Original Article

Alterations of regional cerebral glucose metabolism using ^{18}F-fluorodeoxyglucose positron-emission tomography/computed tomography and electroencephalography analysis during mindfulness breathing in Anapanasati meditation: A preliminary analysis

ABSTRACT

Anapanasati is a core meditation of a breath-centered practice in the Buddhist Theravada tradition, which may have some neurological mechanism effects on the brain. To gain insight into the neurological mechanisms involved in Anapanasati meditation, we measured the alterations of regional cerebral glucose metabolism during Anapanasati meditation using positron-emission tomography/computed tomography (PET/CT) and electroencephalography (EEG) analysis. This prospective study was conducted in six right-handed volunteer participants (two men, four women; aged: 32–67 years) who underwent ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/CT scans to compare the alterations of regional cerebral glucose metabolism during normal consciousness and Anapanasati meditation states. Spectral EEG analysis was performed throughout the investigations. Statistical parametric mapping was used for the ^{18}F-FDG PET/CT image analyses. The visual analysis demonstrated moderate-to-marked increased metabolism in posterior cingulate cortex in all six patients, while mild-to-moderate increased uptake in the whole frontal lobe was also observed in four patients and precuneus in four patients. Meanwhile, the semiquantitative analysis yielded an increase of regional cerebral glucose metabolism in the right mid-to-posterior cingulate gyrus ($P < 0.000$), with visible alpha waves on the frontal of the EEG findings. Our semiquantitative analysis showed a significantly increased metabolism only in the posterior cingulate cortex, but visually, there was also an increased metabolism in the whole frontal lobe in most of the patients correlating with EEG findings.

Keywords: Anapanasati meditation, cerebral metabolic rate of glucose, electroencephalography, positron-emission tomography

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INTRODUCTION

The mindfulness of breathing, or Anapanasati meditation, is one of the most common forms of meditation in Theravada Buddhism, with the breath-centered practice that practitioners continually focus on the sensations of their own breathing. Meditation has been shown to be useful for improving mental health and brain function.[1] A number of studies have also demonstrated that the

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effects of meditation are related to complex neuronal mechanisms.[2]

Neuroimaging techniques including functional magnetic resonance imaging and positron-emission tomography (PET) have been applied to study the brain function of people in meditative states.[3,4] However, the study to understand neurological mechanism effects on the body and brain during Anapanasati meditation practice remains lacking and limited. Thus, we aimed to compare the alterations of regional cerebral glucose metabolism during normal consciousness and mindful breathing of Anapanasati meditation states, using PET/computed tomography (PET/CT) and electroencephalography (EEG) analysis.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee of our institute on March 23, 2010. The project code was 012/2552. Before the study, written informed consent was obtained from all participants for participation in the study of data for research and educational purposes.

Participants

Six right-handed volunteers (two men, four women; aged: 31–67 years; median age: 50.5 years), with experience in Anapanasati meditation ranging from 1 month to 50 years (median 0.54 years), participated in the study. No participants had a history of psychological or neurological diseases or use of psychotropic drugs.

Procedures

All participants underwent a resting-state ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET/CT scan of normal consciousness within 2 weeks before the performed scan during Anapanasati meditation. On the day of Anapanasati meditation study, each participant had an intravenous catheter placed in the antecubital vein before starting Anapanasati meditation, while EEG was continuously recorded. They were not interrupted during the meditation. The participants sat with both eyes closed in a quiet room without covering their ears and meditated the Anapanasati meditation, focusing on mindful awareness of breathing while inhaling and exhaling. Once effective meditation, as described in the EEG section, was detected by EEG software, ^{18}F-FDG was immediately injected via the intravenous catheter. Then, the participants continued their awareness of breathing for 30 min to allow brain uptake of the ^{18}F-FDG before the PET/CT scan. This was in accordance with the reference from EANM procedure guidelines for PET brain imaging, ^{18}F-FDG, version 2, which recommended the uptake phase at least 20 min.[5]

On the day of normal consciousness, all of them underwent the same procedure during their performing of Anapanasati meditation. They were only asked to sit with both eyes closed while the ^{18}F-FDG was injected.

