Effects of heart rate variability biofeedback training on anxiety reduction and brain activity: a randomized active-controlled study using EEG

Ryuji Saito[1], Kazuki Yoshida[2], Daisuke Sawamura[2], Akihiro Watanabe[1], Yukina Tokikuni[1], Shinya Sakai[2]

1. Graduate School of Health Sciences, Hokkaido University, Sapporo, Japan
2. Department of Rehabilitation Science, Faculty of Health Sciences, Hokkaido University, Sapporo, Japan

Corresponding Author

Kazuki Yoshida, Ph.D.

Address: N12, W5, Kita-ku, Sapporo, Hokkaido, 060-0812, Japan

Phone/Fax number: +81-11-706-3414

Email: ot-k-yoshida@huhp.hokudai.ac.jp

ORCID ID

Ryuji Saito: https://orcid.org/0000-0003-1925-7044

Kazuki Yoshida: https://orcid.org/0000-0002-1701-6274

Daisuke Sawamura: https://orcid.org/0000-0003-3180-3474

Shinya Sakai: https://orcid.org/0000-0003-4863-083X

Abstract

Heart rate variability biofeedback (HRVBF) is a promising anxiety-reducing intervention that increases vagally-mediated heart rate variability (vmHRV) through slow-paced breathing and feedback of heart rhythm. Several studies have reported the anxiety-reducing effects of HRVBF; however, some studies have reported such training as ineffective. Furthermore, the effects of training and underlying brain activity changes remain unclear. This study examined the anxiety-reducing effects of HRVBF training and related brain activity changes by randomly assigning participants, employing an active control group, and measuring anxiety-related attentional bias using the emotional Stroop task and electroencephalography (EEG). Fifty-five healthy students with anxiety were randomly assigned to the HRVBF or control groups, and 21 in the HRVBF group and 19 in the control group were included in the analysis. Both groups performed 10 training sessions of 20 min each within 3 weeks. They were assessed using resting vmHRV, event-related potential (ERP), time-frequency EEG, attentional bias, and the State-Trait Anxiety Inventory-JYZ (STAI-JYZ) before and after training. The results demonstrated increased resting vmHRV in the HRVBF group compared to the control group after training. However, no differences were observed in ERP, time-frequency EEG, attentional bias, and STAI-JYZ. Participants with higher pre-training resting vmHRV achieved higher heart rhythm coherence in HRVBF training and had reduced attentional bias. This study suggests that individuals with higher resting vmHRV are more likely to be proficient in HRVBF training and benefit from its anxiety-reducing effects. The findings contribute to participant selection to benefit from HRVBF training and modification of the training protocols for non-responders.

Clinical trial registration

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Keywords

heart rate variability biofeedback, anxiety, attentional bias, heart rate variability, electroencephalography

Statements and Declarations

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Introduction

Heart rate variability (HRV), a physiological index reflecting the regulation of autonomic balance, is the variability of the continuous heart interbeat interval. Specifically, vagally-mediated heart rate variability (vmHRV), reflecting cardiac vagal activity, is indicative of good autonomic control (Berntson et al., 1997; Laborde et al., 2017; Malik, 1996). The primary parameters of resting vmHRV include the root mean square of successive differences (RMSSD), a time-domain parameter, and high frequency (HF), a frequency-domain parameter (Berntson et al., 1997; Laborde et al., 2017; Malik, 1996). The neurovisceral integration model explains that neural networks involved in cognitive and emotional processing, consisting of brain regions such as the prefrontal cortex, insula, cingulate cortex, and amygdala, play a systemic role in autonomic nervous system (ANS) regulation, suggesting that high resting vmHRV is associated with superior cognitive performance and emotional regulation such as anxiety reduction (Smith et al., 2017; Thayer et al., 2009). Considering such a model and theoretical background, a recent review has proposed that decreases in HRV lead to a decline in brain functions, resulting in psychopathological symptoms (Cattaneo et al., 2021). Meta-analyses have also indicated that resting vmHRV is reduced in depression (Koch et al., 2019) and anxiety disorders (Cheng et al., 2022). However, the review has emphasized that further research is needed to explore the detailed relationship between decreases in HRV and psychopathological symptoms via decline in brain functions (Cattaneo et al., 2021). Thus, the relationships among resting vmHRV, brain activity, and emotional regulation remain unclear, and direct evidence supporting the notion that changes in resting vmHRV are associated with emotional regulation and related changes in brain activity is currently lacking.

One common intervention that modulates vmHRV is heart rate variability biofeedback (HRVBF). HRVBF is a breathing training designed to increase vmHRV by providing feedback on the correspondence between slow breathing and HRV waveform (Lehrer et al., 2013). HRVBF is effective for emotional, cognitive, and physical disabilities, both in continuous and one-time training sessions, and the effect is pronounced on anxiety and depression (Lehrer et al., 2020). Meta-analyses have reported a reduction in self-reported anxiety and stress (Goessl et al., 2017) and an improvement in depressive symptoms (Pizzoli et al., 2021), indicating the clinical usefulness of HRVBF. The mechanism underlying the effect of HRVBF has been postulated to be its effect on emotion and cognition centers through vagal afferent tracts facilitated by baroreceptors and pulmonary stretch receptors. In addition, the enhancement of the network involved in emotional regulation is achieved through oscillations in brain regions synchronized with heart rate oscillations (Gerritsen & Band, 2018; Laborde et al., 2022; Mather & Thayer, 2018; Noble & Hochman, 2019; Sevoz-Couche & Laborde, 2022). However, considering not only the postulated mechanisms of HRVBF training but also the neurovisceral integration model (Smith et al., 2017; Thayer et al., 2009) and the relationship between decreases in HRV and psychopathological symptoms via decline in brain functions (Cattaneo et al., 2021), it remains unclear whether the changes in resting vmHRV caused by HRVBF training are related to the observed effects of improved emotional regulation, such as anxiety reduction, and changes in brain activity corroborating these effects. This study aimed to investigate whether the changes in resting vmHRV resulting from HRVBF training are associated with anxiety reduction and related changes in brain activity.

