Activation of the anterior prefrontal cortex and serotonergic system is associated with improvements in mood and EEG changes induced by Zen meditation practice in novices

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**ABSTRACT**

To gain insight into the neurophysiological mechanisms involved in Zen meditation, we evaluated the effects of focused attention (FA) on breathing movements in the lower abdomen (Tanden) in novices. We investigated hemodynamic changes in the prefrontal cortex (PFC), an attention-related brain region, using 24-channel near-infrared spectroscopy during a 20-minute session of FA on Tanden breathing in 15 healthy volunteers. We found that the level of oxygenated hemoglobin in the anterior PFC was significantly increased during FA on Tanden breathing, accompanied by a reduction in feelings of negative mood compared to before the meditation session. Electroencephalography (EEG) revealed increased alpha band activity and decreased theta band activity during and after FA on Tanden breathing. EEG changes were correlated with a significant increase in whole blood serotonin (5-HT) levels. These results suggest that activation of the anterior PFC and 5-HT system may be responsible for the improvement of negative mood and EEG signal changes observed during FA on Tanden breathing.

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1. Introduction

A recent review by Lutz et al. (2008) specifies two styles of meditation: focused attention (FA) meditation and open monitoring (OM) meditation. FA meditation involves the voluntary focusing of attention on a chosen object, such as a subset of localized sensations caused by breathing. OM meditation involves nonreactive monitoring of the content of experience from moment to moment.

Although both FA and OM practices are combined over the course of Zen meditation training, FA on breathing movements in the lower abdomen (Tanden) is considered a fundamental technique that is commonly practiced by Zen monks, who breathe more slowly during meditation practice and spend more time breathing out than breathing in (Austin, 2006). The focusing of attention on Tanden breathing is practiced particularly intensively in the initial stages of Zen meditation training by monks.

To gain insight into the neurophysiological mechanisms related to Zen meditation, we examined novices during FA on Tanden breathing (Fumoto et al., 2004). Novices were chosen as a study group to avoid the effects of experience in expert meditators, in accord with Lutz et al. (2008). To easily focus participants’ attention on breathing movements in the lower abdomen, participants were instructed to observe and confirm the contraction of their abdominal muscles by viewing their abdominal electromyography (EMG) signal on an oscilloscope. This method is referred to as “FA on Tanden breathing with visual feedback” and is mainly related to FA meditation.

A number of recent imaging studies reported that attention-related brain regions, including the prefrontal cortex (PFC), were activated during FA meditation (Cahn and Polich, 2006; Brefczynski-Lewis et al., 2007; Lutz et al., 2008). Furthermore, Manna et al. (2010) found that FA meditation elicited neural activation in the anterior PFC (BA10) and anterior cingulate cortex. In the present study, we sought to evaluate activation in the PFC during FA on Tanden breathing with visual feedback, using 24-channel near-infrared spectroscopy (NIRS).

NIRS is a recently developed functional brain imaging technique, which has been used to assess PFC activation in a number of exercise studies (Rooks et al., 2010). Unlike functional magnetic resonance imaging (fMRI), NIRS cannot be used to investigate the involvement of deep brain structures (Pagnoni and Cekic, 2007; Luders et al., 2009). However, NIRS is able to measure brain activity continuously during FA movements in a more natural setting and body position than is possible with fMRI, which requires participants’ movement to be highly constrained during data collection.