^{18}F-fluorodeoxyglucose imaging procedure

All participants fasted for 6 h before being scanned using a Siemens/Biograph 16 scanner in three-dimensional mode. Imaging was then performed 30 min after intravenous injection of 5 MBq/kg ^{18}F-FDG during normal consciousness and mindfulness of breathing in Anapanasati meditation. The level of plasma glucose was determined prior to the ^{18}F-FDG PET/CT study. The plasma glucose level of < 200 mg/dl was considered as acceptable for all patients. Image acquisition was performed for 10 min per bed position, matrix size $= 256 \times 256$, zoom $= 1$, and a Gaussian filter of full-width at half-maximum (FWHM) $=2.0$. Image reconstruction was performed using the ordered subset expectation maximization (OSEM) with four iterations and eight subsets.

Electroencephalography

EEGs were recorded during normal consciousness and Anapanasati meditation with a BIOSEMI™ ActiveTwo 32-channel EEG recording system, equipped with Ag/AgCl active electrodes. A sampling rate of 1024 Hz was applied together with LabVIEW software (LabVIEW 2015, National Instruments Corporation, Austin, Texas, USA) developed in-house to detect meditative states in the brain in real time. For this real-time quantitative evaluation, effective meditation should occur when the following condition was satisfied three times:

$(PSD_{F3} + PSD_{F4} + PSD_{Fp1} + PSD_{Fp2}) > 0.7 (PSD_{P03} + PSD_{P04} + PSD_{03} + PS_{D04})$,[1] Where PSDi denotes the sum of the Welch power spectral density[6] of frequency between 8 and 13 Hz from channel i according to the 10–20 system of electrode placement.[7] The threshold of 0.7 was arbitrarily selected to enable early detection of effective meditation.

The idea of this equation can be explained by the assumption that the brain would focus on some specific concentrations during effective meditation. The subjects of every recorded EEG channels were likely to produce low frequencies (deactivation, 8–13 Hz) in the cortex area. Therefore, the spectrums of the lower frequency band tended to be increased in all channels. Naturally, at the normal state, the low-frequency EEG would not efficiently be observed in the frontal area. Consequently, the increasing of lower frequency spectrums in the frontal area compared with the parietal and the occipital areas was used as the index.

Data and Statistical analysis

PET imaging data processing and analysis was conducted using Statistical Parametric Mapping version 2 (SPM2, Wellcome Department of Cognitive Neurology, University College, London), running on MATLAB 6.5.1 (MathWorks, Inc., Sherborn, MA, USA). Images in DICOM format were converted to analyze format using MRIcro (www.mricro.com) and transferred to SPM2. Data were then standardized to the Montreal Neurological Institute (MNI) atlas, using a 12-parameter affine transformation, followed by trilinear interpolation. The resulting voxel dimensions were 2 mm × 2 mm × 2 mm. The standardized data were smoothed with a Gaussian filter of a FWHM of 16 mm to increase the signal-to-noise ratio and to blur individual variations in the gyral anatomy. The two brain states within the same person (normal consciousness and Anapanasati meditation) were normalized for whole-brain count and compared using paired *t*-test. Areas showing statistically significant differences were identified at the voxel level with a height threshold of corrected $P < 0.05$ and a cluster size larger than 32 voxels (voxel extent threshold, kE). The anatomical location from the resulting MNI coordinates was identified using the MNI2Tal and Talairach Daemon database (http://ric.uthscsa.edu/projects/talairachdaemon.html).

The imaging data were also analyzed by MIM neuroanalysis software (MIM Software Inc., 25800 Science Park Drive-Suite 180, Cleveland, Ohio, 44122, USA), including a cluster subtraction workflow (P = 0.05) of FDG metabolism difference between normal consciousness and Anapanasati meditation. This analysis applied the Chulabhorn Hospital normal neuro-database for the comparison of FDG metabolisms.

The visual assessment was separately interpreted by two experienced nuclear physicians in multiplanar images, using the Syngovia Dicom viewer software (Siemens Healthineers, Munich, Germany).

RESULTS

Patient characteristics are shown in Table 1.

