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A Functional Near-Infrared Spectroscopy Study of High-Frequency Yoga Breathing Compared to Breath Awareness

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Background: High-frequency yoga breathing (breath rate of 2.0 Hz) has been associated with changes in oxy-hemoglobin in the prefrontal region of the brain. The present study assessed the effects of high-frequency yoga breathing (HFYB) at 1.0 Hz on frontal oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb).

Material/Methods: Forty healthy male participants were recruited for the study. The experimental group consisted of 20 participants 23–40 years old (group mean \pm S.D., 26.4 \pm 4.7 years) with at least 3 months of experience performing HFYB (group mean \pm S.D., 16.3 \pm 9.8 months). The control group consisted of 20 participants ages 23–38 years (group mean age \pm S.D., 27.4 \pm 4.1 years), who were seated quietly for the same duration and their average experience of yoga practice was (\pm S.D.) 4.3 \pm 2.7 months. Each participant in the experimental group was assessed at 2 sessions (HFYB and breath awareness [BAW]) on alternate days. Hemodynamic changes were assessed using a functional near-infrared spectroscopy sensor placed over the forehead. Data were analyzed using repeated-measures analyses of variance followed by post hoc Bonferroni adjustment.

Results: A significant reduction was observed in oxy-Hb during and after HFYB on the left and right sides compared to values before. We also found a significant reduction in deoxy-Hb during and after the quiet sitting control session compared to pre-session values on left and right sides.

Conclusions: The decrease in oxy-Hb during and after HFYB suggests that there was no frontal activation during HFYB when practiced at the rate of 1.0 Hz.

MeSH Keywords: **Cerebral Arteries • fNIRs • Hemodynamics • Meditation • Yoga**

Abbreviations: **fNIRs** – near-infrared spectroscopy; **oxy-Hb** – oxy-hemoglobin; **deoxy-Hb** – deoxy-hemoglobin; **HFYB** – high-frequency yoga breathing; **BAW** – breath awareness; **HRV** – heart rate variability

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Background

Voluntary regulation of breathing is an integral part of yoga. Voluntary breath regulation may involve changes in depth and rate of breathing, the nostril breathed through, and a period of breath holding, among other factors [1]. The breath rate is increased in a specific practice called high-frequency yoga breathing (HFYB) or *kapalabhati* (*kapala* means forehead and *bhati* means shining in Sanskrit). Commentaries on this practice state that practicing HFYB influences the function of the brain [2]. The influence of HFYB on the brain can occur through improvement in specific functions and change in cerebral blood flow.

Earlier studies on HFYB at 2.0 Hz and 1.0 Hz showed that HFYB can improve specific functions of the brain, such as attention. When HFYB was practiced for a short duration of 1 min with 120 breaths/min (2.0 Hz), there was improved performance in a task requiring selective and sustained attention as well as ability to shift attention [3]. This was observed in individuals 20–60 years old. Similar effects were also seen when the effects of HFYB practiced at 2.0 Hz were studied on the event-related potential, P300 [4]. Neuroelectric events that underlie the P300 are related to interaction between the frontal lobe, hippocampus, and temporoparietal lobe [5]. Changes in the P300 suggested that HFYB reduced the time needed to perform a task requiring selective attention.

HFYB practiced at 1.0 Hz for 5 min at a time up to 15 min has been shown to increase fine motor skills and visual discrimination [6] as well as visual perception, with a decrease in degree of optical illusion [7]. Changes in fine motor skills and visual discrimination and improved visual perception all indirectly indicate that HFYB at 1.0 Hz can increase attention.

The influence of HFYB on specific functions of the brain can be connected with a change in cerebral blood flow. The cerebral blood flow can depend on at least 2 factors: (i) the vasodilatory effect of increased arterial carbon dioxide levels on cerebral blood vessels [8] and (ii) the increase in sympathetic drive that increase the mean blood flow velocity through the larger vessels such as the middle cerebral artery [9], which could in turn influence focal blood flow changes.

HFYB at 2.0 Hz and 1.0 Hz influence the autonomic nervous system differently. HFYB practiced at 2.0 Hz was shown to shift the autonomic balance, with a significant increase in low-frequency power and the ratio between low-frequency and high-frequency power [10]. This suggested a shift in autonomic balance towards reduced vagal activity. However, the variables in frequency domain analysis are recognized to be less clear indicators of sympathetic-parasympathetic activity compared to time domain variables. In contrast, HFYB practiced at 1.0

Hz showed a significant decrease in time domain variables such as NN50, pNN50, and mean RR interval during and after HFYB [11]. These results suggest reduced parasympathetic activity during and after HFYB at 1.0 Hz, with no change in sympathetic activity. The effects on HRV could influence cerebral blood flow [12].