In addition, a number of electroencephalography (EEG) studies have examined electrophysiological activity during Zen meditation, reporting the appearance of alpha and theta waves (Kasamatsu and Hirai, 1966; Murata et al., 1994). A previous study in our laboratory (Fumoto et al., 2004) showed that increases in EEG low frequency activity during FA practice were correlated with increased levels of whole blood serotonin (5-HT) concentrations. These results suggest that activation of the anterior PFC and 5-HT system may be responsible for the improvement of negative mood and EEG signal changes observed during FA on Tanden breathing.
Several observations suggest that EEG changes during Zen meditation or FA on Tanden breathing may be induced by serotonin (5-HT) activity. First, Jones and Mühlthaler (1998) revealed in an animal study that an appearance of relatively low-frequency EEG activity, that is, cortical suppression, was induced by the local application of 5-HT into the basal forebrain where cholinergic neurons project to broad cortical areas. Second, animal studies (Jacobs and Fornal, 1993) have reported that the activity of 5-HT neurons is enhanced by voluntary rhythmic behaviors, including locomotion, mastication and breathing. We consider FA on Tanden breathing to constitute a voluntary rhythmic breathing behavior. Based on these findings, we hypothesized that augmentation of the 5-HT system in the brain during FA on Tanden breathing would elicit cortical suppression through the basal forebrain, resulting in the appearance of alpha band activity.

In the current study, we tested whether measurable augmentation of the 5-HT system could be observed during FA on Tanden breathing. To this end, we measured the 5-HT concentration in whole blood before and after FA on Tanden breathing. A recent animal study in our laboratory confirmed that this method was appropriate for the assessment of 5-HT augmentation in the brain (Nakatani et al., 2008), revealing that when 5-HT levels were increased within the brain, 5-HT was able to cross the blood-brain barrier (BBB) into the basal forebrain where cholinergic neurons project to broad cortical areas. Therefore, animal studies (Jacobs and Fornal, 1993) have reported that the activity of 5-HT neurons is enhanced by voluntary rhythmic behaviors, including locomotion, mastication and breathing. We consider FA on Tanden breathing to constitute a voluntary rhythmic breathing behavior. Based on these findings, we hypothesized that augmentation of the 5-HT system in the brain during FA on Tanden breathing would elicit cortical suppression through the basal forebrain, resulting in the appearance of alpha band activity.

A number of previous studies have indicated that meditation is related to not only the focusing of attention, but also emotional regulation (Lutz et al., 2008; Goldin and Gross, 2010). In the current study, we thus assessed mood state changes before and after FA on Tanden breathing using the Profile of Mood States (POMS) assessment.

2. Materials and methods

2.1. Subjects

Fifteen healthy, right-handed subjects (aged 38 ± 15.9 years, 14 males and 1 female) volunteered to take part in this study. Subjects were screened to exclude those with mental, neurological, or respiratory illness; history of head injury; or medications that could affect EEG recordings, regional cerebral blood flow or 5-HT measurements. Verbal 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. It was made clear to the subjects that they were free to withdraw from the study at any time if they did not wish to continue.

2.2. Focused attention (FA) on Tanden breathing

No subject had previously practiced any form of meditation technique. To focus subjects’ attention on their breathing movements in the lower abdomen (Tanden), we used a breathing exercise with visual feedback, as follows. EMG was recorded to monitor abdominal muscle contraction, using a pair of skin-taped silver cup electrodes placed near the right anterior superior iliac spine. The electrodes were spaced at a distance of 3 cm. The EMG signals were amplified and filtered (0.03–1 kHz) using a bioelectric amplifier (Nihon Kohden EEG-4217, Japan). Subjects were able to observe and confirm the contraction of the abdominal muscles by viewing the abdominal EMG on an oscilloscope, which was placed approximately 1 m in front of them. It should be noted that the contraction of abdominal muscles is not usually observed during spontaneous breathing at rest. Subjects were instructed to produce strong and prolonged contractions of the abdominal muscles during the breathing exercise, so that they could monitor their abdominal muscle contraction on the oscilloscope during the breathing exercise. This technique was defined as focusing of attention on breathing movements in the lower abdomen, referred to as “FA on Tanden breathing” in this study.