[18]F-fluorodeoxyglucose positron-emission tomography/computed tomography

Visual analysis demonstrated moderate-to-marked increased metabolism in the posterior cingulate cortex in all six patients and mild-to-moderate increased uptake in the whole frontal lobe in four patients and in precuneus in four patients on the meditation PET image. The results of the visual analysis were compared during the meditation and normal consciousness in each individual participant, as shown in Table 2 and Figures 1-6, respectively.

Statistical parametric mapping analysis indicated that glucose consumption during Anapanasati meditation was significantly higher than the state of normal consciousness in the right mid-to-posterior cingulate gyrus ($P < 0.000$ uncorrected for multiple comparisons). In addition, we observed an increased uptake in the left superior frontal gyrus, with no statistically significant difference from the state of normal consciousness.

MIMneuro software gave similar results, with higher activity observed in the mid-to-posterior cingulate gyrus during Anapanasati meditation. The results are shown in Figures 7-9.

Electroencephalography

The normal pattern of consciousness which naturally occurred in every individual and the sample pattern of concentration during Anapanasati meditation from the group of participants are shown in Figures 10 and 11, while the timing of detected pattern of concentration during meditation by the proposed index is demonstrated in Table 3. The index was selected according to the fundamental of the EEG pattern. To further analyze

Table 1: Participants' characteristics

Characteristics	blank
Sex, *n* (%)	
Male	2 (33)
Female	4 (67)
Age, years	
Median	50
Range	31-67
Experience in Anapanasati meditation, years	
Median	0.54
Range	0.08-50

Table 2: The hypermetabolic brain regions by visual assessment comparing between normal consciousness and during meditation

Participant	The hypermetabolism brain regions during the meditation, compared with normal consciousness
1	Posterior cingulate gyrus, cuneus, whole frontal
2	Posterior cingulate gyrus, precuneus
3	Posterior cingulate gyrus, precuneus, cuneus, whole frontal
4	Posterior cingulate gyrus, precuneus
5	Whole frontal, posterior cingulate gyrus
6	Posterior cingulate gyrus, precuneus, whole frontal

Table 3: Time for effective meditation

Participant	Time when the effective meditation was detected
1	18 min 4 s
2	7 min 46 s
3	26 min 56 s
4	36 min 36 s
5	48 min 52 s
6	23 min 53 s

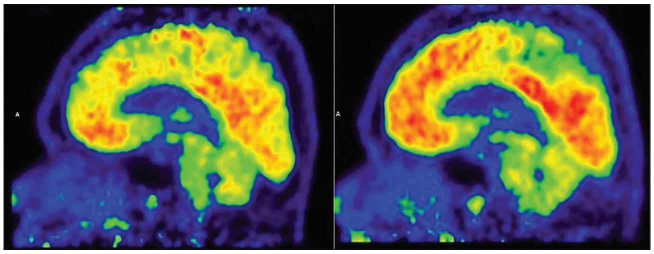

Figure 1: Participant 1 had ^{18}F-fluorodeoxyglucose positron-emission tomography sagittal images in the same level of at 0%–85% of imaging contrast, (right) during meditation, (left) normal consciousness. The hypermetabolism areas were identified at posterior cingulate gyrus, cuneus and whole frontal lobe during the meditation

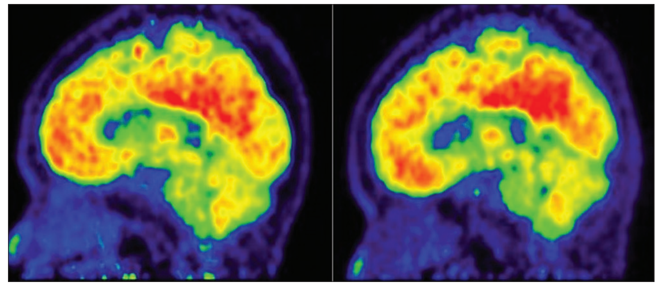

Figure 2: Participant 2 had ^{18}F-fluorodeoxyglucose positron-emission tomography sagittal images in the same level of at 0%–85% of imaging contrast, (right) during meditation, (left) normal consciousness. The hypermetabolism areas were identified at posterior cingulate gyrus and precuneus during the meditation