Recent neuroimaging studies have examined the association between brain activity and HRVBF, providing insights into the mechanisms of HRVBF training. In functional magnetic resonance imaging (fMRI) studies, temporal changes in the standard deviation of normal-to-normal intervals (SDNN) induced by continuous HRVBF training have been correlated with changes in resting-state functional connectivity between the ventral medial prefrontal cortex and the anterior insula, cingulate cortex, and amygdala (Schumann et al., 2021). An increase in downregulation in the somatosensory brain region, including the right insula, while viewing emotional pictures after continuous HRVBF training has also been reported (Nashiro et al., 2023). Electroencephalography (EEG) studies show an increase in alpha band power in the anterior cingulate cortex, a decrease in beta band power in the posterior cingulate cortex (Sherlin et al., 2010), and an increase in the theta/beta ratios at Fz, Cz, and Pz after one-time HRVBF training in healthy participants (Prinsloo et al., 2013).

Thus, many HRVBF studies have reported its effect on anxiety and brain activity. However, there are also reports showing no effectiveness of HRVBF, with no significant changes in alpha and beta band power before and after training (Lin, 2018) and fewer changes in mu suppression than when combined with neurofeedback (Goodman et al., 2018), and with no significant anxiety reduction compared to the control (Munafò et al., 2016; Patron et al., 2013; Saito et al., 2021). Thus, the effects of HRVBF on anxiety and brain activity are reportedly mixed. Furthermore, the fMRI and EEG studies mentioned above have not examined the impact of changes in resting vmHRV induced by HRVBF training on the direct association between anxiety reduction and associated changes in brain activity. In addition, a recent systematic review has highlighted the lack of methodological consensus on HRVBF training (Lalanza et al., 2023). A previous study has suggested the importance of employing an active control group with sham training for appropriate evaluation of training effects (Ratajczak et al., 2021). These findings suggest that previous studies on HRVBF may not have appropriately evaluated the training effects due to study designs featuring passive control groups and inconsistent training protocols lacking reproducibility. Therefore, to elucidate the mechanisms of HRVBF training on anxiety reduction, well-designed studies with an active control group are necessary to examine the relationship between anxiety-reducing effects and changes in brain activity induced by HRVBF training.

It is necessary to detect anxiety using a behavioral task and measure changes in brain activity indicative of reduced anxiety using event-related potential (ERP) and time-frequency EEG as the outcome of HRVBF training to address this research problem. Attentional bias refers to the tendency for attention to be drawn to negative information in highly anxious individuals (Bar-Haim et al., 2007). The emotional Stroop task is one of the behavioral tasks to measure attentional bias. It has been shown to be useful in detecting attentional bias for negative stimuli in patients with anxiety disorders and major depression (Joyal et al., 2019). A recent study has reported a relationship between a reduction in late positive potential (LPP) and a decrease in anxiety-related attentional bias measured by the emotional face-color Stroop task (Pan et al., 2019). LPP, an ERP component, reflects attention allocation to emotional stimuli (Hajcak & Foti, 2020). A review has reported that higher resting vmHRV is associated with more adaptive and functional top-down and bottom-up cognitive modulation of emotional stimuli (Park & Thayer, 2014). It has also been reported that LPP for emotional facial stimuli is related to respiratory sinus arrhythmia (RSA) activity, reflecting cardiac vagal activity (Woody et al., 2019). Based on these findings, it is possible that increased resting vmHRV is related to a decrease in anxiety-related attentional bias in the emotional face-color Stroop task and a corresponding reduction in LPP. In addition, a reduction in vertex positive potential (VPP) is observed in parallel with a decrease in attentional bias in the emotional face-color Stroop task (Pan et al., 2019), and the frontal resting theta/beta ratio (TBR) is inversely correlated with the control of attentional bias (Angelidis et al., 2018; van Son et al., 2018). VPP, an ERP component, reflects face processing (Chen et al., 2022), and frontal resting TBR, a parameter derived from time-frequency EEG, is a useful electrophysiological marker for emotional regulation (Putman et al., 2010). Thus, a decrease in VPP and resting TBR may be observed in a decrease in anxiety-related attentional bias. Furthermore, regarding HRVBF training, the study with the highest effect size (Paul & Garg, 2012) in the meta-analysis showed a high anxiety reduction effect by the training (Goessl et al., 2017) conducted 10 training sessions of 20 min each. In addition, a previous study that reported an increase in theta band power and changes in alpha asymmetry with continuous HRVBF training conducted 10 training sessions of 20 min each within 3 weeks (Dziembowska et al., 2016). From the data of these previous studies, we determined attentional bias for anxiety parameters and LPP, VPP, and resting TBR for brain activity parameters, as well as 10 training sessions of 20 min each within 3 weeks for the training volume of HRVBF.

This study aimed to appropriately assess the anxiety-reducing effects of HRVBF training by randomly assigning participants and establishing an active control group. Additionally, the study sought to investigate whether changes in resting vmHRV caused by the training are associated with anxiety reduction and changes in related brain activity using LPP, VPP, and resting TBR. We evaluated resting vmHRV and resting TBR, attentional bias measured by the emotional face-color Stroop task, and its associated LPP and VPP before and after 10 sessions of continuous HRVBF training conducted within 3 weeks among a cohort of relatively anxious undergraduate and graduate students. Based on the presumed relationship outlined earlier between resting vmHRV, anxiety-related attentional bias, and LPP, we hypothesized that anxiety-related attentional bias and LPP would decrease as resting vmHRV (RMSSD and HF) increased with HRVBF training. Also, in parallel with the reduction of attentional bias, it was assumed that VPP and resting TBR would decrease.

Methods

Sample size estimation

The required sample size was calculated using G*power 3.1 (Faul et al., 2009). Pan et al. (2019) reported a correlation coefficient ($r = 0.54$) between a decrease in anxiety-related attentional bias and a reduction in LPP. The sample size was estimated based on significance probability ($\alpha = 0.05$) and statistical power ($1-\beta = 0.80$), resulting in 22 participants in one group. Considering a 15% dropout rate and outliers, we assigned 52 participants to the HRVBF and control groups, with 26 participants each.

Participants

Considering the effect of age on HRV (Tiwari et al., 2021), right-handed, healthy undergraduate and graduate students aged 18 to 30 were recruited. Participants completed the Trait Anxiety Scale of the State-Trait Anxiety Inventory-JYZ (STAI-JYZ) (Hidano et al., 2000) before participating in this study. The exclusion criteria were as follows: 1) low anxiety (under the 30th percentile of the normative score: males score 42 or under and females score 39 or under) on the Trait Anxiety Scale (Hidano et al., 2000); 2) medical history of psychiatric disorder, or brain, heart, or respiratory diseases; 3) history of dizziness and consciousness disorder due to a controlled respiratory rate; 4) consumption of drugs affecting the ANS; and 5) neurological or visual disability that interferes with a behavioral task. Participants were stratified based on sex and Trait Anxiety scores and randomly assigned to the HRVBF or control groups.