There have been attempts to assess blood flow when HFYB was practiced at 2.0 Hz and 1.0 Hz. A previous study assessed 18 healthy persons and 18 persons with diagnosed schizophrenia using fNIRs (functional near-infrared spectroscopy) [13]. Participants with normal health showed an increase in bilateral oxy-hemoglobin (oxy-Hb) and total hemoglobin (total-Hb) compared to the baseline during HFYB, whereas persons with schizophrenia showed a significant decrease in deoxy-hemoglobin (deoxy-Hb) on the right side. These findings were interpreted as suggesting there is less prefrontal cortical activation in persons with schizophrenia, although HFYB did increase oxy-Hb bilaterally in normal persons. In contrast to this, a pilot study on HFYB practiced at 1.0 Hz using fNIRs showed frontal bilateral reduction in oxy-Hb during 15 min of HFYB and for 10 min after HFYB practice [14].

Since the results were the opposite to that of HFYB at 2.0 Hz, and the design of the pilot study [14] was inadequate (it lacked a control group), the present study was planned to reassess the findings of the earlier study on HFYB at the rate of 1.0 Hz [14] by adding a control group. This study was considered important due to the large numbers of people practicing HFYB.

Material and Methods

Participants

In this study, 40 healthy male volunteers were recruited as participants. The experimental group consisted of 20 participants ages 23–40 years (group mean age \pm S.D., 26.4 \pm 4.7 years) who had experience in the practice of yoga breathing (mean experience \pm S.D., 16.3 \pm 9.8 months) and an average of 14.4 years of education. In the control (quiet sitting) group, there were 20 participants with ages ranging between 23 years and 38 years (group mean age \pm S.D., 27.4 \pm 4.1 years), experience in yoga practice (mean experience \pm S.D., 4.3 \pm 2.7 months), and 13.2 average years of education. Participants were staying at a residential yoga center located in the north of India. The inclusion criteria were: (i) the participants should have at least 3 months of experience in the yoga breathing practices evaluated in this study (HFYB and breath awareness), (ii) ability to practice HFYB at the rate of 1.0 Hz (range, 0.8 to 1.2 Hz) for the required time, (iii) male participants alone were studied as there are gender differences in the cerebral blood flow in the frontal region [15], and (iv) normal health based on a routine

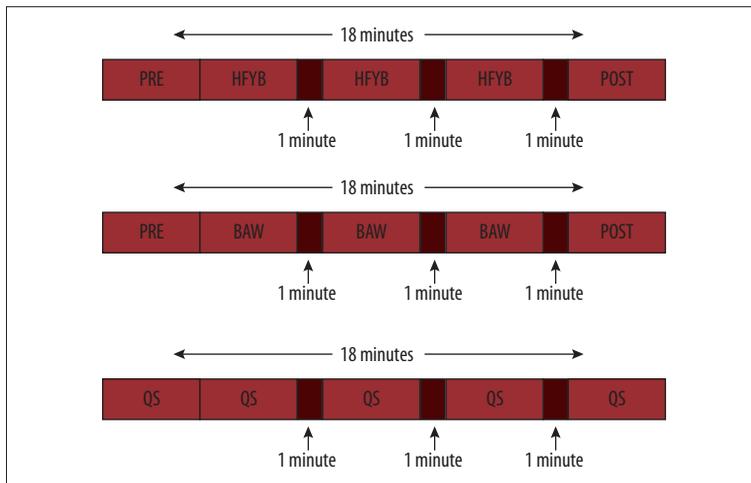


Figure 1. Schematic representation of the study design. ■ – experimental group tested on two days; HFYB – high frequency yoga breathing; BAW – breath awareness and quiet sitting control (QS) tested once; ■ – one-minute duration between practice epochs.

clinical examination. The exclusion criteria were: (i) a history of smoking, (ii), any history of epilepsy, and recent chest or abdominal surgery were contraindicated (because the intervention was high-frequency breathing), (iii) taking medication or using other wellness strategies, and (iv) those who were using stimulants or intoxicating substances. Participants were recruited by notices in the institution's notice boards. They did not receive any incentive to take part in the study. Their practice of the breathing techniques was observed and approved by an experienced yoga instructor. There was no attempt to statistically calculate the sample size prior to the experiment. However, post hoc calculations using G Power Software Version 3.1 and based on changes in oxy-Hb (during HFYB versus pre-HFYB, effect size=0.42 [medium]) showed that the power was high (0.945) [16]. The variables to be recorded and the study design were described to the participants and their signed informed consent to participate in the study was obtained. The study received approval by the Ethics Committee of Patanjali Research Foundation, Haridwar, India on February 15, 2014 (approval number PRF/14/0086).