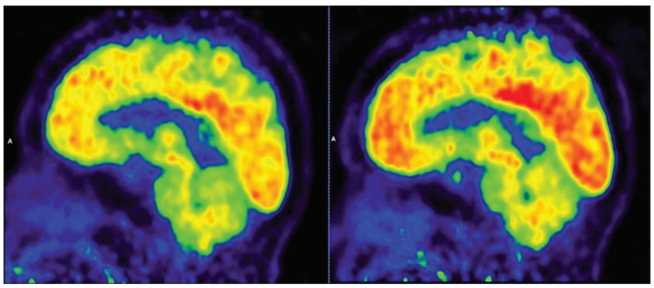

Figure 3: Participant 3 had ^{18}F-fluorodeoxyglucose positron-emission tomography sagittal images in the same level of at 0%–85% of imaging contrast, (right) during meditation, (left) normal consciousness. The hypermetabolism areas were identified at posterior cingulate gyrus, precuneus, cuneus, and whole frontal lobe during the meditation

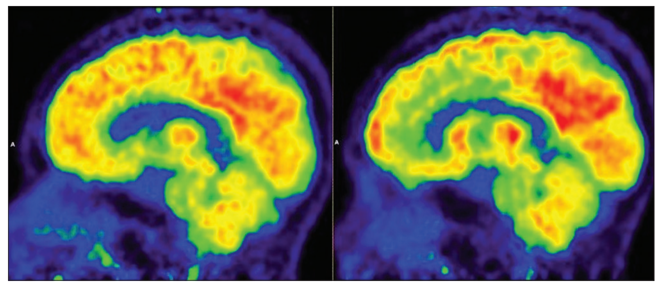

Figure 4: Participant 4 had ^{18}F-fluorodeoxyglucose positron-emission tomography sagittal images in the same level of at 0%–85% of imaging contrast, (right) during meditation, (left) normal consciousness. the hypermetabolism areas were identified at posterior cingulate gyrus and precuneus during the meditation

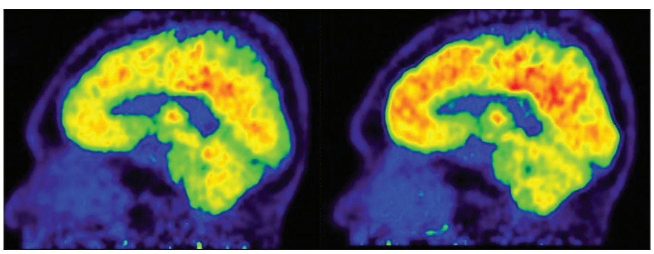

Figure 5: Participant 5 had ^{18}F-fluorodeoxyglucose positron-emission tomography sagittal images in the same level of at 0%–85% of imaging contrast, (right) during meditation, (left) normal consciousness. The hypermetabolism areas were identified at the whole frontal lobe and posterior cingulate gyrus during the meditation

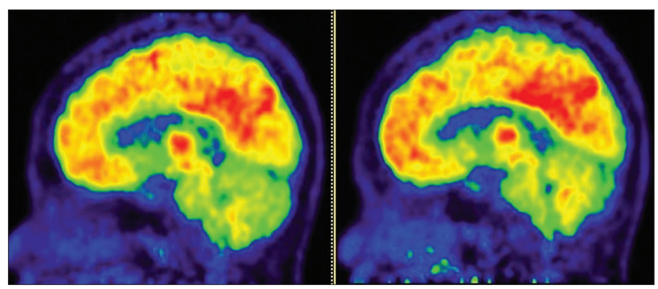

Figure 6: Participant 6 had ^{18}F-fluorodeoxyglucose positron-emission tomography sagittal images in the same level of at 0%–-85% of imaging contrast, (right) during meditation, (left) normal consciousness. The hypermetabolism areas were identified at the posterior cingulate gyrus, precuneus, and whole frontal lobe during the meditation

the recorded multichannel EEG signals, time-domain EEGs, we applied the standardized low-resolution brain electromagnetic tomography (sLORETA) source localization[8] and topographical brain maps of the EEG signals before and during effective meditation, with a modified explanation. Furthermore, the records during the normal consciousness and the sample pattern of concentration during the meditation from the group of participants are represented in Figures 10 and 11, with the normal alpha activity pattern during the normal consciousness [Figure 10a] and the low-frequency alpha activity in the occipital and parietal regions [Figure 11a] (rows A1, A30, A4, and A27 in the frontal channels Fp1, Fp2, F3, and F4, respectively; A14, A18, A15, and A17 in the parietal and occipital channels PO3, PO4, O1, and O2, respectively); A14, A18, A15, and A17 in the parietal and occipital channels PO3, PO4, O1, and O2, respectively).