The study was conducted with the approval of the Ethics Committee of the Faculty of Health Sciences, Hokkaido University (approval number: 21-92). It was registered at the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) (registered number: UMIN000047096). Written informed consent was obtained from all participants before the commencement of the study.

Breathing training

Breathing training was performed using the emWave Pro (HeartMath LLC, Boulder Creek, CA, USA). Pulse wave data measured by the sensor was displayed on the screen as feedback for the HRV waveform. The emWave Pro provided a coherence score, representing heart rhythm coherence, a sine wave-like pattern in the HRV waveform. This score is calculated as 'Peak power/(Total power – Peak power)' (McCraty et al., 2009). Peak power is determined by identifying the highest peak in the 0.04-0.26 Hz range and calculating the integral in a window of 0.030 Hz width, centered on the highest peak in that region. Total power is calculated for the 0.0033–0.4 Hz range. In this study, the average coherence score was calculated by averaging the coherence scores of all training sessions, defining it as a parameter for training proficiency level.

In the HRVBF group, HRVBF training was performed according to a previously published protocol (Lehrer et al., 2013). First, the resonance frequency, which is the respiratory cycle that maximizes low frequency (LF, 0.04-0.15Hz) (ms^2), was determined from the range of 6.5, 6.0, 5.5, 5.0, and 4.5 breaths per minute. LF is a parameter that reflects changes in baroreceptor reflex caused by HRVBF training (Lehrer et al., 2013; Shaffer & Meehan, 2020) and is related to resting vmHRV (Kromenacker et al., 2018). Subsequently, participants performed inhalations and exhalations based on the resonance frequency with the guidance of a breathing pacer. They adjusted the HRV waveform to achieve a sine wave-like pattern, receiving visual feedback of the waveform. Participants were instructed to perform pursed-lip breathing, abdominal breathing, inhalation through the nose, and exhalation through the mouth, with the aim of achieving higher coherence scores.

In the control group, sham-paced breathing training was performed as according to a previous study (Breach, 2012). First, referring to the previous study that examined the effect of the respiratory cycle on several HRV parameters, including LF (Song & Lehrer, 2003), the respiratory cycle with the least effect on LF was determined from the range of 14, 13, 12, 11, 10 breaths per minute. Next, participants performed inhalations and exhalations based on the determined respiratory cycle with the guidance of a breathing pacer but without visual feedback of the HRV waveform. Participants were instructed to perform inhalation through the nose and exhalation through the mouth and aim for higher coherence scores.

Referring to previous studies that reported high effect size (Goessl et al., 2017; Paul & Garg, 2012) and an increase in theta band power and changes in alpha asymmetry (Dziembowska et al., 2016) by continuous HRVBF training, we set a goal of 10 training sessions of 20 min each to be performed within 3 weeks. No practice sessions were conducted. Participants who attended 8 or more training sessions were included and those who attended 7 or less training sessions were excluded to ensure at least 80% training volume. The average number of training sessions per week was 4.0 ± 1.0 in the first week, 3.0 ± 1.1 in the second week, and 2.6 ± 1.2 in the third week.

Behavioral task and analysis

Attentional bias was measured using an emotional face-color Stroop task (Isaac et al., 2012; Lee et al., 2009; Pan et al., 2019). With attentional bias, the higher the anxiety, the more delayed the response to negative facial expressions, and the more positive the correlation with trait anxiety (Lee et al., 2009). Therefore, attentional bias is regarded as an objective indicator of trait anxiety.

The timeline of one trial in the emotional face-color Stroop task and an example of facial expression stimuli are shown in Fig. 1. Forty face pictures (16 angry, 16 disgusted, and 8 neutral) were selected from the facial expression database (Fujimura & Umemura, 2018). Participants were asked to respond to the color (red, blue, yellow, or green) of colored facial expression stimuli (angry, disgusted, or neutral) presented after fixation by pressing a key (“D,” “F,” “J,” and “K”) on a keyboard as quickly and accurately as possible. The color-key correspondence was counterbalanced. Finger placement for a key press (“D,” “F,” “J,” and “K” with left middle, left index, right index, and right middle fingers, respectively) was instructed. Participants were seated 60 cm away from a 23-inch monitor with a 1920 × 1080 pixel resolution. Fixation was randomly presented with equal probability in 2000, 2500, or 3000 ms. Subsequently, 5.4° × 4.1° of the visual angle-colored facial expression stimuli were presented for 500 ms. Reaction time (RT) was calculated from the beginning of the stimuli presentation to 1200 ms, and the interstimulus interval (ISI) was 2700, 3200, or 3700 ms. Seventy-two trials were included in one block, and a total of 360 trials were performed in five blocks. The rest period between blocks was 30 s. Before the formal experiments, participants performed 24 practice trials to familiarize themselves with the task. Facial stimuli in the practice trials differed from those in the formal experiments. This task was created using PsychoPy (Peirce et al., 2019).

An incorrect keypress, no response, and a response shorter than 200 ms or longer than 1200 ms were defined as errors. RTs exceeding mean ± 3SD per participant were excluded from the analysis. Attentional bias (ms) was calculated by subtracting the RTs for neutral faces (neutral face RT) from the RTs for negative (angry and disgusted) faces (negative face RT). Participants with an accuracy of less than 80% were excluded from the analysis. Data exclusion based on RTs and task performance was based on a previous study that measured attentional bias (Gillioz et al., 2023).

HRV recording and analysis

We recorded electrocardiogram (ECG) data using a portable ECG device, Check My Heart (Daily Care Biomedical Inc., Taiwan). ECG data were recorded for 5 min in the resting sitting position, and RR interval data were extracted at a sampling frequency of 250 Hz. RR interval data were analyzed using Kubios HRV Premium version 3.4.2 (Kubios Oy, Kuopio, Finland). In the pre-processing, the RR interval data were detrended by the smoothness priors method ($\lambda = 500$) and artifact-corrected by an automatic correction algorithm. The RR interval data with a percentage of corrected beats exceeding 5% were defined as invalid, and participants with invalid data were excluded from the analysis. Subsequently, the RR interval data were re-sampled at 4 Hz, and Fast Fourier Transform (FFT) was applied, with a window width of 300 s and a window overlap of 50%. Thus, we calculated RMSSD (ms) as time-domain parameters and HF (0.15-0.4 Hz) (ms^2) as a frequency-domain parameter. The RMSSD reflects cardiac vagal activity, and HF reflects it only during 9-24 breaths per minute (Berntson et al., 1997; Laborde et al., 2017; Malik, 1996). These two are recommended as resting vmHRV indicators corresponding to clearly identified physiological systems (Laborde et al., 2017). Therefore, we adopted the RMSSD and HF for the analysis.