Subjects were instructed to breathe at a rate of roughly 3–4 breaths/min, with an expiratory period and inspiratory period of approximately 9–12 s and 6–8 s, respectively. Subjects were instructed not to be overly rigid in adhering to these guidelines, but to make breathing as relaxed as possible. This breathing pattern is mainly controlled by voluntary contraction of the abdominal muscles during the expiratory phase, leading to an increase in expiratory tidal volume, compared with spontaneous breathing. The prolonged duration of expiration compensates for this augmentation of expiratory tidal volume, so that neither hyperventilation nor hypoventilation occurs. This FA on Tanden breathing method was mastered easily by novice subjects.

2.3. EEG measurement

EEG was recorded from three scalp loci, at Cz, Pz, and Oz, according to the international 10/20 system. Reference electrodes were placed on the left and right earlobes (A1 and A2) for monopolar recording. The EEG signals were amplified and filtered (0.3–60 Hz) using a bioelectric amplifier (Nihon Kohden EEG-4217, Japan), and were continuously monitored on the recorder. These signals were then digitized at a sampling rate of 200 Hz and stored on a microcomputer for off-line analysis.

Electrooculography (EOG) was monitored with two silver–silver chloride electrodes, attached above and below the external corner of the right eye, to monitor eye-movement artifacts. In addition, electrocardiography (ECG) was recorded to monitor heart rate: two skin-taped silver cup electrodes were placed on the right clavicle and left eighth rib. Both EOG and ECG signals were amplified and filtered (0.03–60 Hz) using the bioelectric amplifier.

2.4. NIRS data acquisition

We used a 24-channel NIRS system (OMM-3000, Shimadzu Corporation, Japan) to detect concentration changes in oxygenated hemoglobin (oxyHb), deoxygenated hemoglobin (deoxygenHb), and their sum (totalHb) using three types of near infrared light (wavelengths: 780, 805, and 830 nm). These parameters were calculated according to the following equations:

\[ \text{oxyHb} = -1.49 \times \Delta A_{780} + 0.597 \times \Delta A_{805} + 1.4847 \times \Delta A_{830} \]
\[ \text{deoxygenHb} = 1.845 \times \Delta A_{780} - 0.2394 \times \Delta A_{805} - 1.0947 \times \Delta A_{830} \]

totalHb = oxyHb + deoxyHb

\( \Delta A_{780}, \Delta A_{805}, \) and \( \Delta A_{830} \) represent detected optical absorbances at 780, 805, and 830 nm, respectively. These were calculated every 130 ms, and cumulative data sampling was performed for 1040 ms (130 ms × 8 points) for analysis.

A 4 × 4 optode probe set (consisting of eight light emitters and eight photo detectors) was placed over each participant’s frontal area as shown in Fig. 1A. Channel 2, i.e. the center of the lowest row of the probes, was located at electrode position Fpz, according to the international 10/20 system for EEG recording. The optodes were spaced at a distance of 3 cm. Before the attachment of optodes, hairs under the optode were carefully brushed away to avoid signal disruption. In addition, we checked whether the photomultiplier values were at an optimal level, which was obtained when the input level was between 0.05 and 4 V. If the input level was over or under...
2.5. Experimental procedures

The experiment was conducted in a sound-attenuated, electrically shielded, dimly lit room, with subjects seated on a straight-backed chair. Prior to the experimental task, subjects undertook a practice session for several minutes to become familiar with the use of their abdominal EMG signal presented on an oscilloscope, so that they could confirm the voluntary movement of their own abdominal muscles. The practice session continued until participants learned to perform FA on Tanden breathing to a satisfactory level, and to adapt their rates of breathing appropriately. To obtain baseline recordings, the subject was asked to rest for 5 min. Then, the control experiment was followed by a 2-min baseline condition period before FA on Tanden breathing. After the task experiment, the subject was asked to rest for 5 min and 30 min after FA on Tanden breathing.

2.6. Measurements of serotonin (5-HT) in the whole blood

For whole blood 5-HT analysis, we applied methods described in detail by Kremer et al. (1990) and Mohri et al. (2005). First, 0.5 ml of blood was suspended in 2.5 ml of water. Then, 30 μl of the internal standard and 10 μl of a 10% (weight per volume) solution of ascorbic acid in water were added to the suspended blood sample. The sample was then frozen and stored at −20 °C until the assay.

