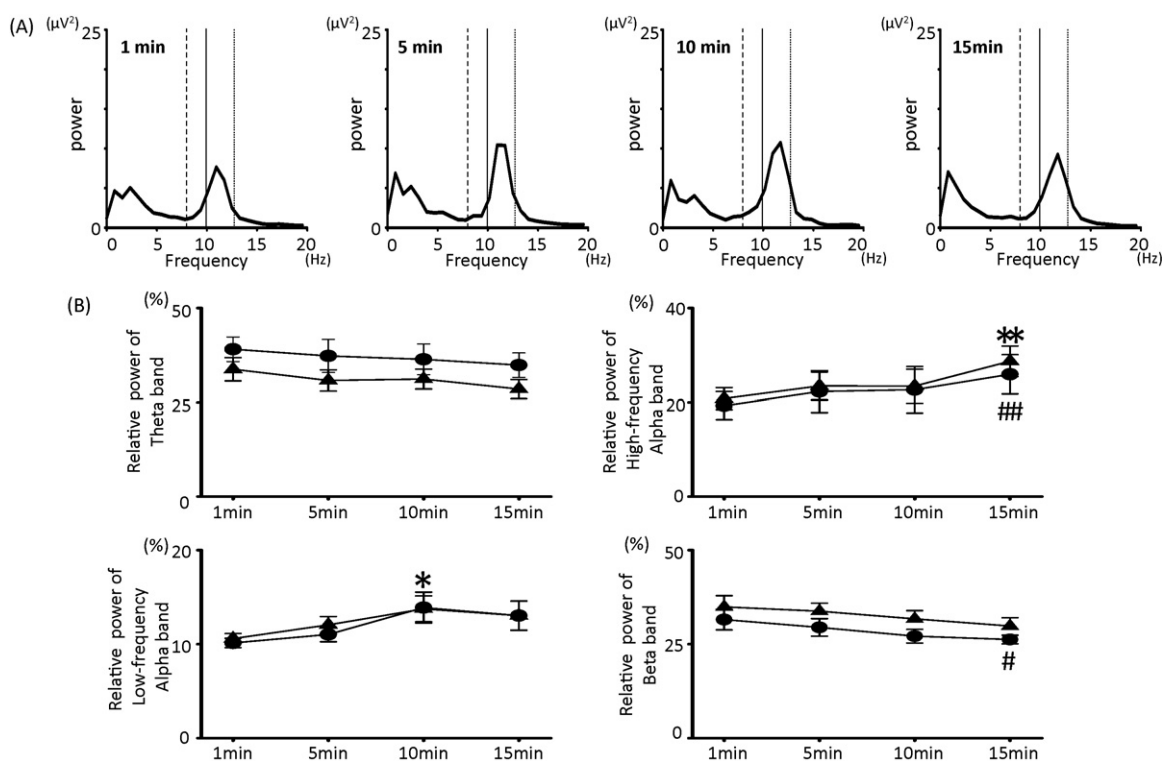


Fig. 7. (A) An example of spectral analysis of EEG in the parietal area during PE. Spectral analyses for 1 min of the EEG were performed during PE: Spectral analyses were performed at 1, 5, 10, and 15 min after the onset of PE. Dashed, solid and dotted vertical lines indicate 8, 10, and 13 Hz, respectively. (B). The changes in mean relative power of theta (4–8 Hz), low-frequency alpha (8–10 Hz), high-frequency alpha (10–13 Hz), and beta (13–30 Hz) bands during PE. Black circle and triangle indicate the relative powers of EEG in Cz and Pz position, respectively. Data are expressed as the mean \pm S.E. ($n = 10$). * $p < 0.05$, ** $p < 0.01$ (for Cz position). # $p < 0.05$, ## $p < 0.01$ (for Pz position).

no significant main effect ($F = 2.29$, $p = 0.10$). For the Pz position, the ANOVA showed a significant main effect ($F = 3.55$, $p < 0.05$). There was a significant post hoc difference between 1 min of PE and 15 min of PE ($p < 0.05$).

3.5. 5-HT levels

The changes in the mean whole blood 5-HT levels before PE, soon after PE, and 30 min after ending PE are illustrated in Fig. 8. The

mean whole blood 5-HT level before PE was 169.61 ± 21.29 ng/ml. The mean 5-HT level increased to 181.84 ± 22.27 ng/ml soon after ending PE but decreased to 168.63 ± 18.95 ng/ml at 30 min after PE. One-way repeated measure ANOVA on the mean whole blood 5-HT level showed a significant main effect of PE ($F = 6.53$, $p < 0.01$). The significant post hoc difference was obtained between before PE and immediately after PE ($p < 0.05$) (Fig. 8).