EEG recording and pre-processing

We recorded EEG data using actiCHamp Plus (BrainProducts GmbH, Munich, Germany) with active electrodes mounted on an elastic cap according to the 10-20 international system. Electrodes were placed at the following 31 sites: Fp1, Fp2, F3, F4, F7, F8, Fz, FC1, FC2, FC5, FC6, FCz, C3, C4, Cz, T7, T8, CP1, CP2, CP5, CP6, TP9, TP10, P3, P4, P7, P8, Pz, O1, O2, and Oz. The reference electrode was placed at the nose tip, and the ground electrode was on the forehead. An electrooculogram (EOG) was placed at the infraorbital and supraorbital regions of the left eye for the vertical EOG and at the outer canthus of each eye for the horizontal EOG. The electrode impedance was kept below 5 kΩ, and the sampling rate was 500 Hz. Participants were instructed to fix their gazes with their eyes open, avoiding eye blinks and head motion, during the EEG recording. All EEG data were processed with the Brainstorm (Tadel et al., 2019). EEG signals were bandpass filtered between 1-40 Hz and re-referenced to the average reference. Independent component analysis (ICA) was applied to remove artifacts due to eye blinks and eye movement.

ERP analysis

Task-related EEG data were epoched using a time window of 200 ms before and 1200 ms after stimulus onset. Baseline correction was conducted with a 200 ms pre-stimuli period. Epochs exceeding ± 80 µV from the baseline and errors of the emotional face-color Stroop task were rejected from the analysis. The cleaned epochs were averaged for both negative and neutral faces.

Based on a previous study (Pan et al., 2019), the following electrodes were selected for EEG analysis: P3, Pz, and P4 for the LPP, and FCz, Cz, CP1, and CP2 for the VPP. LPP is a sustained positive deflection in the central-parietal sites after emotional stimulus onset and reflects the allocation of attention to emotional stimuli (Hajcak & Foti, 2020). VPP is a positive peak recorded in central sites after face stimulus onset and reflects face processing (Chen et al., 2022). It has been shown that LPP (Schienle et al., 2022) and VPP (Yin et al., 2021) amplitudes increase when negative faces are presented compared to neutral faces. Thus, these ERP amplitudes probably increase with a delay in RTs to negative faces because of attentional bias. The LPP and VPP amplitudes were averaged over electrode clusters. We defined time windows for VPP (150-190 ms) and LPP (300-600 ms) based on visual inspection of grand-average waveforms and previous studies (Gu et al., 2019; Jia et al., 2021; Pan et al., 2019). We measured the mean amplitude of LPP and the baseline-to-peak amplitude of VPP and obtained LPP difference (μV) and VPP difference (μV) by subtracting ERP amplitudes for neutral faces from those for negative faces. This subtraction corresponded to the calculation of attentional bias.

EEG time-frequency analysis

We analyzed 5 min of all recordings in resting-state EEG data and 5 min recordings that had the first and last 2.5 min removed from a 10-min training in the training phase EEG data. The EEG data were epoched at an interval of 2000 ms with a 50% temporal overlap. Epochs exceeding ± 80 μV from the baseline were rejected from the analysis. Each cleaned epoch was then averaged.

Time-frequency decomposition of the epoched data was conducted using Morlet wavelets. Subsequently, the frontal resting TBR was calculated. TBR is the ratio of theta to beta band powers, and frontal resting TBR is inversely correlated with the control of attentional bias (Angelidis et al., 2018; van Son et al., 2018). The analysis was conducted with the following frequency bands for theta band power (4-7 Hz) and beta band power (14-30 Hz), and electrodes of interest F3, Fz, F4, and FCz (Angelidis et al., 2018; van Son et al., 2018).

Procedure

The overview of the study procedure is shown in Fig. 2. Measurements were conducted between 12:00 and 20:00 hours, considering the influence of time of day on autonomic activity. Post-measurements were conducted within 1 week of the final breathing training. Participants were asked to get sufficient sleep the day before the measurements, not consume alcohol or caffeine on the day of the measurements, and not engage in intense exercise. For questionnaires, a demographic questionnaire included information on age, body mass index (BMI), smoking status, exercise time, and sleeping time because they are known to affect HRV (Tiwari et al., 2021). A visual analog scale (VAS) (cm) was conducted to investigate the confidence of the group assignment (0 = "I definitely belong to a control group," 5 = "I don't know," and 10 = "I definitely belong to an intervention group"). Participants completed the State Anxiety Scale of STAI-JYZ in pre-measurement since the Trait Anxiety Scale of STAI-JYZ had been completed at the time of recruitment and the State and Trait Anxiety Scale of STAI-JYZ in post-measurement. For physiological and behavioral measurements, EEG and HRV were recorded during the resting state for 5 min and EEG during the emotional face-color Stroop task for approximately 20 min. For greater effect from the breathing training in post-measurement, HRVBF or control training and an EEG during the training was conducted simultaneously for 10 min before the emotional face-color Stroop task.

Statistical analysis

Demographic data and pre-training STAI-JYZ scores were compared between the two groups using an unpaired *t*-test.

The training effects were examined using a 2 × 2 mixed-design analysis of variance (ANOVA) with groups (HRVBF and control) and time (pre-and post-training) as the between- and within-participant factors, respectively. We applied this analysis to resting vmHRV (RMSSD and HF), ERP (LPP difference and VPP difference), time-frequency EEG (resting TBR), attentional bias, and STAI-JYZ scores (state and trait anxiety). When the interaction was significant, a post-hoc analysis with Bonferroni correction was performed. We compared TBR during HRVBF and control training, in post- measurements, training-related data (number of training sessions, days from final training to post-measurement, and the average coherence score through all training sessions), and VAS scores for confidence in a group assignment, using an unpaired *t*-test. We calculated partial eta-squared (η_p^2) for the ANOVA and Cohen's *d* for an unpaired *t*-test to estimate the effect sizes.