Design

Each participant in the experimental group was assessed on 2 separate days for 2 different yoga breathing practice sessions at the same time of day. For both practices, participants were seated erect, with their spine straight and eyes closed. On one day they practiced HFYB and on another day they practiced breath awareness (BAW). The sequence of the practice was reversed for every alternate participant. The total duration of each session was 28 min, with 5 min before the practice, 18 min during the practice, and 5 min after the practice. This was true for HFYB, BAW, and quiet sitting sessions. Assessments were taken continuously in the pre-session, session 1, session 2, session 3, and post-session periods of 5 min each (Figure 1). No recordings were made during the three 1-min rest periods.

Recording conditions

The assessments were done before, during, and after HFYB or BAW sessions. The participants were seated in a sound-attenuated and dimly lit room and monitored on a closed-circuit television from an adjacent room. All participants were in a sitting position throughout the measurements and to prevent artifacts they were asked to avoid movements. Instructions were given through an intercom. During recording days, average air temperature and humidity were $22.5 \pm 2.1^\circ\text{C}$ and 63.3 ± 10.0 percent, respectively. The sound level was approximately 26 db, which was measured using a Digital Sound Level Meter, Model 407730, Extech Instruments, USA.

Assessment

Functional near-infrared spectroscopy

Measurements for hemodynamic changes such as the concentration of oxy-hemoglobin (oxy-Hb) and deoxy-hemoglobin (deoxy-Hb) in the prefrontal area were assessed using a continuous-wave 16-channel functional near-infrared spectroscopy (Model no. 1000, COBI Studio Software, USA). The fNIRs system was connected to a flexible sensor made of foam and wire. The sensor consists of 4 light sources or emitters with 2 wavelengths (730 ± 15 nm; 850 ± 15 nm), sampling at 25 kHz, and 10 detectors with a fixed-source detector separation of 2.5 cm (voxel 1–8=left hemisphere; 9–16=right hemisphere). The sensor was fixed to the participant's forehead by elastic straps. For horizontal fixation, the lower edge of the sensor set was fixed 1 cm above the nasion. The fNIR sensor is shown in Figure 2.

When the light source was turned on, the scattered light was collected by the 10 detectors. The 4 light sources were controlled by a computer and turned on sequentially, so the fNIRs system had 16 voxels, which were the midpoint between emitter-detector

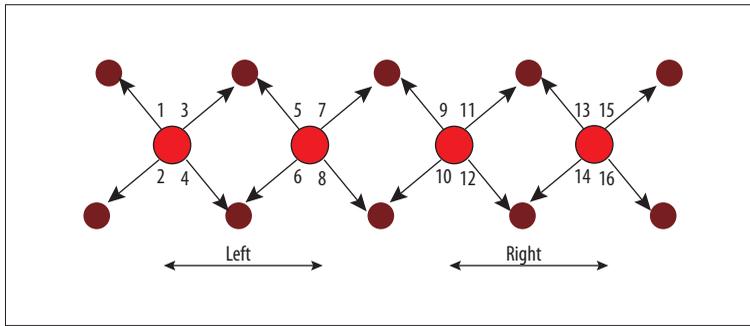


Figure 2. fNIR sensor showing the position of 16 voxels, 10 detectors, and 14 light sources or emitters.

● – detector, which detects the light (n=10); ● – light source or emitter, which emits the light (n=4).

pairs. The detector's output signal, which was proportional to the light intensity, was amplified through the data box, then the signal was given as the input to the computer after conversion by an analog-to-digital converter. Light intensity changes in wavelengths of 730 nm and 850 nm can be calculated by the fNIRs software attached to the system and then converted to concentration changes of oxy-Hb using the modified Beer-Lambert law. fNIRs imaging shows changes in concentration of oxy-Hb and deoxy-Hb as values relative to the values at baseline.

Intervention

Two yoga techniques were performed by the participants in the experimental group: (i) HFYB and (ii) BAW. The total time for an intervention session was 18 min, with 1 min of rest after each 5 min of practice.

In HFYB, participants were asked to practice high-frequency yoga breathing at approximately 1.0 Hz with forceful exhalation, for 3 periods of 5 min each. After each 5-min period, there was a 1-min gap during which they breathed normally; thus, the duration was 18 min. Throughout the practice, the participants were asked to sit in a comfortable posture (the same posture for both practices), keeping their spine erect and neck aligned. The participants were requested to keep their eyes closed throughout the assessment period.

In breath awareness sessions, the participants were asked to be aware of the flow of air as it enters and passes through the nasal passages. They were told to be aware of their breath without modifying the rate or depth or any other aspect of breathing. They assumed the same posture as for high-frequency yoga breathing, which is seated with spine erect and eyes closed. The participants practiced this technique for 18 min, which consisted of 3 epochs of 5 min, with a 1-min rest in between.

In the quiet sitting control group, the participants allowed random thoughts to pass through their mind without modifying them and they avoided modifying their breath or being aware of their breathing. They were asked to sit with their spine erect and eyes closed. There was no other activity.