4. Discussion

4.1. Changes in oxyHb levels in the PFC during PE

The present study revealed that PE evoked a significant increase in oxyHb levels in the ventral PFC (BA 9–10) as compared with those in the dorsal PFC (BA 8–9). This finding indicates that such a regional difference in oxyHb levels within the PFC regions is not caused by the augmentation of systemic circulation during exercise but by local oxygen demand or activity. There is a previous report [18] demonstrating an increase in oxyHb levels in the PFC during PE, using a one-channel NIRS probe, though the precise location of the probe in the PFC was not defined. The present result indicates that PE causes increased activity in a restricted region within the PFC, namely the ventral PFC.

Regarding the functional difference between the ventral PFC and dorsal PFC, it has been documented that the dorsal PFC (BA 8–9) is mainly involved in working memory [12,26]. As for the regional activation in the ventral and dorsal PFC, there are several studies indicating that the ventral PFC may be related to mood or emotional state. For example, Drevets et al. [9] reported abnormally decreased activity in the ventral PFC in both familial bipolar depressives and familial unipolar depressives. Kronhaus et al. [22] demonstrated a negative correlation between depression severity and ventral PFC activity in patients with bipolar depression. These previous studies

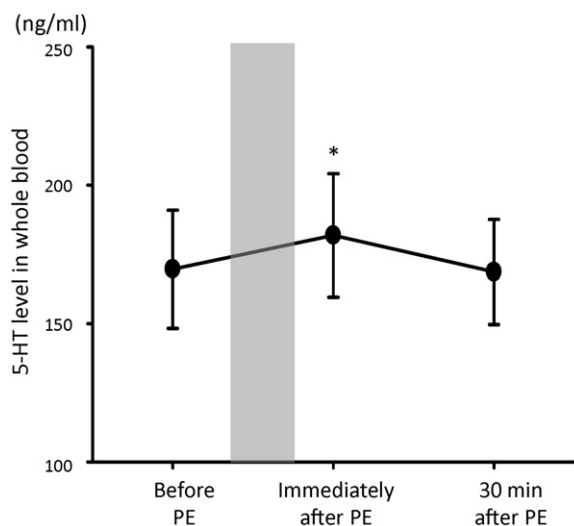


Fig. 8. Changes in mean whole blood 5-HT level before, immediately after, and 30 min after ending PE. The gray bar represents a period of pedaling exercise (PE). Data are expressed as the mean \pm S.E. ($n = 10$). * $p < 0.05$ as compared with the whole blood 5-HT level before PE.

indicate that the decreased activity in the ventral PFC may be linked with an enhanced negative mood. Although this is hypothetical, we thus presumed conversely that the increased activity of the ventral PFC observed in this study may produce a decreased negative mood.

Concerning a relationship between mood and activation of PFC, a hypothesis on frontal cortex asymmetry has been reported by Davidson [7,8]. He reported that prefrontal hemispheric asymmetry is related to positive or negative affective responses and approach or withdrawal behaviors. In this regard, several investigators [30,33] reported frontal asymmetry of the alpha frequency range in EEG after exercise; however, in this study, we did not obtain such a difference between left and right PFC activity using NIRS. This may be explained by the methodological difference (i.e., EEG vs. NIRS). A future study is needed to explore frontal asymmetry using the NIRS method.

4.2. Increases in the power of alpha band in EEG after PE and during PE

The present study revealed a significant increase in the relative power of high-frequency alpha band after PE as compared with that in the resting condition before PE. The relative power of high-frequency alpha band also showed a significant increase during PE. There are many reports demonstrating an increase in the alpha band of EEG during and after PE [6]. We would like to emphasize that this study revealed that the relative power of high-frequency alpha band was significantly exaggerated after PE as well as during PE. Interestingly, Cantero et al. [4] reported that the activity of high-frequency alpha band, but not of low-frequency alpha band, is linked with a state of wakefulness. This finding appears compatible with the present result, i.e., the decreased theta activity after PE. In other words, the arousal state of subjects did not shift to a drowsy state or cortical inactivation. In addition, the present results concerning mood state showed a tendency of increased vigor-activity after PE, which is contrary to the sleep state, suggesting that the development of high-frequency alpha band during and after PE in this study may be linked with a state of wakefulness or a feeling of vigor-activity.

4.3. The mechanism underlying the occurrence of high-frequency alpha band during PE

As for the mechanism underlying the occurrence of high-frequency alpha band with a feeling of vigor-activity, we hypothesized that the 5-HT system within the brainstem may play a significant role based on the following experimental data. Jones and Mühlethaler [20] demonstrated that the microinjection of 5-HT into the basal forebrain resulted in cortical attenuation in rats. Because cholinergic neurons in the basal forebrain are known to project to broad areas in the cortex, this 5-HT-induced inhibition of the cholinergic pathway may be responsible for the cortical attenuation that was characterized by the occurrence of high-frequency alpha band.