Pearson's correlation analysis was conducted for each group with regard to changes in resting vmHRV, LPP difference, VPP difference, resting TBR, attentional bias, and STAI-JYZ scores before and after training (post-training values minus pre-training values) to examine whether changes in resting vmHRV resulting from HRVBF training are associated with anxiety reduction and changes in LPP difference, VPP difference, and resting TBR. All statistical analyses were performed using SPSS version

26.0 (IBM Corp., Armonk, NY, USA). The statistical significance level was set at $p < 0.05$.

Results

A flow diagram of the Consolidated Standards of Reporting Trials (CONSORT) is shown in Fig. 3. We recruited 55 participants and included 21 in the HRVBF group (mean age = 21.7 ± 2.4 years, 13 females) and 19 in the control group (mean age = 21.9 ± 2.3 years, 12 females) in the analysis.

Participant characteristics

No significant differences were found between the groups with regard to demographic data or STAI-JYZ scores ($p > 0.05$) (Table 1A, B). The only one smoker was excluded from the analysis; thus, data on smoking status was not shown.

Effects of Training

A 2 × 2 mixed-design ANOVA revealed significant interactions between groups and time in RMSSD (F (1, 38) = 4.13, p = 0.049, η_p^2 = 0.10) and HF (F (1, 38) = 9.24, p = 0.004, η_p^2 = 0.20) with regard to resting vmHRV. It showed that RMSSD and HF increased in the HRVBF group while they decreased in the control group from pre- to post-measurement. A significant effect of time was found in the trait anxiety scores (F (1, 38) = 7.73, p = 0.008, η_p^2 = 0.17), which showed that trait anxiety decreased in both groups before and after training. However, no significant interactions were found for any of the LPP difference, VPP difference, resting TBR, or attentional bias ($p > 0.05$) (Table 2). An unpaired t-test revealed no significant differences in the TBR during the training in either group ($p > 0.05$).

In the training-related data, no significant group differences were found in the number of training sessions or days from final training to post-measurement ($p > 0.05$), and the average coherence score was significantly higher in the HRVBF group than in the control group (t (38) = 11.40, p = 0.000, d = 3.61) (Table 1C). No significant group differences were found in the VAS scores ($p > 0.05$) (Table 1D).

Correlation between physiological and behavioral data

The change values (Δ) represent differences between post-training and pre-training values. For resting vmHRV and attentional bias, a significant positive correlation between RMSSD (Δ) and attentional bias (Δ) was found in the HRVBF group (r = 0.478, p = 0.029), but not in the control group (r = 0.222, p = 0.360). For resting vmHRV and LPP difference, VPP difference or resting TBR, and attentional bias and LPP difference, VPP difference or resting TBR, no significant correlations were found among any parameters in either group (p > 0.05).

Additional exploratory analysis

The positive correlation between RMSSD (Δ) and attentional bias (Δ) was contrary to our hypothesis. Given that attentional bias is the difference between negative and neutral face RTs, we analyzed further the correlation between RMSSD (Δ) and negative and neutral face RT (Δ) using Pearson's correlation analysis. This analysis revealed a negative correlation between RMSSD (Δ) and neutral face RT (Δ) in the HRVBF group (r = -0.526, p = 0.014) but not in the control group (r = 0.097, p = 0.694). No significant correlation was found between the RMSSD (Δ) and negative face RT (Δ) in either group (p > 0.05).

Significant effects of HRVBF training were observed on resting vmHRV (RMSSD and HF). However, there were no significant effects of training on the LPP difference, VPP difference, resting TBR, and attentional bias. Thus, to explore which participants benefited more from the anxiety-reducing effects of HRVBF training, Pearson's correlation analysis was performed among the pre-training resting vmHRV, attentional bias (Δ), and average coherence score. We found that pre-training RMSSD was significantly negatively correlated with attentional bias (Δ) (r = -0.434, p = 0.0495) and significantly positively correlated with the average coherence score (r = 0.669, p = 0.001) in the HRVBF group but not in the control group (r = -0.256, p = 0.290; r = 0.209, p = 0.390, respectively). Pre-training HF was also significantly positively correlated with the average coherence score in the HRVBF group (r = 0.635, p = 0.002) but not in the control group (r = 0.242, p = 0.317) (Table 3).

Discussion

In this study, we employed random assignment of participants and included an active control group for appropriate evaluation of HRVBF training effects. We examined the anxiety-reducing effects of

HRVBF training assessed by the emotional face-color Stroop task. We investigated whether alterations in resting vmHRV resulting from the training are associated with anxiety reduction and changes in related brain activity, as measured by LPP, VPP, and resting TBR. Although we observed significant training effects of HRVBF on resting vmHRV, no corresponding effects were found on anxiety-related parameters such as attentional bias, STAY-JYZ scores, LPP, VPP, and resting TBR. Contrary to our hypothesis, anxiety-reducing effects of HRVBF training and changes in LPP, VPP, and resting TBR were not confirmed. Additional analyses were conducted to examine these results, bearing important implications for understanding the effectiveness of HRVBF training.