Data extraction

The pre-session (5 min), session (15 min, as the 3 min of rest were not included), and post-session (5 min) data were extracted separately. Data extraction of hemodynamic changes in the prefrontal area such as concentration change in oxy-Hb and deoxy-Hb was done using COBI Studio software in the 16-channel fNIRs system. fNIRs data epochs for practice and rest periods were extracted from the continuous data using time synchronization markers. Blood oxygenation changes within each of the 16 voxels were calculated using the modified Beer-Lambert law. Movement artifacts were noted during the recording and were removed during data extraction.

Data analysis

Repeated measures analysis of variance (RM-ANOVA) followed by post hoc Bonferroni adjustment were done to compare data recorded during and after the sessions with data recorded before the 2 practices, using SPSS Version 18.0. There were 2 within-subject factors: sessions (HFYB and breath awareness) and states (before, during, and after sessions). The data from the quiet sitting control group were analyzed using the paired *t* test. These data were not included in the repeated-measures ANOVA, since the subjects assessed with ANOVA were of a single group with 2 sessions, whereas the control group subjects were separate.

Results

Oxy-hemoglobin (oxy-Hb)

In the HFYB session, a significant reduction was observed on the left side when pre-session values were compared to (i) session (phase 2) ($p < .01$), (ii) session (phase 3) ($p < .01$), both at voxel 3 and (i) session (phase 2) ($p < .05$), (ii) session (phase 3) ($p < .05$), both at voxel 5. On the right side a significant reduction was observed when pre-session values were compared to (i) session (phase 2) ($p < .05$), (ii) session (phase 3) ($p < .05$), both at voxel 13, and (i) session (phase 2) ($p < .001$), (ii) session (phase 3) ($p < .01$), (iii) post-session ($p < .01$) all at voxel 15.

Table 1. Changes in oxy-hemoglobin in HFYB, BAW and quiet sitting control sessions. Values are group mean ±S.D.

Session	Voxel	Pre	During			Post
			During 1	During 2	During 3	
HFYB	Voxel 1	-0.15±1.60	-0.45±1.78	-1.22±2.14	-1.32±2.36	-1.04±2.24
	Voxel 2	-1.12±2.33	-1.92±6.25	-2.51±6.21	-2.47±6.27	-2.51±6.32
	Voxel 3	-0.48±1.30	-0.98±1.48	-1.79±1.80**	-1.94±1.85**	-1.38±1.67
	Voxel 4	-0.77±1.40	-1.10±2.00	-1.71±2.20	-1.90±2.61	-1.42±2.13
	Voxel 5	-0.46±1.39	-0.80±1.75	-1.65±2.07*	-1.87±2.26*	-1.51±2.26
	Voxel 6	-0.76±1.78	-0.97±2.56	-1.63±3.21	-1.66±3.62	-1.06±3.01
	Voxel 7	-0.53±1.06	-0.56±1.55	-1.17±2.10	-1.37±2.56	-1.08±2.58
	Voxel 8	-0.20±1.60	-0.47±1.97	-1.48±2.53	-1.61±2.80	-1.70±3.02
	Voxel 9	-0.46±1.33	-0.59±1.78	-1.38±2.23	-1.61±2.47	-1.04±2.61
	Voxel 10	-0.10±1.37	-0.36±1.83	-1.21±2.44	-1.41±2.85	-1.07±2.96
	Voxel 11	-0.15±1.24	-0.55±1.56	-1.25±2.33	-1.37±2.50	-0.94±2.28
	Voxel 12	-0.45±1.82	-0.46±2.26	-0.94±2.81	-0.79±3.00	-0.16±2.45
	Voxel 13	-0.35±1.46	-0.89±1.57	-1.61±2.08*	-1.75±2.17*	-0.90±1.94
	Voxel 14	-1.14±2.63	-1.44±2.82	-2.13±2.58*	-2.12±3.07	-1.28±2.09
	Voxel 15	-0.32±1.57	-0.79±1.76	-1.80±1.98***	-1.93±2.01**	-1.27±1.87**
	Voxel 16	-0.55±1.43	-0.75±1.62	-1.72±1.84	-1.56±2.03	-0.63±1.74
BAW	Voxel 1	-1.90±6.71	-1.26±5.75	-1.62±6.54	-1.31±6.96	-0.69±7.11
	Voxel 2	0.12±1.23	-0.06±1.43	-0.10±1.33	0.29±1.61	1.32±2.13
	Voxel 3	0.21±0.57	-0.02±1.00	-0.17±1.16	0.11±1.40	0.26±1.57
	Voxel 4	0.11±1.35	0.00±1.65	0.11±1.77	0.48±2.00	0.31±6.00
	Voxel 5	0.27±0.70	0.12±1.20	-0.06±1.46	0.27±1.88	0.43±1.98
	Voxel 6	0.20±1.48	0.06±1.97	0.14±2.32	0.50±2.38	0.31±5.23
	Voxel 7	0.22±0.66	0.09±1.19	0.02±1.45	0.41±1.90	1.05±2.02
	Voxel 8	0.20±0.84	0.22±1.08	0.39±1.62	0.78±2.13	0.98±2.14
	Voxel 9	0.23±0.69	0.27±0.91	0.29±1.09	0.63±1.38	1.14±1.79
	Voxel 10	0.18±0.79	0.17±0.84	0.08±1.20	0.25±1.74	0.73±2.37
	Voxel 11	0.18±0.61	0.22±0.93	0.06±1.32	0.31±1.63	0.74±2.29
	Voxel 12	0.20±0.91	-0.08±1.36	-0.38±1.55	-0.13±2.14	0.66±3.15
	Voxel 13	0.24±0.72	0.07±1.26	-0.01±1.56	0.21±1.95	0.75±1.91
	Voxel 14	0.21±0.93	0.24±1.69	0.34±2.00	0.74±2.25	1.43±2.57
	Voxel 15	0.06±0.80	-0.22±1.34	-0.34±1.50	-0.07±1.69	0.52±1.45
	Voxel 16	0.34±1.27	0.32±2.17	0.43±2.77	0.85±3.15	1.69±2.86