Whole blood 5-HT levels were measured within 2 weeks after the experiment. The blood sample was thawed and 167 μl of methanol was added to 1 ml of the blood sample to remove proteins. The blood sample was then centrifuged at 4670 × g for 10 min at 4 °C. The 500 μl supernatant of the 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, Japan) and 20% methanol at pH 6.0. The blood sample was injected into a high-performance liquid chromatography (HPLC) system (HPLC-ECD, EICOM 300, EICOM, Japan) to determine 5-HT levels. The working electrode was a graphite carbon electrode set at a detector potential of +400 mV against an Ag/AgCl2 reference electrode. 5-HT was separated on a reversed phase column (EICOMPAK CA-5 ODS, EICOM). The flow rate was set at 0.22 ml/min and the analysis temperature was 35 °C.

2.7. Data analysis

For statistical analysis of NIRS data, we used changes in oxyHb for assessment of regional cortical activation, based on previous reports (Strangman et al., 2002; Rasmussen et al., 2007). Among the three NIRS parameters, changes in oxyHb concentration were found to be the most sensitive for task-related hemodynamic changes, and provided the strongest correlation with the blood oxygen–hemoglobin level dependent (BOLD) signal.

The NIRS raw data were originally relative values, which cannot be averaged directly across subjects or channels. We thus converted the raw oxyHb data from each channel into a z-score (‘converted oxyHb’), because this score could be averaged regardless of units (Matsuda and Hiraki, 2006). The z-score was calculated using the mean value and standard deviation of oxyHb before the FA on Tanden breathing session. Therefore, the mean value and the standard deviation of oxyHb during the 2-min baseline condition period before FA on Tanden breathing were converted to z-scores, “0” and “1” in each channel, respectively.
To analyze local changes in PFC activation, we divided the 24 PFC regions into six combined regions (termed the LAL: left anterior-lateral region, AM: anterior-medial region, RAL: right anterior-lateral region, LDL: left dorsal-lateral region, DM: dorsal-medial region, and RDL: right dorsal-lateral region), as shown in Fig. 1B. For examining converted oxyHb levels (z-score) in the six combined regions (LAL, AM, RAL, LDL, DM and RDL), we performed a one-way repeated-measures analysis of variance (ANOVA) with region as a within-subjects factor, followed by a Scheffe’s post hoc test for further comparison.

In addition, we analyzed the difference between two combined regions, i.e., the anterior PFC region (a combined region of the LAL, AM and RAL) and the dorsal PFC region (a combined region of the LDL, DM and RDL). Channels 11, 12, 13 and 14 were excluded from this analysis because of a boundary zone between the anterior PFC and dorsal PFC. Differences in oxyHb level between the anterior and dorsal PFC regions were compared using paired t-tests.

For analysis of EEG data, spectral analyses of EEGs for 1 min were performed using ATAMAP II software (Kissei Comtec, Japan) immediately before, during and immediately after FA on Tanden breathing. Spectral analyses during FA on Tanden breathing were performed at 5, 10, 15 and 20 min following the onset of FA on Tanden breathing. A fast Fourier transform method was used to obtain the mean power amplitudes in the theta (4–8 Hz), alpha (8–13 Hz), and beta (13–30 Hz) bands. A logarithmic transformation of log(x/100–x) was used to achieve nearly Gaussian distribution for all relative power measures (x), where x denotes relative power in each frequency band (John et al., 1980). The effect of FA on Tanden breathing on each EEG power band (theta, alpha, and beta) was assessed using a two-way repeated measures ANOVA with EEG electrode position and time course as within-subjects factors, followed by a Scheffe’s post hoc test for further comparison.

The mean 5-HT level in whole blood was analyzed using one-way repeated measures ANOVA with analysis period as a within-subjects factor, followed by Dunnett’s post hoc test for further comparison. Pearson’s correlation test was used for correlation analyses among three parameters, namely, 5-HT level, EEG power and oxyHb level, in a combined PFC region.