The lack of observed effects of HRVBF training on anxiety reduction, LPP, VPP, and resting TBR in our study may be related to the participants' anxiety levels. Low-anxiety individuals, based on the trait anxiety scores, were excluded, and those with moderate or higher anxiety levels were recruited. Furthermore, participants in our study had similar levels of state and trait anxiety to those in previous studies that reported anxiety reduction (Castro Ribeiro et al., 2023; Deschodt-Arsac et al., 2018) and an increase in theta band power and changes in alpha asymmetry (Dziembowska et al., 2016) through continuous training as well as one-time training (Prinsloo et al., 2013; Sherlin et al., 2010). However, it is worth noting that in a previous study where attentional bias modification (ABM) led to a reduction in anxiety-related attentional bias measured by the emotional face-color Stroop task and a subsequent reduction in LPP, participants in the top 20% of Social Interaction Anxiety Scale (SIAS) scores from a large sample pool were recruited (Pan et al., 2019). A meta-analysis of attentional bias and anxiety showed that attentional bias was observed in anxious non-clinical populations but not in non-anxious populations (Bar-Haim et al., 2007). Thus, the participants' anxiety levels in our study may have been lower than those in the previous study (Pan et al., 2019). In addition, the normative data of the STAI-JYZ based on a large sample of university students shows that the mean and standard deviation on the State Anxiety Scale is 47.27 ± 10.45 for males and 45.94 ± 10.25 for females, and on the Trait Anxiety Scale is 48.82 ± 10.03 for males and 47.65 ± 9.96 for females (Hidano et al., 2000). Compared to the normative data, the trait anxiety score was higher, whereas the state anxiety score was lower in our data. This contrast suggested that participants in our study had lower state anxiety than general students despite recruiting participants with higher trait anxiety levels. Collectively, individuals with relatively high anxiety levels may be more likely to benefit from anxiety-reducing effects and related changes in LPP, VPP, and resting TBR induced by HRVBF. Similarly, a systematic review investigating the effects of HRVBF training on executive function suggested that patient populations or individuals with particular profiles (i.e., exposed to stressful environments) are more likely to benefit from the effects of training (Tinello et al., 2022). Therefore, the anxiety-reducing effect of HRVBF training can be influenced by the participants' anxiety levels prior to the training; this may provide practical information on the anxiety levels to be targeted. Overall, individuals with relatively high anxiety may be more likely to benefit from the anxiety-reducing effects of HRVBF training. However, since this is the first study to examine the effectiveness of HRVBF training for anxiety-related attentional bias, further studies are needed to examine the impact of participants' anxiety levels on anxiety-reducing effects.

Factors other than anxiety levels need to be considered to interpret the results. The HRVBF group showed significantly increased resting vmHRV compared to the control group after the training. In addition, the emotional face-color Stroop task created based on a previous study (Pan et al., 2019) had sufficient emotional valence and arousal in the facial expression stimuli in the task (Umemura & Fujimura, 2018). Therefore, our HRVBF training and emotional face-color Stroop task were appropriate for examining the anxiety-reducing effect of HRVBF training. We also assessed the correlation between physiological and behavioral data. Correlation analysis of the changes in values of each parameter showed that attentional bias increased significantly with increasing resting vmHRV before and after HRVBF training. This result contradicted our hypothesis. However, several interpretations are possible to explain this result. The first is regarding the vigilance-avoidance hypothesis, which is one of the models involved in the interpretation of attentional bias. The vigilance-avoidance hypothesis proposes that individuals with high anxiety rapidly orient attention to threatening stimuli and then later avoid them compared to individuals with low anxiety. It is considered the strategic process to reduce arousal induced by threatening stimuli (Mogg et al., 2004). Some studies have reported that lower resting vmHRV was associated with attentional avoidance of negative stimuli (Grol & Raedt, 2020; Katahira et al., 2014). In addition, it has been suggested that chronic use of attentional avoidance may reduce emotional arousal in the short term but may maintain and exacerbate anxiety and related symptoms over the long term (Bardeen & Daniel, 2017). Given these findings, the positive correlation between increased resting vmHRV and increased attentional bias may suggest that the HRVBF training suppressed the attentional avoidance of negative stimuli and facilitated adaptation to them by habituation. This interpretation is supported by previous studies showing that suppressed attentional avoidance of negative information and greater attentional bias facilitated learning that apparent threatening situations do not necessarily lead to an aversive outcome (Lester et al., 2019; Vassilopoulos, 2005). The second is regarding the calculation of attentional bias. Focusing on the fact that attentional bias is the difference between negative and neutral face RTs, we conducted an additional correlation analysis with RTs for each facial expression. The results showed that neutral face RTs were significantly faster with increasing resting vmHRV before and after HRVBF training. Neutral face RTs are RTs for face stimuli without emotional valence and can be considered attentional control parameters (Ranjbar et al., 2020). In addition, a meta-analysis of fMRI studies has shown that subcortical and cortical regions (e.g., the inferior frontal gyrus and insula) are less activated in neutral face processing than in emotional face processing (Qiu et al., 2022), suggesting that neutral face RTs are parameters with relatively little brain activity related to emotion. Therefore, our results suggest that attentional control may improve with increased resting vmHRV before and after HRVBF training. This interpretation is supported by a meta-analysis suggesting an association between high resting vmHRV and good attentional control (Magnon et al., 2022). In contrast, changes in resting vmHRV and negative-face RT before and after HRVBF training were not significantly correlated. Possibly, the correlation between increased resting vmHRV and attentional bias before and after HRVBF training may have resulted from the significant acceleration of neutral face RTs. Therefore, from these interpretations, the suppressed attentional avoidance of negative stimuli and/or acceleration of neutral face RTs may have affected the anxiety-reducing effects validated by measuring attentional bias. However, since this is the first study to examine the effect of resting vmHRV modulation on attentional bias, future studies are needed to validate these findings.

Regarding the decrease in trait anxiety in both HRVBF and control groups, both groups performed abdominal breathing, breathing in through the nose and out through the mouth at a constant pace. Since abdominal breathing has been reported to reduce anxiety (Chen et al., 2017; Magnon et al., 2021), a decrease in trait anxiety may have been observed not only in the HRVBF group but also in the control group. This result may suggest the anxiety-reducing effect of constantly paced abdominal breathing; the adoption of an active control group, as in our study, may be important in properly determining the effectiveness of HRVBF training without overestimating it.

From the fact that no anxiety-reducing effects were observed in HRVBF training regardless of increased resting vmHRV, the benefit of the training effect may depend on individual differences in resting vmHRV. Thus, we conducted additional correlational analyses to explore which participants benefited more from the anxiety-reducing effects of HRVBF training. The results showed that participants with higher pre-training resting vmHRV had a higher proficiency average for the 10 training sessions and reduced attentional bias before and after the training. Considering that higher resting vmHRV has been associated with better emotion regulation (Brown et al., 2022), these findings suggest that high pre-training resting vmHRV may contribute to training proficiency and anxiety reduction. Additionally, as another possibility, we focused on individual differences in the awareness of changes inside the body, the so-called interoception. Interoception is the sensing of the internal state of the body (e.g., heartbeat, visceral sensations, hunger, and thirst) (Schmitt & Schoen, 2022) and is quantified mainly by interoceptive tasks (Hickman et al., 2020) and heartbeat-evoked potential (HEP) (Coll et al., 2021). A high resting vmHRV is positively correlated with interoceptive task accuracy (Lischke et al., 2021; Owens et al., 2018). Furthermore, the neural pathways of interoception and the postulated mechanism underlying the effect of HRVBF are common, being integrated into the anterior cingulate cortex and insula via the afferent vagal nerves (Sevoz-Couche & Laborde, 2022). Therefore, highly interoceptive individuals tend to exhibit superior cardiac vagal reactivity, which may be activated by HRVBF training. This possibility is supported by a previous study reporting increased interoceptive task accuracy being positively correlated with increased 0.1 Hz HRV after resonance frequency breathing underlying HRVBF training (Leganes-Fonteneau et al., 2021). In summary, individuals with a high resting vmHRV at the start of HRVBF training may be more proficient in training and benefit from the anxiety-reducing effects. Interoception may be one of the responder characteristics for HRVBF training, and we suggest the need for validation of the relation between interoception and training proficiency and its effects.