In contrast, the sample of concentration patterns during meditation from participants is illustrated in Figure 11. In addition to the parietal and occipital regions, the alpha activity during effective meditation (detected at 26 min, 56 s) was also clearly observed in the frontal regions. The alpha activity led to high-amplitude EEGs, which are visualized in Figures 10c and 11c as topographical brain maps (high amplitudes in red and low amplitudes in blue). To further analyze the differences in EEG between the two brain states,

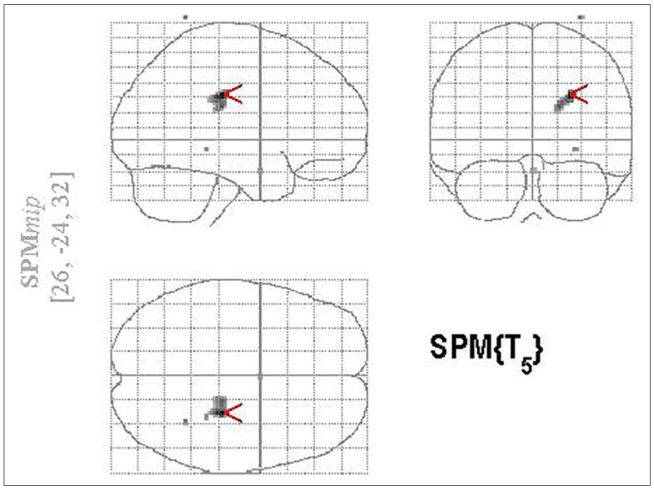

Figure 7: Voxels showing significant hypermetabolism in the right mid-to-posterior cingulate gyrus during Anapanasati meditation compared with the normal resting state. Glass brain activations are rendered by statistical parametric mapping. A cluster with Talairach (x, y, z) co-ordinates of (26, –24, 32) and voxel T-score of 9.38, P uncorrected at voxel level = 0.000. This was the only cluster significantly activated more during Anapanasati meditation than during the normal resting state