The present study revealed that PE induced an increase in 5-HT level in whole blood. This result indicates that increases in 5-HT level in whole blood after PE are likely derived from augmentation of brain 5-HT. Several recent *in vitro* studies [3,32] have revealed the presence of 5-HT transporter mRNA in vascular endothelial cells within the brain, indicating that the blood–brain barrier may act as an efflux transport system for 5-HT. Our recent study [27] revealed that augmented 5-HT level within the brain can cross the BBB to the peripheral blood through the 5-HT transporter in rats that had undergone gastrointestinal and kidney resections along with liver inactivation (organs contributing to increasing blood 5-HT after 5-HTP administration). It is important to note that we measured 5-HT in whole blood, not in platelet-poor plasma, in this study. 5-HT

released across the BBB is thought to be taken up quickly to the platelets, and thus we must evaluate 5-HT both in the plasma and the platelets, i.e. the whole blood.

Activation of the 5-HT system during PE may be linked with the observations in an animal experiment reported by Jacobs and Fornal [19]. They revealed that augmentation of the 5-HT system in the brainstem can be elicited by various repetitive rhythmical behaviors, such as locomotion, mastication, and breathing; the increased activation of 5-HT neurons in the brain was obtained several seconds after the onset of exercise. Fujino et al. [10] also showed that the increased brain 5-HT level appeared after 3 min of swimming using an *in vivo* microdialysis method. PE in the present study is considered a repetition of the rhythmical behavior of locomotion. Although the rhythmical behavior of PE is considered to activate the brain 5-HT system, a future study is needed to explore the precise mechanism for the possible effect of PE on the brain 5-HT system.

4.4. The source of an augmentation of the 5-HT system

The activation of the 5-HT system in the brainstem may be linked with the increased activity of PFC based on the following previous observations. Hajós et al. [15] demonstrated that there are reciprocal projections from the PFC region to 5-HT neurons in the dorsal raphe nucleus. In addition, Celada et al. [5] showed that electrical stimulation of the PFC region increased activity in 5-HT neurons in the dorsal raphe nucleus. The present study revealed a significant increase in oxyHb levels in the ventral PFC during PE. It is likely that the increased activity of PFC caused the activation of the 5-HT system in the dorsal raphe nucleus, which was evidenced by the increased 5-HT levels in whole blood. This is supported by a recent report [14] demonstrating that the ventral PFC sends robust projections to the dorsal raphe nucleus.

4.5. Methodological consideration

We used two electrodes (Cz and Pz) for EEG recording in this study. Concerning the different changes in EEG activity in specific brain areas, several investigators reported frontal asymmetry of the alpha frequency range [30,33]. But in our experimental settings, the subject's frontal region was covered with the NIRS holder to fixate the 24-channel optodes. Because our priority was NIRS measurement of the frontal region in this study, we did not put EEG electrodes on the frontal region. As for the temporal and occipital regions, we also could not place EEG electrodes because the bands to fixate the NIRS holder were positioned on the temporal and occipital regions. Note that in our previous report we performed EEG recording using five electrodes at Fz, C3, Cz, C4, and Pz [11], and we obtained similar results.

To evaluate the change in activity within PFC regions, we measured oxyHb levels in this study based on previous reports concerning NIRS experiments. Strangman et al. [31] showed a strong correlation between the blood oxygen level-dependent signal of functional MRI (fMRI) and oxyHb level by using a simultaneous recording of fMRI and NIRS in humans. Hoshi et al. [17] suggested that oxyHb is the most sensitive indicator of changes in blood flow among the three NIRS parameters (oxyHb, deoxyHb, and totalHb) using a perfused rat brain model. We therefore evaluated oxyHb levels as the best indicator of changes in blood flow in PFC and analyzed regional differences in oxyHb levels within the PFC regions in this study.

As for the comparison of whole blood 5-HT level before and after PE, the present study did not include a control experiment; however, our previous study [25] included a control experiment where the subjects stayed at rest for 20 min. We found that there were no significant changes in 5-HT levels in whole blood after 20 min in the control experiment.

In conclusion, the present study demonstrated that PE induces increased activity in the ventral PFC region, which may be responsible for the accompanying feeling of decreased negative mood with increased vigor-activity. PE also induces significant increases in the relative powers of high-frequency alpha bands in EEG. We hypothesize that such EEG changes are linked with the increased blood 5-HT levels or an augmentation of the 5-HT system in the brain-stem because the 5-HT-induced inhibition of the basal forebrain would cause cortical attenuation. The activation of the ventral PFC during PE may also be responsible for the augmentation of the 5-HT system based on reciprocal projections from the PFC region to 5-HT neurons in the raphe nucleus.

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