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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.

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.,

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2004) demonstrated increased alpha band (8–13 Hz) activity during FA on Tanden breathing, but did not examine theta (4–8 Hz) and beta band (13–30 Hz) activity. Therefore, in the present study we used EEG to examine theta and beta frequency bands, as well as the alpha frequency band 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 Mühlethaler (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 systemic circulation through the 5-HT transporter in rats. Since 5-HT released from the brain into systemic circulation is quickly taken up by platelets (Pletscher, 1987), 5-HT augmentation would be expected to be manifest in both plasma and platelets (*i.e.* in whole blood).

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 (deoxyHb), 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:

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

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

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

A_{780}, A_{805}, and A_{830} represent 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