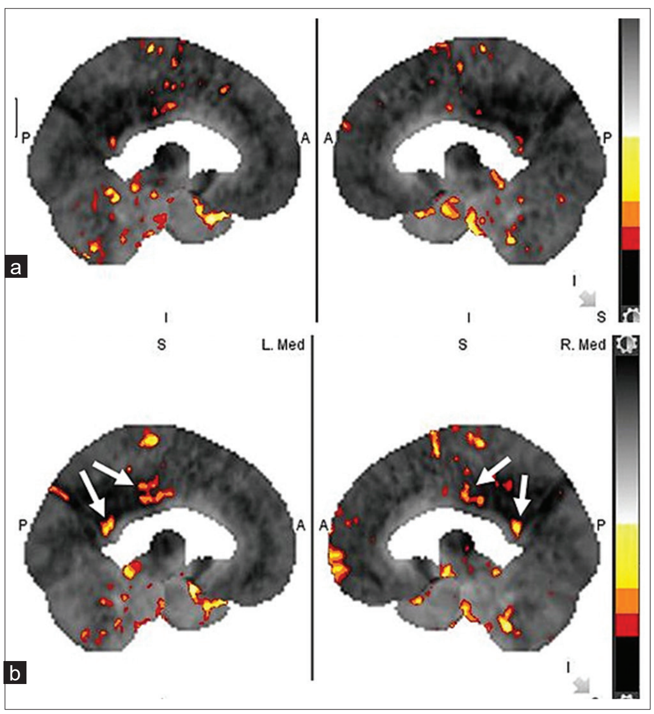

Figure 9: MIM software output image showing the medial three-dimensional stereotactic surface projection normalized by the cerebellum. The SUVr is compared with the Chulabhorn hospital normal database of fluorodeoxyglucose uptake. The areas with a higher difference than 2.5 z-score, indicating with color scale. (a) Normal resting-state: The three-dimensional stereotactic surface projection does not indicate any areas that had significantly higher uptake than they do in the normal database. (b) Anapanasati meditation: The three-dimensional stereotactic surface projection indicates significantly higher uptake in bilateral mid-to-posterior cingulate gyrus (arrow) than in the normal database. Thus, glucose metabolism in the mid-to-posterior cingulate is higher during Anapanasati meditation than during normal rest. SUVr, standard uptake value ratio

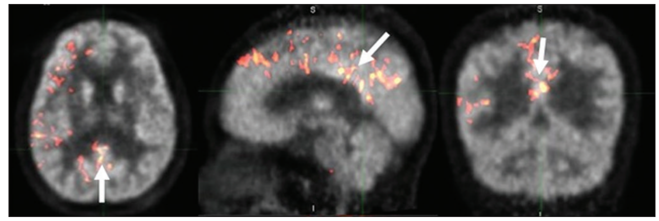

Figure 8: Cluster subtraction (*P* = 0.05) between the normal resting state and Anapanasati meditation using MIM software fluorodeoxyglucose examination. The subtraction shows significantly higher signal intensity in the posterior cingulate gyrus (arrow)

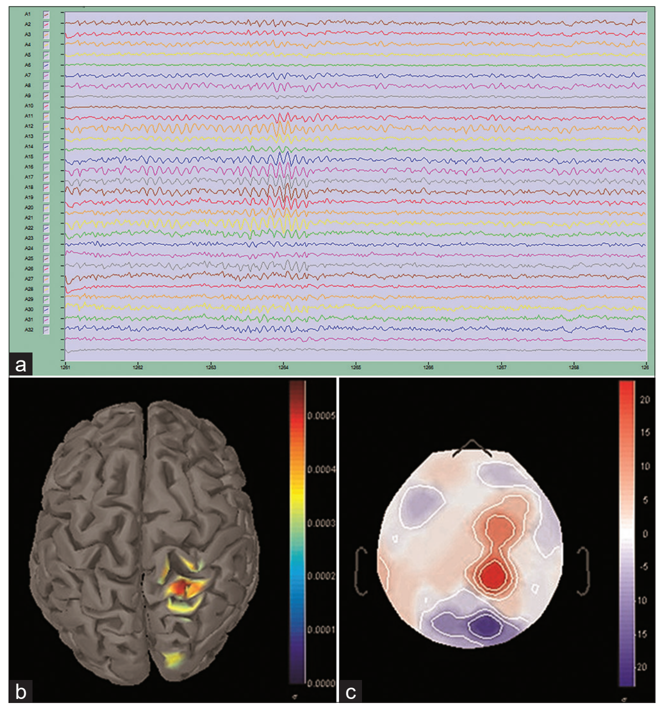

Figure 10: (a) Normal alpha activity during resting state of participant 3. Rows A1, A30, A4, and A27 represent channels Fp1, Fp2, F3, and F4, respectively. Rows A14, A18, A15, and A17 represent channels PO3, PO4, O1, and O2, respectively. (b) Electroencephalography source localization. (c) Topographical brain mapping. High amplitude is depicted in the shades of red and low amplitude in blue

we examined the neural activity in the brain by taking the inverse of the 32-channel EEG using sLORETA. Figure 11b shows the neural activity in the frontal areas during effective meditation but absent during the normal consciousness state [Figure 10b].