The statistical significance of differences in the POMS questionnaire score between before and after FA on Tanden breathing was tested using paired t-tests.

Effects were considered significant if p-values were less than 0.05. All data were expressed as mean ± S.E.

3. Results

3.1. NIRS during FA on Tanden breathing

To assess regional cortical activation, we grouped the 24 PFC regions into six combined regions (LAL, AM, RAL, LDL, DM and RDL) shown in Fig. 1B. Fig. 2 shows the grand average of the time course of converted oxyHb levels in the six combined PFC regions. The black lines indicate the mean converted change in oxyHb, while the gray lines indicate the standard error. The vertical dashed bars at 2 and 27 min represent the start and end points of the FA on Tanden breathing session, respectively.
among the six combined PFC regions during FA on Tanden breathing ($F_{5,84} = 5.34, p < 0.001$). Changes in the mean converted oxyHb level in RAL ($p < 0.05$) and AM ($p < 0.05$) were significantly greater than the change in DM. The change in mean converted oxyHb level in LAL was not significantly different compared to changes in LDL ($p = 0.406$), DM ($p = 0.185$) and RDL ($p = 0.415$), respectively. There were no significant differences in mean converted oxyHb levels in the right, medial, or left regions within the anterior PFC, or in dorsal PFC regions (Fig. 3).

These analyses revealed that mean converted oxyHb levels in each of the LAL, AM, and RAL regions were greater than those in each of the LDL, DM, and RDL regions. We thus compared the mean converted oxyHb level in the anterior PFC region (a grouped region combining LAL, AM and RAL) to that in the dorsal PFC region (a grouped region combining the LDL, DM and RDL) during FA on Tanden breathing, as illustrated in Fig. 4A. The results revealed that the mean converted oxyHb level in the anterior PFC region was significantly greater ($t = 4.38, p < 0.001$) than that in the dorsal PFC region, as shown in Fig. 4B.

3.2. EEG during FA on Tanden breathing

Fig. 5 illustrates the time course of mean log relative power in the theta, alpha and beta bands before, during and after FA on Tanden breathing.

The ANOVA examining log relative theta power (first panel in Fig. 5) showed a significant main effect of the FA on Tanden breathing task (comparison of log relative theta power before and during FA on Tanden breathing: $F = 9.85, p < 0.001$). The time course×electrode position interaction was not significant ($F = 0.08, p = 1.000$). There was a significant post hoc difference between log relative theta power before FA on Tanden breathing and at 15 min ($p < 0.001$), 20 min ($p < 0.001$) during FA on Tanden breathing, and after FA on Tanden breathing ($p = 0.032$). In addition, there was a significant difference in log relative theta power at Cz, Pz, and Oz ($F = 54.43, p < 0.001$). The log relative theta power at Cz was significantly greater than that at Pz ($p < 0.001$), and Oz ($p < 0.001$). Moreover, the log relative theta power was significantly greater at Pz than at Oz ($p < 0.001$).

The ANOVA examining log relative alpha power (second panel in Fig. 5) showed a significant main effect of the FA on Tanden breathing task (comparing log relative alpha power before and during FA on Tanden breathing: $F = 9.31, p < 0.001$). The time course×electrode position interaction was not significant ($F = 0.16, p = 0.999$). There was a significant post hoc difference in log relative alpha power before FA on Tanden breathing and at 5 min ($p = 0.018$), 10 min ($p = 0.015$), 15 min ($p < 0.001$), and 20 min ($p < 0.001$) during FA on Tanden breathing, and after FA on Tanden breathing ($p = 0.014$). There was a significant difference in log relative alpha power at Cz, Pz, and Oz ($F = 7.75, p < 0.001$), and log relative alpha power at Cz was significantly less than that at Pz ($p < 0.001$).