Table 1 continued. Changes in oxy-hemoglobin in HFYB, BAW and quiet sitting control sessions. Values are group mean \pm S.D.

Session	Voxel	Pre	During			Post
			During 1	During 2	During 3	
Quiet sitting control	Voxel 1	-0.17 \pm 1.00	-0.24 \pm 1.22	-0.26 \pm 1.18	-0.12 \pm 1.49	0.20 \pm 2.14
	Voxel 2	-0.24 \pm .92	-0.34 \pm 1.24	-0.40 \pm 1.33	-0.32 \pm 1.65	-0.04 \pm 2.32
	Voxel 3	-0.13 \pm 0.96	-0.19 \pm 1.27	-0.19 \pm 1.36	0.02 \pm 1.56	0.25 \pm 1.99
	Voxel 4	-0.23 \pm 0.91	-0.31 \pm 1.39	-0.38 \pm 1.44	-0.37 \pm 1.69	-0.07 \pm 2.19
	Voxel 5	0.00 \pm 0.81	-0.29 \pm 0.95	-0.35 \pm 1.17	-0.11 \pm 1.34	0.03 \pm 1.74
	Voxel 6	-0.50 \pm 0.83	-0.83 \pm 1.44	-0.89 \pm 1.39	-0.78 \pm 1.55	-0.33 \pm 2.22
	Voxel 7	0.00 \pm .87	-0.30 \pm 0.81	-0.35 \pm 0.89	-0.23 \pm 1.24	0.44 \pm 1.81
	Voxel 8	0.03 \pm 0.91	-0.38 \pm 1.03	-0.50 \pm 0.96	-0.45 \pm 1.34	-0.50 \pm 2.16
	Voxel 9	0.15 \pm 0.72	-0.16 \pm .98	-0.09 \pm 1.12	-0.00 \pm 1.43	0.18 \pm 1.95
	Voxel 10	-0.38 \pm 0.97	-0.36 \pm 0.83	-0.54 \pm 0.75	-0.65 \pm 0.95	-0.90 \pm 1.43
	Voxel 11	0.21 \pm 0.51	0.11 \pm 0.75	0.24 \pm 0.95	0.39 \pm 1.16	0.43 \pm 1.43
	Voxel 12	-0.33 \pm 1.56	-0.38 \pm 1.53	-0.36 \pm 1.54	-0.31 \pm 1.89	-0.26 \pm 2.45
	Voxel 13	-0.05 \pm 0.89	-0.33 \pm 1.06	-0.33 \pm 1.23	-0.23 \pm 1.39	-0.12 \pm 2.01
	Voxel 14	-0.04 \pm 1.03	-0.03 \pm 1.36	-0.10 \pm 1.59	-0.09 \pm 1.74	-0.23 \pm 2.40
	Voxel 15	-0.23 \pm 1.15	-1.59 \pm 5.37	-1.59 \pm 5.36	-1.53 \pm 5.64	-1.38 \pm 5.76
	Voxel 16	-0.14 \pm 0.91	-0.24 \pm 1.06	-0.10 \pm 1.34	-0.15 \pm 1.53	.14 \pm 2.30

No significant changes were observed during and after the BAW and quiet sitting sessions.

The details of changes in oxy-Hb are given in Table 1.

Deoxy-hemoglobin (deoxy-Hb)

In the HFYB session, no significant difference was observed in deoxy-Hb during or after HFYB when compared with pre-session values. Also, there was no significant difference in deoxy-Hb during or after the BAW session when compared with pre-session values.