DISCUSSION

Different meditation techniques may have different effects on the brain.[9,10] Directive meditation appears to be related to increased brain activity, while nondirective meditation is associated with decreased brain activity.[11] A previous ^{18}F-FDG PET/CT study demonstrated alterations in regional metabolism after prolonged meditation practice for 8 weeks.[12] Body/mind-relaxation

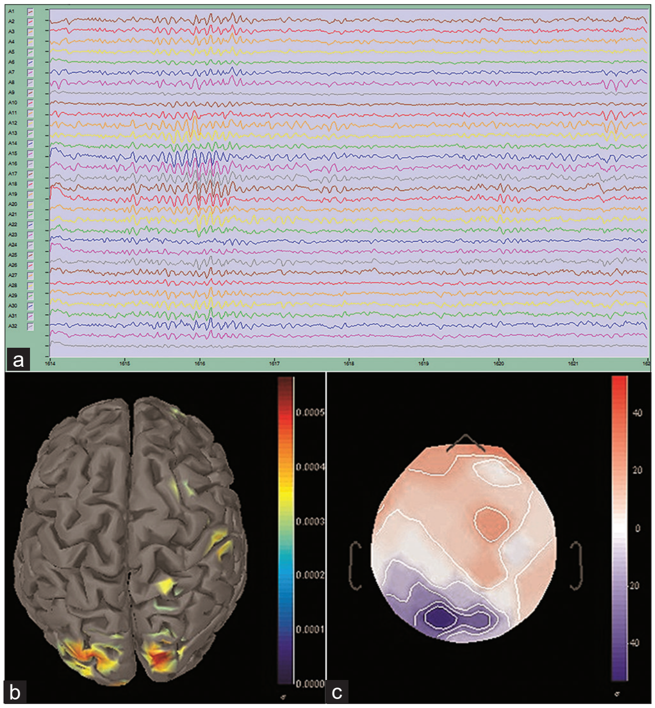

Figure 11: (a) Alpha activity when effective meditation was detected at 26 min, 56 s. Rows A1, A30, A4, and A27 represent channels Fp1, Fp2, F3, and F4, respectively. Rows A14, A18, A15, and A17 represent channels PO3, PO4, O1, and O2, respectively. (b) Electroencephalography source localization. (c) Topographical brain mapping. High amplitude is depicted in the shades of red and low amplitude in blue

meditation could affect the brain activity in the prefrontal cortex[13] and yoga yielded the effects to the cerebellum, right temporal, right inferior frontal, left superior frontal, left associative visual, and right posterior cingulate cortices (PCCs).[14]

Our study is the first report of alterations in regional cerebral glucose metabolism and EEG during Anapanasati meditation. The increased glucose metabolism in the right mid-to-posterior cingulate gyrus was statistically significant at the voxel level when not corrected for multiple comparisons. Meanwhile, we found a nonsignificant increase in the left superior frontal gyrus. EEG analysis also indicated the frontal area alpha activity only during the meditation. Following the visual assessment in each individual, we found the hypermetabolism area during the meditation at posterior cingulate gyrus, compatible to the semiquantitative analysis. Meanwhile, the hypermetabolism was also identified at frontal, precuneus, or cuneus in some participants but not statistically significant in the semiquantitative analysis. This could be likely due to the limitation of our quantitative analysis, such as the selection of suboptimal brain area for signal intensity normalization, conversion of each individual brain to the MNI space template due to variation of individual brain morphology, adjustment of imaging smoothing parameters, statistical setting for voxel-wise comparison or variation from anatomical mapping with MNI2Tal, and Talairach Daemon database for identification of regional brain.[15,16]

Our findings were compatible to those of Newberg *et al*., which used single-photon emission computed tomography (SPECT) to demonstrate the increased cerebral blood flow in the right posterior cingulate gyrus and left superior frontal regions of meditators.[14] Our results were also consistent with Khalsa *et al*., using SPECT to examine the alterations in brain physiology during the chanting meditation called Kirtan Kriya. The study reported the increased regional cerebral blood flow in the posterior cingulate gyrus.[17] Meanwhile, Herzog *et al*. reported that yoga meditation led to the increased cerebral glucose metabolism.[18]

Initially, the PCC is involved in very complex pathways, and several studies have reported its involvement in meditation practice. For instance, Brewer *et al*. reported the increased functional connectivity between the ventral PCC and the frontoparietal control network in expert meditators.[19] Additional support for the role of PCC in meditation was demonstrated in Manuello *et al*., with the increased posterior cingulate gyrus connectivity with the default mode network in novices who practiced mindfulness or meditation and the decreased connectivity in expert practitioners.[20]

Importantly, the stage of concentration or the optimal/successful meditation as defined in Buddhist Anapanasati meditation depends on each individual's mindfulness, not the different brain functions in a wide range of ages. As well, meditation in Buddhism mainly relies on the state of mind during each session of meditation. In the meantime, it was unable to define expert and novice practitioners in our study due to no single standardized parameter for measurement, such as the amount of time for meditation in practice to differentiate the level of expertise in meditation.