In the analysis of log relative beta power (third panel in Fig. 5), the ANOVA did not reveal a significant main effect of the FA on Tanden breathing task ($F = 0.96, p = 0.440$) on the time course. There was a
The present results demonstrated that FA on Tanden breathing induced a significant increase in oxyHb levels in the anterior PFC (BA10 and 9), compared with levels in the dorsal PFC (BA8 and 9). This finding indicates that the regional difference in oxyHb levels within the PFC was not produced by the augmentation of systemic circulation during FA on Tanden breathing, but by local oxyHb demand or activity within the PFC.

The present results regarding changes in local blood flow in the PFC (activation of BA10 and 9) were in partial agreement with several previous reports. Brefczynski-Lewis et al. (2007) reported that novices and expert meditators showed activation in a large overlapping network of attention-related brain regions including the PFC (BA9) during FA meditation while focusing on an external visual point. Manna et al. (2010) recently found that FA meditation activated BA10 and anterior cingulate cortex. These previous results are in accord with the suggestion from the present findings that FA meditation causes activation in the anterior PFC (BA10 and 9).
In terms of anterior PFC function, a number of reports indicate that the anterior PFC may contribute to attention control, although a meta-analysis by Gilbert et al. (2006) showed that the anterior PFC is involved in various tasks such as attention, working memory, episodic retrieval, mentalizing and multitasking. For example, Braver and Bongiolatti (2002) proposed that the anterior PFC is part of an attentional network that coordinates and integrates subgoal processing during working memory task. A review by Pollmann (2004) suggested that the anterior PFC may be at the top of a cascade of processes that control the allocation of attention. It is thus likely that the anterior PFC plays a significant role in the control of attention.

We hypothesized that the present experimental task may be linked to the control of attention, because the subjects focused attention on their breathing movements in the lower abdomen (Tanden). This experimental task elicited activation in the anterior PFC (BA10 and 9), as discussed above. Therefore, we concluded that the activation in the anterior PFC during FA on Tanden breathing observed in this study was caused by the experimental task being related to the control of attention.

On the other hand, it is established that directing attention to one’s internal body sensations is a major component of most meditation traditions (Khalsa et al., 2008). Raffone and Srinivasan (2009) proposed that activation of the anterior PFC might be correlated with endogenous attention during FA meditation. Thus, we suggest that the activation in the anterior PFC observed in this study was evoked by directing attention to the Tanden breathing movement as an internal body sensation.

### 4.2. The mechanisms underlying EEG changes during FA on Tanden breathing

The present results, in accord with previous studies (Fumoto et al., 2004), demonstrated that FA on Tanden breathing evoked an increase in alpha band activity. Although the present study did not include a control task, we showed in a previous study that there was no significant change in alpha band activity during a control task where subjects rested for 20 min. The present study further revealed that FA on Tanden breathing elicited a reduction in theta band activity, but little change in beta band activity. In contrast, Kasamatsu and Hirai (1966) reported that both alpha and theta band activity were increased during Zen meditation by experienced monks. Similarly, Murata et al. (1994) reported that expert Zen meditators exhibited increased alpha and theta band activity, while novices showed decreased theta band activity accompanied by increased alpha band activity during Zen meditation. These results suggest that changes in theta band activity may correlate with the degree of Zen experience. The participants in our present and previous experiments were all novices, possibly explaining the absence of a theta band activity increase during FA on Tanden breathing.