In the quiet sitting control session, a significant reduction was observed in deoxy-Hb when pre-session values were compared to during and after at (i) voxel 1 (left side), (ii) voxel 2 (left side), (iii) voxel 4 (left side), (iv) voxel 9 (center right side), (v) voxel 11 (right side), and (vi) voxel 12 (right side). The details of changes in deoxy-Hb are given in Table 2.

Discussion

The present study assessed hemodynamic changes of the pre-frontal area of the brain using functional near-infrared spectroscopy (fNIRS) during and after HFYB sessions at 1.0 Hz. There was a bilateral decrease in oxy-hemoglobin (oxy-Hb) during HFYB and a bilateral decrease in deoxy-hemoglobin (deoxy-Hb) during quiet sitting.

Hemodynamic changes are correlated with neural activity, so that in neural foci that are active, there would generally be a decrease in oxy-hemoglobin. This neurovascular coupling can be due to at least 2 factors: arterial CO₂ and sympathetic drive.

An earlier study on fNIRS in participants practicing HFYB at 1.0 Hz showed frontal bilateral reduction in oxy-hemoglobin during 15 min of HFYB and for 10 min after HFYB practice [14]. These results are comparable to the present findings. Both studies on HFYB practiced at 1.0 Hz resulted in similar finding (no evidence of frontal activation based on hemodynamic changes), which were in contrast to the results of a study on HFYB practiced at the rate of 2.0 Hz [13]. In the study on HFYB at 2.0 Hz, a comparison was made between healthy persons and those diagnosed with schizophrenia, with healthy

Table 2. Changes in deoxy-hemoglobin in HFYB, BAW and quiet sitting control session. Values are group mean \pm S.D.

Session	Voxel	Pre	During			Post
			During 1	During 2	During 3	
HFYB	Voxel 1	-0.09 \pm 2.11	0.31 \pm 1.50	0.91 \pm 1.78	1.42 \pm 2.28	1.59 \pm 2.32
	Voxel 2	0.20 \pm 0.87	0.37 \pm 1.35	0.55 \pm 1.43	0.85 \pm 1.61	1.15 \pm 1.99
	Voxel 3	-0.33 \pm 2.08	0.12 \pm 1.26	0.59 \pm 1.61	0.77 \pm 1.83	0.96 \pm 1.68
	Voxel 4	-0.29 \pm 1.95	-0.18 \pm 1.13	0.18 \pm 1.37	0.13 \pm 1.54	0.87 \pm 1.50
	Voxel 5	-0.51 \pm 1.86	-0.02 \pm 1.16	0.41 \pm 1.54	0.54 \pm 1.82	0.60 \pm 1.99
	Voxel 6	-0.28 \pm 1.95	0.11 \pm 1.10	0.55 \pm 1.63	0.57 \pm 1.57	1.09 \pm 1.52
	Voxel 7	-0.09 \pm 2.33	0.46 \pm 1.58	0.75 \pm 2.41	0.74 \pm 2.98	0.84 \pm 2.56
	Voxel 8	-0.56 \pm 2.50	-0.21 \pm 2.62	0.49 \pm 3.03	0.56 \pm 2.84	1.25 \pm 3.00
	Voxel 9	-0.21 \pm 2.28	0.51 \pm 1.44	1.05 \pm 1.82	1.20 \pm 2.06	1.27 \pm 1.89
	Voxel 10	-0.75 \pm 2.44	-0.27 \pm 2.74	0.34 \pm 3.43	0.38 \pm 3.66	0.73 \pm 3.79
	Voxel 11	-0.54 \pm 2.05	0.00 \pm 1.41	0.37 \pm 1.75	0.48 \pm 1.82	0.61 \pm 1.78
	Voxel 12	-0.81 \pm 1.81	-0.66 \pm 1.33	-0.26 \pm 1.90	-0.26 \pm 2.22	0.35 \pm 2.05
	Voxel 13	-0.47 \pm 2.23	0.03 \pm 1.49	0.43 \pm 1.92	0.54 \pm 2.01	0.64 \pm 2.01
	Voxel 14	-0.38 \pm 2.05	-0.13 \pm 1.40	0.18 \pm 1.77	0.20 \pm 2.13	0.76 \pm 2.07
	Voxel 15	-0.05 \pm 2.34	0.58 \pm 1.43	1.22 \pm 1.73	1.49 \pm 1.99	1.94 \pm 2.07
	Voxel 16	-0.36 \pm 2.06	0.03 \pm 1.29	0.52 \pm 1.64	0.66 \pm 1.95	1.13 \pm 1.91
BAW	Voxel 1	0.24 \pm 1.76	-0.05 \pm 2.06	0.15 \pm 1.95	-0.01 \pm 2.34	-0.43 \pm 3.11
	Voxel 2	-0.03 \pm 0.71	-0.33 \pm 1.41	-0.13 \pm 1.31	-0.28 \pm 1.72	-0.34 \pm 2.12
	Voxel 3	-0.36 \pm 0.54	-0.74 \pm 1.07	-0.60 \pm 0.91	-0.86 \pm 1.18	-0.65 \pm 2.00
	Voxel 4	-0.25 \pm 0.75	-0.51 \pm 1.27	-0.50 \pm 1.18	-0.67 \pm 1.55	-1.41 \pm 5.00
	Voxel 5	-0.43 \pm 0.69	-0.80 \pm 1.26	-0.64 \pm 1.40	-0.81 \pm 1.51	-0.56 \pm 2.28
	Voxel 6	-0.44 \pm 0.92	-0.79 \pm 1.45	-0.68 \pm 1.37	-0.83 \pm 1.90	-1.64 \pm 5.80
	Voxel 7	-0.32 \pm 0.60	-0.76 \pm 1.25	-0.60 \pm 1.24	-0.77 \pm 1.35	-0.55 \pm 2.31
	Voxel 8	-0.36 \pm 0.77	-0.56 \pm 1.17	-0.38 \pm 1.45	-0.45 \pm 1.82	-0.11 \pm 2.47
	Voxel 9	-0.36 \pm 0.54	-0.77 \pm 1.17	-0.62 \pm 1.07	-0.74 \pm 1.03	-0.47 \pm 1.97
	Voxel 10	-0.33 \pm 0.57	-0.51 \pm 1.00	-0.30 \pm 1.15	-0.21 \pm 1.59	0.18 \pm 2.60
	Voxel 11	-0.27 \pm 0.56	-0.56 \pm 1.09	-0.44 \pm 1.29	-0.64 \pm 1.58	-0.42 \pm 2.36
	Voxel 12	-0.28 \pm 0.47	-0.48 \pm 1.10	-0.28 \pm 1.33	-0.32 \pm 2.04	0.16 \pm 3.55
	Voxel 13	-0.34 \pm 0.48	-0.70 \pm 1.15	-0.55 \pm 0.98	-0.76 \pm 1.18	-0.64 \pm 1.90
	Voxel 14	-0.40 \pm 0.56	-0.99 \pm 1.56	-0.84 \pm 1.53	-0.98 \pm 1.61	-0.90 \pm 2.35
	Voxel 15	-0.18 \pm 0.64	-0.55 \pm 1.15	-0.35 \pm 0.87	-0.62 \pm 0.85	-0.45 \pm 1.69
	Voxel 16	-0.17 \pm 0.97	-0.53 \pm 1.63	-0.44 \pm 1.46	-0.65 \pm 1.53	-0.65 \pm 2.11