Our study has a few limitations. The first relates to the anxiety-related outcomes measured. Many HRVBF studies have used subjective questionnaires, such as STAI, as the primary outcome of anxiety (Goessl et al., 2017). However, our study is the first to use a behavioral parameter, attentional bias, as a primary outcome of anxiety. Other HRVBF studies have used salivary amylase (Hunter et al., 2019) and salivary cortisol (Makaracı et al., 2023) as markers, and salivary cortisone and cortisol levels have been correlated with STAI state anxiety scores (Bae et al., 2019). Future studies should comprehensively evaluate the anxiety-reducing effects of HRVBF training using subjective questionnaires as well as objective measures such as behavioral and physiological parameters. The second limitation concerns participant selection. We emphasize the importance of participant selection for greater effectiveness of HRVBF training in terms of higher resting vmHRV. Considering the impact of age (Tiwari et al., 2021) and psychiatric symptoms such as anxiety (Cheng et al., 2022) and depression (Koch et al., 2019) on resting vmHRV, since we included only young, healthy students, generalization of our results to a broader population should be performed with caution. Further research in larger populations is needed to examine the differences in training effects according to participant characteristics.

Conclusion

We tested the anxiety-reducing effects of HRVBF training from multiple perspectives by measuring anxiety-related attentional bias using an emotional face-color Stroop task, the STAI-JYZ, and EEG. The results indicate increased resting vmHRV after HRVBF training but no anxiety-reducing effects. However, our study suggests that individuals with higher resting vmHRV are more likely to be proficient in HRVBF training and benefit from its anxiety-reducing effects. Participant selection for HRVBF training may be critical and may contribute to the modification of training protocols for non-responders.

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Author Contributions

Ryuji Saito: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Visualization; Writing – original draft. **Kazuki Yoshida:** Conceptualization; Formal analysis; Methodology; Project administration; Resources; Supervision; Visualization; Writing – review & editing. **Daisuke Sawamura:** Conceptualization; Methodology; Resources; Writing – review & editing. **Akihiro Watanabe:** Investigation. **Yukina Tokikuni:** Investigation. **Shinya Sakai:** Conceptualization; Funding acquisition; Methodology; Resources; Supervision.

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Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest disclosure

The authors declare that they have no conflict of interest.

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Table 1. Group statistics for demographic data, STAI-JYZ scores, training-related data, and VAS questionnaire

Parameters	Groups		*P* value
	HRVBF group (n = 21)	Control group (n =19)	
	Mean (SD)		
A. Demographic data			
Age (years)	21.7 (2.4)	21.9 (2.3)	0.808
BMI (kg/m^2)	21.0 (2.8)	21.0 (2.1)	0.965
Average exercise time per week (hours)	2.4 (2.9)	2.5 (3.1)	0.859
Average sleeping time per day (hours)	6.7 (1.0)	6.9 (1.0)	0.576
B. Pre-training STAI-JYZ scores			
State anxiety score	41.2 (8.4)	42.5 (6.3)	0.591
Trait anxiety score	51.5 (8.0)	50.6 (7.7)	0.708
C. Training-related data			
Number of training sessions	9.6 (0.7)	9.4 (0.9)	0.451
Days from final training to post-measurement	1.2 (1.6)	1.4 (1.6)	0.799
Average coherence score through all training sessions	4.2 (0.8)	1.8 (0.4)	0.000*
D. VAS questionnaire			
Confidence in the group assignment (cm)	5.0 (1.6)	4.6 (1.1)	0.322

BMI, body mass index; HRVBF, heart rate variability biofeedback; SD, standard deviation; STAI-JYZ, State-Trait Anxiety Inventory-JYZ; VAS, visual analog scale

*$p < 0.001$

Table 2. ANOVA results for physiological, behavioral, and psychological variables

Parameters	Groups				Source of variation								
	HRVBF group (n = 21)		Control group (n = 19)		Group			Time			Group × Time		
	Pre	Post	Pre	Post	*F*	*P*	η_p^2	*F*	*P*	η_p^2	*F*	*P*	η_p^2
	Mean (SD)												
RMSSD	33.10 (10.91)	38.00 (14.44)	39.71 (18.26)	36.00 (17.24)	0.28	0.602	0.01	0.08	0.779	0.00	4.13	0.049^{*}	0.10
HF	463.05 (261.08)	772.62 (586.21)	763.84 (727.66)	593.89 (579.73)	0.15	0.703	0.00	0.78	0.382	0.02	9.24	0.004^{**}	0.20
Attentional bias	7.13 (17.61)	5.36 (15.27)	15.22 (12.03)	3.88 (17.62)	0.85	0.364	0.02	3.52	0.068	0.08	1.88	0.179	0.05
State anxiety	41.19 (8.36)	39.57 (6.70)	42.47 (6.35)	39.58 (5.01)	0.13	0.722	0.00	3.83	0.058	0.09	0.31	0.584	0.01
Trait anxiety	51.52 (8.05)	47.67 (8.59)	50.58 (7.75)	47.32 (7.70)	0.09	0.770	0.00	7.73	0.008^{**}	0.17	0.05	0.818	0.00
LPP difference	-0.06 (0.38)	-0.09 (0.40)	-0.09 (0.39)	0.09 (0.54)	0.66	0.423	0.02	0.54	0.466	0.01	1.10	0.301	0.03
VPP difference	0.05 (0.62)	-0.01 (0.51)	0.10 (0.66)	0.15 (1.03)	0.43	0.516	0.01	0.00	0.975	0.00	0.10	0.749	0.00
Resting TBR	0.97 (0.80)	1.26 (1.36)	1.08 (0.92)	0.94 (1.07)	0.23	0.637	0.01	0.09	0.766	0.00	0.76	0.387	0.02