The electrophysiological mechanisms underlying EEG changes during FA on Tanden breathing are currently not fully understood. One factor that may have influenced the EEG changes observed in our study is the activity of the serotonergic system in the brain stem. This possibility is supported by the finding that decreased alpha band activity and increased theta band activity (i.e. opposite EEG changes to those observed during FA on Tanden breathing in the present study) were observed after the administration of a 5-HT1A agonist, buspirone, in healthy participants (Murasaki et al., 1989; Anderer et al., 2000). Although the systemic administration of the drug in these previous studies makes the localization of its effects difficult, McAllister-Williams et al. (2007) suggested that such EEG changes may be mediated by somatodendritic 5-HT1A receptors, which are expressed on 5-HT cells in the dorsal and median raphe nuclei and act as autoreceptors that inhibit 5-HT cell firing. These 5-HT cells send ascending 5-HT fibers to many parts of the forebrain, and decreases in their rate of firing attenuate 5-HT release in their projection areas throughout the brain (Nichols and Nichols, 2008). The authors therefore proposed that inhibition of the 5-HT system may be responsible for the EEG changes observed with buspirone administration, which are characterized by decreased alpha band activity and increased theta band activity. These previous findings suggest that the opposite EEG changes we observed during FA on Tanden breathing in
the current study (i.e., increased alpha band activity and decreased theta band activity) may have been caused by activation of the 5-HT system. On the basis of these findings, we propose that FA on Tanden breathing augments 5-HT release in projection areas in the forebrain.

Among the 5-HT projection areas in the forebrain, the basal forebrain is particularly likely to play an important role in EEG changes during FA on Tanden breathing. In an animal study, Jones and Mühlethaler (1998) demonstrated that the local application of 5-HT into the basal forebrain inhibited cholinergic neurons projecting to broad cortical areas, and diminished cortical arousal. These findings suggest that the increased release of 5-HT into the basal forebrain induced by FA on Tanden breathing in the current study might result in cortical attenuation, which could be expressed as increased alpha band activity, as observed in the present results.

To investigate this possibility, we examined the correlation between alpha band activity and 5-HT level measured during FA on Tanden breathing. Linear regression analysis showed a significant correlation between increases in alpha band activity and increments in the level of 5-HT. This finding indicates that increased levels of 5-HT in the brain may induce augmented alpha band activity during FA on Tanden breathing.

The findings of an animal experiment reported by Jacobs and Fornal (1993) are also in accord with the notion that FA on Tanden breathing involves 5-HT system activation. Their findings revealed a unique pattern of activity of 5-HT neurons that was enhanced by voluntary rhythmic behaviors, including locomotion, mastication and breathing. We previously reported that the 5-HT system in humans could be activated by FA on Tanden breathing induced by pedaling exercise (Fumoto et al., 2010), an example of voluntary rhythmic locomotion behavior, and gum chewing (Mohri et al., 2005), an example of voluntary rhythmic mastication behavior. Because FA on Tanden breathing can be considered a voluntary rhythmic breathing behavior, it seems reasonable to expect that it may also elicit augmentation of 5-HT system activity.

To evaluate the augmentation of 5-HT release in the brain in this study, we measured 5-HT levels in the whole blood before and after FA on Tanden breathing. The rationale for this method was based on several recent findings. Wakayama et al. (2002) demonstrated that 5-HT transporter mRNA is localized on both the abluminal and luminal sides of the BBB. Moreover, when augmented 5-HT within the brain was induced in rats that had undergone gastrointestinal and kidney resections along with liver inactivation (organs contributing to increasing blood 5-HT after administration of a 5-HT precursor), 5-HT levels were found to be elevated in whole blood. Moreover, elevated blood 5-HT levels were abolished by the inhibition of the 5-HT transporter with a selective serotonin reuptake inhibitor (SSRI), indicating that 5-HT crosses the BBB into the systemic circulation via the 5-HT transporter (Nakatani et al., 2008). We propose that the increased level of 5-HT in whole blood observed after FA on Tanden breathing in the current study arose from augmented 5-HT levels within the brain.

4.3. The mechanism of augmentation of the 5-HT system

The augmentation of 5-HT system activity we observed in the present study may have been caused by the activation of anterior PFC. This possibility is supported by several previous findings. Goncalves et al. (2009) reported the existence of robust projections from the PFC to the dorsal raphe nucleus (DRN), mainly derived from the ventral part of the median PFC. In addition, Vázquez-Borsetti et al. (2009) demonstrated that DRN neurons receive excitatory inputs from pyramidal cells located within the PFC. Thus, it is likely that increased PFC activity causes the activation of the 5-HT system in the DRN, as evidenced by the increased levels of whole blood 5-HT in the current study. That is, FA on Tanden breathing appears to have induced a significant increase in oxyHb levels in the anterior PFC, which would be expected to elicit activation in the 5-HT system in the DRN.