Table 2 continued. Changes in deoxy-hemoglobin in HFYB, BAW and quiet sitting control session. Values are group mean \pm S.D.

Session	Voxel	Pre	During			Post
			During 1	During 2	During 3	
Quiet sitting control	Voxel 1	-0.00 \pm 0.83	-0.21 \pm 1.15	-0.32 \pm 1.42	-0.37 \pm 1.32*	-0.51 \pm 1.41*
	Voxel 2	-0.11 \pm 0.67	-0.36 \pm 1.03	-0.47 \pm 1.08**	-0.49 \pm 1.14*	-0.72 \pm .99***
	Voxel 3	-0.28 \pm 0.64	-0.48 \pm 1.00	-0.58 \pm 1.23	-0.34 \pm 1.34	-0.62 \pm 1.47
	Voxel 4	-0.23 \pm 0.69	-0.55 \pm 1.07*	-0.60 \pm 1.17*	-0.46 \pm 1.20	-0.62 \pm 1.33*
	Voxel 5	-0.29 \pm 0.96	-0.36 \pm 1.20	-0.44 \pm 1.53	-0.17 \pm 1.66	-0.37 \pm 1.83
	Voxel 6	0.05 \pm 0.82	-0.24 \pm 1.42	-0.39 \pm 1.47	-0.15 \pm 1.53	-0.21 \pm 1.84
	Voxel 7	-0.21 \pm 0.70	-0.34 \pm 1.12	-0.48 \pm 1.33	-0.45 \pm 1.56	-0.55 \pm 1.99
	Voxel 8	0.01 \pm 1.27	-0.07 \pm 1.78	-0.06 \pm 1.80	-0.14 \pm 2.20	0.15 \pm 2.91
	Voxel 9	-0.53 \pm 0.86	-0.78 \pm 1.31	-1.04 \pm 1.59*	-1.26 \pm 1.90*	-1.57 \pm 2.20**
	Voxel 10	-0.04 \pm 1.67	-0.52 \pm 1.71	-0.61 \pm 2.07	-0.63 \pm 2.50	-0.70 \pm 3.05
	Voxel 11	-0.57 \pm 0.91	-0.81 \pm 1.42	-1.16 \pm 1.76*	-1.39 \pm 2.14*	-1.85 \pm 2.40**
	Voxel 12	-0.06 \pm 2.39	-0.52 \pm 2.29*	-0.69 \pm 2.47*	-0.71 \pm 2.92	-0.93 \pm 3.20*
	Voxel 13	-0.36 \pm 0.65	-0.48 \pm 0.78	-0.69 \pm 0.90	-0.86 \pm 1.17	-1.23 \pm 1.56*
	Voxel 14	-0.24 \pm 0.75	-0.55 \pm 0.98	-0.47 \pm 1.33	-0.59 \pm 1.42	-0.93 \pm 1.81
	Voxel 15	-0.30 \pm 0.68	-0.45 \pm 1.42	-0.66 \pm 1.31	-0.69 \pm 1.62	-1.16 \pm 2.02
	Voxel 16	-0.34 \pm 0.63	-0.53 \pm 0.81	-0.62 \pm 1.07	-0.73 \pm 1.25	-0.94 \pm 1.59

persons showing a bilateral increase in frontal oxy-hemoglobin suggestive of frontal activation. The differences in results when HFYB was practiced at 1.0 Hz compared to 2.0 Hz could be due to several factors. An HRV study on the frequency domain measures after HFYB at 2.0 Hz suggested a shift in the autonomic balance towards sympathetic dominance. In contrast, an HRV study on HFYB at 1.0 Hz, which used both frequency and time domain measures, showed changes suggesting that HFYB at 1.0 Hz did not increase sympathetic activity.

Sympathetic drive as described above can influence cerebral blood flow despite cerebrovascular autoregulation. It is possible that the higher rate of breathing (2.0 Hz) did cause such a change. Sympathetic activation has been associated with increased activity of the ventromedial prefrontal cortex and the orbitofrontal cortex [17]. In the cited study [17] the skin conductance level was assessed during functional magnetic resonance imaging (fMRI) while participants preformed bio-feedback arousal and relaxation tasks. Neural activity within the ventromedial prefrontal cortex and the orbitofrontal cortex co-varied with skin conductance levels. These findings suggest an association between sympathetic activation and increased activity of the ventromedial prefrontal cortex and the orbitofrontal cortex. HFYB at 2.0 Hz appears to increase

sympathetic activity [10] whereas HFYB at 1.0 Hz does not [11]. This difference may explain the absence of frontal activation with HFYB at 1.0 Hz.

The role of PaCO₂ in regulating cerebral blood flow is well known [8]. It may be expected that HFYB at 2.0 Hz would lower PaCO₂ levels due to CO₂ wash-out. However, HFYB at 1.0 Hz or 2.0 Hz has not been associated with symptoms of hyperventilation based on negative findings in the Nijmegen questionnaire for hyperventilation [18]. Also, although blood gas levels were not measured during HFYB at 1.0 Hz or 2.0 Hz, in a study of high-frequency breathing at 232 cycles per min (about 4.0 Hz) the arterial O₂ and CO₂ tension remained normal [19]; therefore, it may be speculated that the increase in oxy-Hb levels in the frontal region detected by fNIRs during HFYB at 2.0 Hz are not related to altered arterial post hoc PaCO₂.

The fNIRs studies on HFYB at 1.0 Hz [14] and at 2.0 Hz [13] also differed with respect to the duration of practice (15 min versus 1 min, respectively) and duration of experience of participants in practicing HFYB (16.3 \pm 9.8 months versus 2 weeks, respectively) in the 2 studies. It is not clear whether these factors influenced the outcome.

The bilateral reduction in deoxy-Hb during the quiet sitting control session appears to suggest activation. The mental state during quiet sitting may be comparable to the state when attention is not directed to a task but there is mind wandering and self-referential processing [20]. Hence, the addition of a control group did provide new information.

The present findings suggest that HFYB practiced at the rate of 1.0 Hz does not cause an increase in cerebral blood flow to the prefrontal region. However, HFYB practiced at 1.0 Hz improves functions indirectly related to attention, such as improved fine motor skills, visual discrimination [6], and improved visual perception [7]. Hence, the increase may be localized to specific regions within the prefrontal cortex. Another advantage of the fact the cerebral blood flow does not increase would be that the practice possibly carries minimal risks of cerebrovascular accidents when practiced at this rate (1.0 Hz). However, these are mere speculations, which require further studies before clear conclusions can be drawn.

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Conclusions

Practicing HFYB at 1.0 Hz in 2 experimental states (HFYB and BAW) compared to a control state revealed: (i) similar results to the earlier study on HFYB at 1.0 Hz [14] and (ii) that quiet sitting is not necessarily a control state.

Competing interests

The authors declare that they have no competing interests.