The increase in glucose metabolism in the right mid-to-posterior cingulate gyrus and a potentially increased metabolism in the left superior frontal gyrus in our study may be explained as follows. The increased activity in the posterior cingulate gyrus was likely because the participants reached a meditative level of compassion with joys and happiness.[21] The posterior cingulate gyrus is known to be activated during happiness.[22] Moreover, Brodmann area 9 (superior frontal gyrus) and the posterior cingulate gyrus can play a very crucial role in memory function during the Anapanasati meditation.[23] Ultimately, the increase in brain activity could be resulted from the required memory for consciousness while practising Anapanasati meditation.

Concerning the EEG analysis, we used the software developed in-house to calculate a proposed real-time index for early meditation. Based on EEG knowledge,[7] the alpha activity (8–13 Hz) increases with the reduced sensory perception and the sensory perception seems to decrease during effective meditation. The location of alpha activity during meditation differs from that during normal consciousness state, in which the alpha activity is only observed in the occipital and parietal areas but also in the frontal areas.

Our EEG findings indicated that effective meditation was associated with an increased alpha signal in the frontal lobe. This specific EEG signal was due to the chemical changes that resulted from the neuron activation. Various reports demonstrated that the alpha waves increase during meditation. For example, Lagopoulos *et al*. revealed that the nondirective meditation techniques could alter the theta and alpha EEG patterns significantly more than the regular relaxation.[24] Similarly, a review by Lee *et al*. reported that several types of meditation are related to the higher global oscillatory activity among experienced meditators than beginners. The increase in frontal lobe alpha activity is also correlated with the length of meditation training.[25] Moreover, Huang and Lo also reported more alpha waves in the frontal regions during mindfulness meditation. Based on this evidence, it is interesting that the meditation practice generates these alpha waves, which are believed to relieve stress, anxiety, and pain.[26]

Valente *et al*. and Kuschinsky[27,28] described the strong correlation between local metabolism, blood flow, and EEG, activating brain function in specific functions. With activation in any brain areas, neurons would increase glucose utilization, leading to the increased cerebral blood flow and later glucose metabolism. Then, EEG signals can be linked to the alterations in glucose metabolism. Interestingly, our results showed that the increased alpha EEG activity was in the frontal lobe, with the significantly increased FDG metabolism in the posterior cingulate. It is assumed that the surface EEG cannot be measured by the occurrence of neural activity below the upper layers of the brain due to the voltage fields which fell off the square of the distance from the source.[29]

Our study was limited by the small number of subjects. This was due to the fact that the meditation in Buddhism is not just a common term of concentration. In particular, the levels of meditation cannot be truly showed off by any individuals. Actually, meditation is an internal and natural manner of each meditator toward truthful knowledge and true enlightenment. Hence, very few volunteers would rather participate, while many others feel quite awkward to join in our study. However, our results were statistically significant though a preliminary study.

Despite these limitations, our findings suggested that the state of Anapanasati meditation could lead to both the increased metabolism in the right mid-to-posterior cingulate gyrus and the increased frontal alpha waves. Thus, Anapanasati meditation practice may be related to the stage of meditative concentration of happiness and peacefulness, with the access concentration. Most importantly, Anapanasati meditation likely activates the PCC circuitry involved in self-control, mood stabilization, and happiness, leading to the well-being and improved health.

CONCLUSION

This study is the first report describing the alterations of regional cerebral glucose metabolism and EEG during Anapanasati meditation. Our semiquantitative analysis showed a significantly increased metabolism only in the posterior cingulate cortex, but visually, there was also an increased metabolism in the whole frontal lobe in most of the patients correlating with EEG findings.

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Conflicts of interest

There are no conflicts of interest.

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