4.4. Changes in the mood state during FA on Tanden breathing

The present results demonstrated that FA on Tanden breathing evoked stronger activation of the anterior PFC (BA10 and 9) compared to the dorsal PFC (BA8 and 9), accompanied by a reduction in feelings of negative mood, including significant decreases in tension–anxiety, depression–dejection, anger–hostility, and confusion. According to a previous meta-analysis of task-related neural activation observed in the PFC (Amadillo and Frith, 2006), the more anterior region of the PFC (BA9 and 10) tends to be activated by tasks involving emotion, while the more posterior region of PFC (BA8 and 9) tends to be activated by cognitive tasks. A number of studies have indicated that FA meditation is related to improvements in emotional regulation (Goldin and Gross, 2010; Davidson, 2010). Taken together, these findings indicate that activation of the anterior PFC during FA on Tanden breathing may contribute to a reduccon in feelings associated with negative mood, as well as the control of attention as described above.

Davidson et al. (2003) suggested that PFC asymmetry may be involved in emotional regulation during FA meditation, based on EEG findings that FA meditation training produced increases in relative left-sided PFC activation that were associated with reductions in anxiety and negative affective responses. However, in the present study we did not observe asymmetry of anterior PFC activation during FA on Tanden breathing. This discrepancy may be explained by the methodological differences between EEG and NIRS.

4.5. Methodological limitations

It should be considered that NIRS can detect oxyHb changes only within a limited range of approximately 3 cm below the scalp. The present study examined BA8, 9 and 10 in the PFC, which were localized superficially. The results revealed significant activation in BA10 (i.e., the frontopolar cortex) during FA on Tanden breathing. We were not able to examine any possible involvement of deep brain structures, including the anterior cingulate cortex, insular cortex, limbic system, and brain stem, in which activation during FA meditation has been previously reported (Cahn and Polich, 2006). On the other hand, the advantage of NIRS is that brain activity can be measured continuously in a more natural seating position during FA on Tanden breathing, compared to other functional brain imaging techniques. We were thus able to precisely evaluate the time course of changes in activity of BA8, 9, and 10 in the PFC during FA on Tanden breathing.

We used only three electrodes (Cz, Pz and Oz) for EEG recording in this study, because our priority was the NIRS measurement of the natural seating position during FA on Tanden breathing, compared to other functional brain imaging techniques. We were thus able to precisely evaluate the time course of changes in activity of BA8, 9, and 10 in the PFC during FA on Tanden breathing.

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4.5. Methodological limitations

It should be considered that NIRS can detect oxyHb changes only within a limited range of approximately 3 cm below the scalp. The present study examined BA8, 9 and 10 in the PFC, which were localized superficially. The results revealed significant activation in BA10 (i.e., the frontopolar cortex) during FA on Tanden breathing. We were not able to examine any possible involvement of deep brain structures, including the anterior cingulate cortex, insular cortex, limbic system, and brain stem, in which activation during FA meditation has been previously reported (Cahn and Polich, 2006). On the other hand, the advantage of NIRS is that brain activity can be measured continuously in a more natural seating position during FA on Tanden breathing, compared to other functional brain imaging techniques. We were thus able to precisely evaluate the time course of changes in activity of BA8, 9, and 10 in the PFC during FA on Tanden breathing.

We used only three electrodes (Cz, Pz and Oz) for EEG recording in this study, because our priority was the NIRS measurement of the natural seating position during FA on Tanden breathing, compared to other functional brain imaging techniques. We were thus able to precisely evaluate the time course of changes in activity of BA8, 9, and 10 in the PFC during FA on Tanden breathing.

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References