HF, high frequency; LPP, late positive potential; RMSSD, root mean square of successive differences; SD, standard deviation; TBR, theta/beta ratio; VPP, vertex positive potential

$^{*}p < 0.05$; $^{**}p < 0.01$

Table 3. Correlation between pre-training resting vmHRV parameters, attentional bias, and average coherence score

Parameters	HRVBF group (n = 21)		Control group (n = 19)	
	Pre-training RMSSD	Pre-training HF	Pre-training RMSSD	Pre-training HF
Attentional bias (Δ)	-0.434*	-0.328	-0.256	-0.103
Average coherence score	0.669**	0.635**	0.209	0.242

HF, high frequency; HRVBF, heart rate variability biofeedback; RMSSD, root mean square of successive differences. (Δ) represents post-training minus pre-training values

*$p < 0.05$; **$p < 0.01$

Figures

Fig. 1 Timeline of one trial in the emotional face-color Stroop task and an example of facial expression stimuli

Facial emotional stimuli (angry, disgusted, or neutral), colored red, blue, yellow, or green, were presented after the fixation cross. Participants were required to press the key corresponding to the color of the facial expression stimulus as quickly and accurately as possible. Facial emotional stimuli were created using a sample image of the facial expression database from the supplemental materials (Fujimura & Umemura, 2018). We obtained permission to use only sample images of neutral facial expressions from the National Institute of Advanced Industrial Science and Technology (AIST); thus, this figure includes only neutral but not angry or disgusted facial expression stimuli.

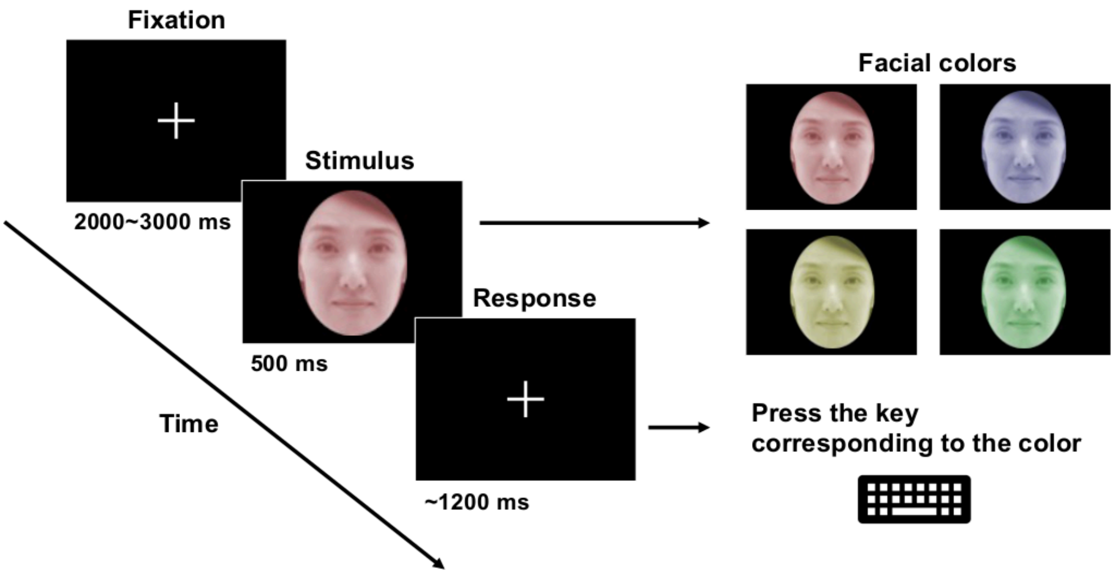

Fig. 2 Overview of the study procedure

EEG, electroencephalography; HRV, heart rate variability; STAI-JYZ, State-Trait Anxiety Inventory-JYZ; VAS, visual analog scale

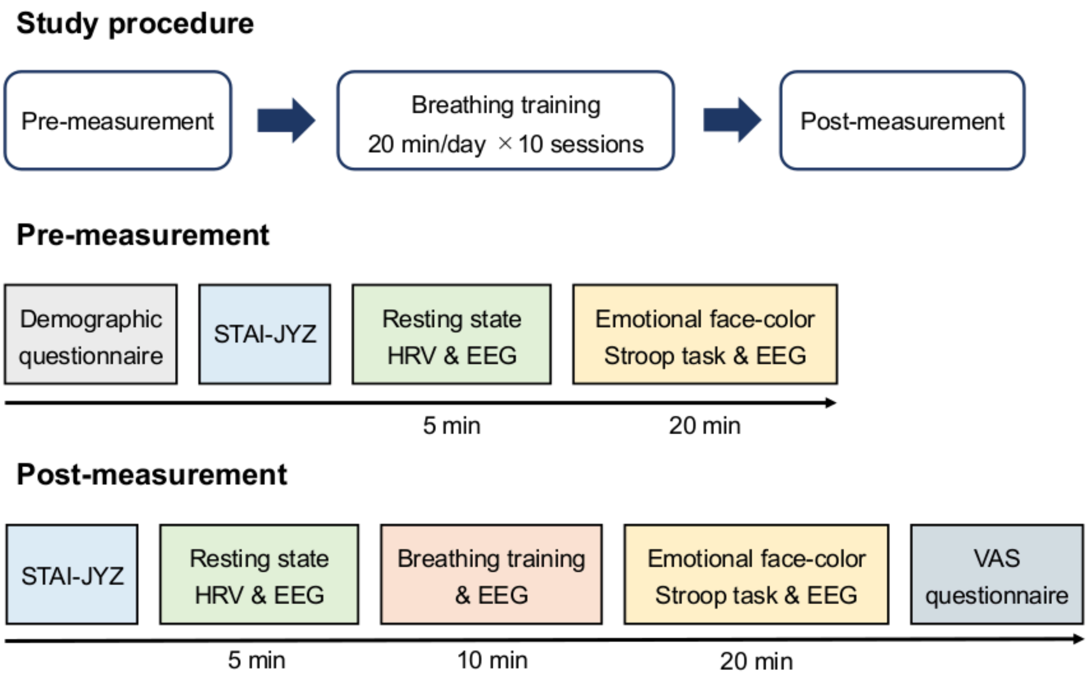

Fig. 3 CONSORT flow diagram

ANOVA, analysis of variance; HRV, heart rate variability; HRVBF, heart rate variability biofeedback

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram