

ARTIGO ORIGINAL

Effect of Brainwave Entrainment on Brainwave Frequency Spectrum and Heart Rate Variability

Efeito do arrastamento de ondas cerebrais no espectro de frequência de ondas cerebrais e na variabilidade da frequência cardíaca

ABSTRACT

Introduction: Brainwave entrainment (BWE) is a method that uses external stimuli, such as sounds, lights, or vibrations at specific frequencies, to guide the brain into specific brainwave patterns and states of consciousness. BWE has gained importance in reducing stress, anxiety and restoring sleep.

Objectives: The aim of this study was to evaluate the effect of BWE on Heart rate variability (HRV) and Brainwave Frequency Spectrum (BFS).

Material and methods: Audio-visual BWE sessions were conducted (n=20) using an BWE device and cell phone application (the Brain-Tap® headset and app). The sessions entailed background music, guided slow deep breathing (SDB), and audio BWE through binaural beats and isochronic tones oscillating from 18 to 0.5 Hz, as well as visual entrainment through light-emitting diode lights at 470 nanometers (nm) flickering at 18 to 0.5 Hz. HRV and BFS were measured before and during the BWE sessions. BFS was assessed by an advanced neuro-fractal analysis.

Results: BWE sessions triggered a state of heightened focus (indicated by increased gamma and beta activity), as well as participants remaining alert and relaxed (marked by increased alpha waves). The study also recorded an increase in the Low Frequency (LF) power of HRV.

Final considerations: This finding suggests that these changes may be vagally mediated. Also, guided SDB might temporarily modulate HRV and act as a physiological method to tap into the cardio-vagal reserve. This study implies that BWE has potential benefits for cardiovascular autonomic regulation.

Keywords: Brainwave Entrainment. Brain waves. Heart Rate Variability. Brainwave frequency spectrum.



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RESUMO

Introdução: Arrastamento de ondas cerebrais (BWE) é um método que usa estímulos externos, como sons, luzes ou vibrações em frequências específicas, para guiar o cérebro em padrões específicos de ondas cerebrais e estados de consciência. O BWE ganhou importância na redução do estresse, ansiedade e na restauração do sono.

Objetivos: O objetivo deste estudo foi avaliar o efeito do BWE na Variabilidade da Frequência Cardíaca (HRV) e no Espectro de Frequência das Ondas Cerebrais (BFS).

Material e métodos: Sessões audiovisuais de BWE foram conduzidas (n=20) usando um dispositivo de BWE e um aplicativo de celular (headset e aplicativo da Braintap®). As sessões envolviam música de fundo, respiração profunda lenta guiada (SDB) e BWE por meio de batidas binaurais, tons isocrônicos e luzes emitidas por um diodo (470 nm) oscilando de 18 a 0,5 Hz. HRV e BFS foram medidos antes e durante as sessões de BWE. BFS foi avaliado por análise neuro-fractal avançada.

Resultados: As sessões de BWE desencadearam um estado de foco intensificado (indicado pelo aumento da atividade gama e beta), enquanto os participantes permaneceram alertas e relaxados (marcado pelo aumento de ondas alfa). O estudo também registrou um aumento na potência de Baixa Frequência (LF) da HRV.

Considerações finais: Estes achados sugerem que essas alterações podem ser mediadas pelo nervo vago. Além disso, SDB guiada pode modular temporariamente a HRV e atuar como um método fisiológico para explorar a reserva cardio-vagal. Este estudo implica que o BWE tem benefícios potenciais para a regulação autonômica cardiovascular.

Palavras-chave: Arrastamento de ondas cerebrais. Ondas encefálicas. Variabilidade do batimento cardíaco. Espectro de frequência de ondas cerebrais

INTRODUCTION

Brain entrainment is a phenomenon in which the brain waves synchronize with external stimuli. This synchronization happens when brain waves are intentionally manipulated to match an external stimulus: visual, such as flashing lights, or auditory, like pulsating tones. This effect is also referred to as “brain entrainment,” “audio-visual entrainment,” “audio-visual stimulation (AVS),” “auditory entrainment,” or “photic stimulation¹. This process works because the brain has a natural propensity to adjust its dominant frequency to align with the rhythm of external periodic stimuli, a principle called frequency following response².

Studies have shown that brainwave entrainment (BWE) can improve subjective ratings of sleep and wakefulness quality, sleepiness, and motivation, notably, in a study conducted with young elite soccer players³. BWE has also been associated with a decrease in migraine frequency⁴, significant improvement in attention⁵, reduced anxiety levels^{6,7}, lessened confusion/bewilderment, fatigue/inertia, and an increase in vigor/activity⁸. Furthermore, heart rate variability (HRV) has also been shown to improve⁹.

Brain waves, specifically delta and alpha waves, generally follow a circadian rhythm, helping maintain homeostasis in the brain. However, this homeostasis can be impaired in certain individuals, causing biological dysfunction. To reestablish this balance, BWE may be used to balance target brainwaves. Neurofeedback works on similar principles, albeit through a self-mediated approach, and doesn't require any external stimulus¹.

Brainwave patterns are typically divided into five different categories or frequency ranges. Each associated with different states of consciousness, by targeting these specific frequencies, BWE is intended to induce such states:

- » Delta Waves (0.5-4 Hz): Delta waves are the slowest brainwaves, typically associated with deep, dreamless sleep and healing. Delta brainwaves are prevalent during deep meditation and restorative sleep¹⁰.
- » Theta Waves (4-8 Hz): Theta waves are associated with light sleep, deep relaxation, and are prominent during dream states and deep meditation. Theta waves can also be connected to creativity, intuition, daydreaming, and recalling emotions and sensations¹¹.

- » Alpha Waves (8-12 Hz): Alpha waves are linked to a state of wakeful relaxation. These waves are dominant during quiet, reflective moments, enhancing creativity and reducing depressive symptoms. Alpha states are often associated with mindfulness meditation¹².
- » Beta Waves (12-30 Hz): Beta waves are associated with a state of awake alertness, logical thinking, and problem-solving. Prolonged presence of beta waves may lead to stress and anxiety¹³.
- » Gamma Waves (30-100 Hz): Gamma waves are associated with high-level information processing and consolidation of information. These waves are linked to consciousness, peak focus, and learning¹⁴.

Recent studies have highlighted the significance of AVS of the brain for a variety of purposes, including enhancing focus, reducing stress, augmenting creativity, and managing symptoms of conditions like anxiety, depression, and attention deficit hyperactivity disorder (ADHD)^{15,2,1,16,17}. However, the evidence supporting these interventions is not fully established, and there are only a few studies on brain entrainment, especially in India. In this context, this paper aimed to research the effect of BWE on HRV and Brainwave frequency spectrum (BFS).

MATERIAL AND METHODS

Study design

This study was a cross-sectional pilot study conducted in the All-India Institute of Medical Sciences (AIIMS), Bhopal - India. The study was structured around a cohort of 20 participants using a purposive sampling method. After due consideration of the Research Review Board of AIIMS Bhopal and detailed discussions in the ethics committee, the Institutional Human Ethics Committee (IHEC) AIIMS Bhopal granted permission to carry out this project vide LOP No. IM0425.

Selection criteria

The study's participants included individuals of any gender who were 18 years or older. Participants had no prior experience with BWE. Furthermore, participants had no hearing disabilities, no severe physical illnesses, were not taking prescribed medications for psychiatric conditions, and were not pregnant at the time of the study.

Procedure

Initially, written informed consent was obtained from all participants. Subsequently, they filled out a questionnaire that sought information about their personal habits and self-perceived stress levels. Following this, each participant was provided with an audio-visual BWE Device (BrainTap® headset - Braintap INC, New Bern, NC, USA), which they were instructed to use in accordance with the guidelines of the cell phone application that operates the device (BrainTap® PRO App - Braintap INC, New Bern, NC, USA). Participants did a twenty minutes alpha/theta session. The sessions entailed background music, guided slow deep breathing meditation (SDB), and audio BWE through binaural beats and isochronic tones oscillating from 18 to 0.5 Hz, as well as visual entrainment through light-emitting diode lights at 470 nanometers (nm) flickering at 18 to 0.5 Hz. HRV.

HRV analysis and BFS were conducted with a NeuralChek Device (BrainTap® INC, NC, USA) before and during a BWE sessions as observed in Figure 1. The software and hardware meet the standards of measurement, physiological interpretation, and clinical use of cardiac intervalometer indices, adopted by the European Society of Cardiology and North American Association of Electrophysiology.

Figure 1 - Participant using the BWE device while HRV and BFS assessment is conducted.



HRV assessment is conducted with the participant sitting down with an electrode placed on each wrist and connected to a laptop with the NeuralChek software installed (BrainTap® INC, New Bern, NC, USA) as observed in Figure 1. The software calculates heart rhythm oscillations using spectral analysis. BFS was assessed by an advanced neuro-fractal analysis that uses a mathematical algorithm extracted from electrocardiogram signals to estimate brainwave activity.

Baseline measurements for the subjects were taken over a period of 5 minutes. An identical 5-minute recording was then made during the BWE session. Various parameters of the autonomic nervous system were recorded, specifically High Frequency (HF), Low Frequency (LF), Very Low Frequency (VLF), and the LF/HF ratio. These measurements were subsequently transferred to a Microsoft Excel® spreadsheet for data cleaning and in-depth analysis.

Statistical analysis

Data from the Microsoft Excel® spreadsheet were cleaned for empty cells, and all continuous data were represented as means and standard deviations. All data were de-personalized and transferred to SPSS® version 27 for further analysis. A paired t-test was performed to analyze the effect of the specified autonomic function parameters and brainwaves before and during the BWE sessions. P values lower than 0.05 were considered statistically significant.

RESULTS

All the recorded parameters were tabulated. The session induced an overall increase in lower-frequency waves with reduced heart rate (indicated by the RR interval). Table 1 shows a tabular representation of all observed parameters.

Table 1 - Effect of Brainwave Entrainment on BFS waves and HRV Parameters.

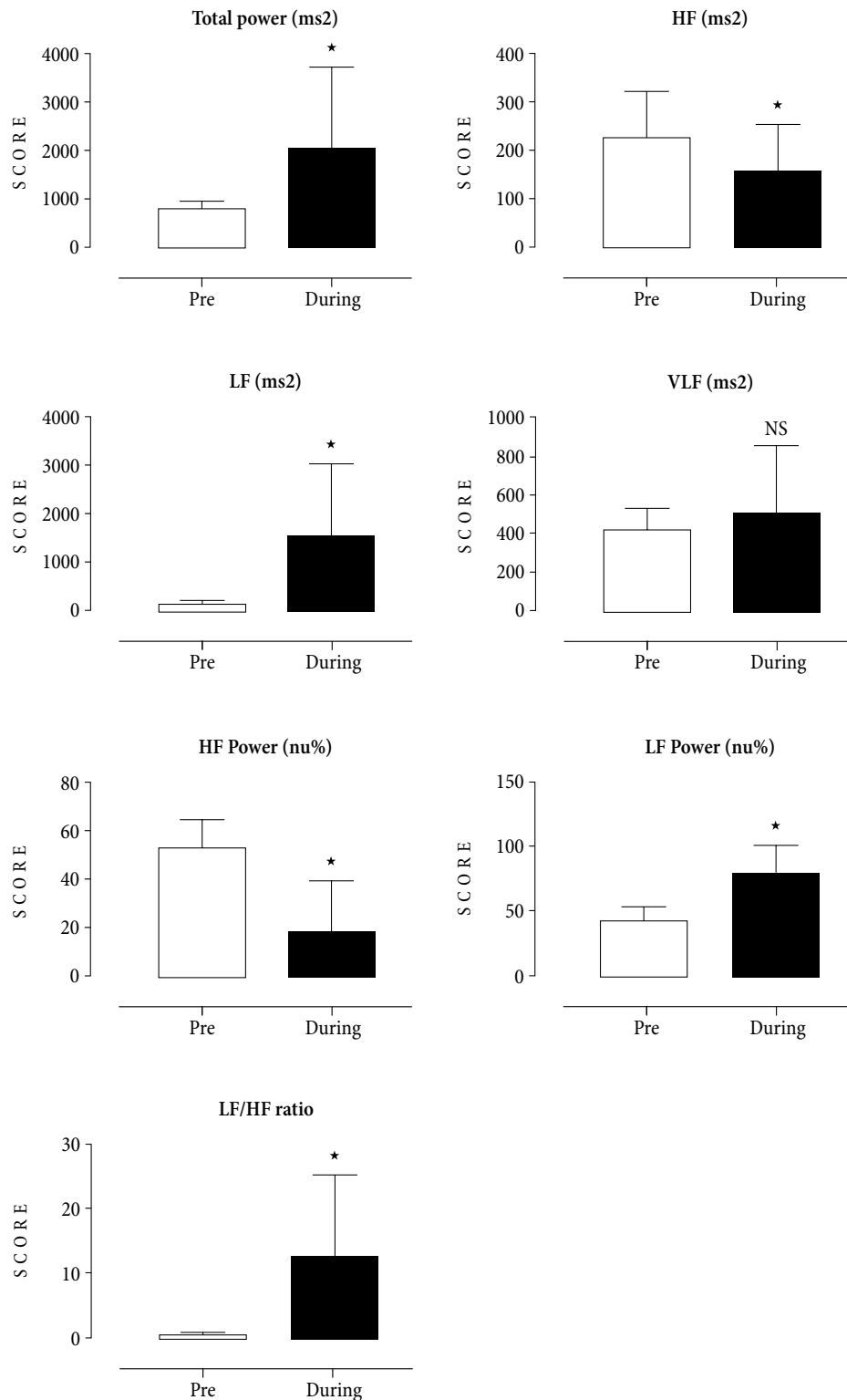
Parameters	df	t	P value	Mean Value		Standard Deviation	
				Pre	During	Pre	During
Delta	20	7.59	<0.00001	65.5	30.4	8.02	19.03
Theta	20	-0.78	0.438109*	13.25	15.65	1.33	13.63
Alpha	20	-4.27	0.000124	11.25	29.5	4.37	18.59
Beta	20	-3.87	0.000406	7	18.9	4.23	13.05
Gamma	20	-5.01	0.000013	2.75	7.15	1.33	3.68
Cervical	20	-5.46	<0.00001	30.5	64.1	7.62	26.43
Thoracic	20	-3.92	0.000349	32	59.5	10.90	29.34
Lumbar	20	-4.38	0.000088	44	67.75	10.48	21.81
Sacral	20	-4.23	0.00014	32.5	58.95	10.89	25.72
Coccygeal	20	-4.55	0.000052	36.25	63.1	9.12	24.71
LF%	20	-4.84	0.000021	24.5	53.6	7.89	25.64
HF%	20	2.14	0.038245	27.25	17	6.38	20.37
VLF%	20	5.11	<0.00001	48.25	29.35	6.70	15.08
<i>Time domain</i>							
Mean heart rate (bpm)	20	0.96	0.342928*	85	82.55	6.02	9.68
SDNN (ms)	20	-4.16	0.00017	29.95	46.205	3.11	17.15
pNN50 (%)	20	-2.74	0.009175	2.25	5.9	2.22	5.51
RMSSD (ms)	20	-2.25	0.30192*	21.45	25.595	4.38	6.96
<i>Frequency domain</i>							
Total power (ms ²)	20	-3.35	0.001813	815.85	2085.7	154.75	1685.88
HF (ms ²)	20	2.33	0.24711*	230.5	160.9	93.41	94.79
LF (ms ²)	20	-4.26	0.000127	172.25	1577.55	47.59	1472.59
VLF (ms ²)	20	-1.04	0.300528*	427.75	512.65	112.09	343.93
HF Power (nu%)	20	6.47	<0.00001	53.6175	19.08	11.37	20.96
LF power (nu%)	20	-7.13	<0.00001	43.9475	80.999	10.23	20.83
LF/HF ratio	20	-4.29	0.000117	0.835	12.923	0.29	12.58

df - degree of freedom, t - distribution is used to calculate the p value, * - not significant at p>0.05.

HRV frequency results are also presented in Figure 2, which is possible to observe an increase in Total Power, LF (ms² and nu%) and LF/HF ratio during the BWE session. Accompanied by a decrease in HF (ms² and nu%). No significant changes were observed in the VLF parameter. Fig-

ure 3 shows the power results for HRV, in which a reduction in VLF (%) can be observed. There were no significant statistical changes in the mean heart rate of participants, however there was an increase in SDNN, pNN50 and RMSSD time domains, as shown in Figure 4.

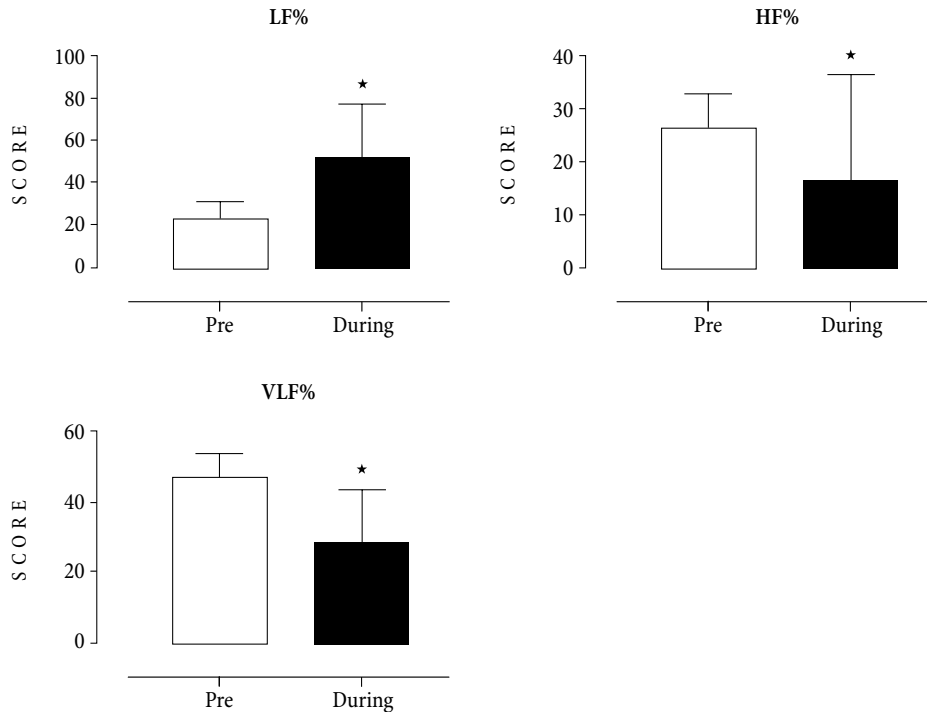
Figure 2 - Frequency results for HRV. Audio BWE session was effective in changing HRV parameters.



Each point represents the mean of 20 participants and vertical lines show the SEM. The Symbol * denotes a significant difference of $*P<0.05$ when compared to pre session eval-

uations. Statistical analysis was performed by t-test. NS – not statistically significant; BWE -Brainwave entrainment; HRV – Heart rate variability; SEM - Standard error of the mean.

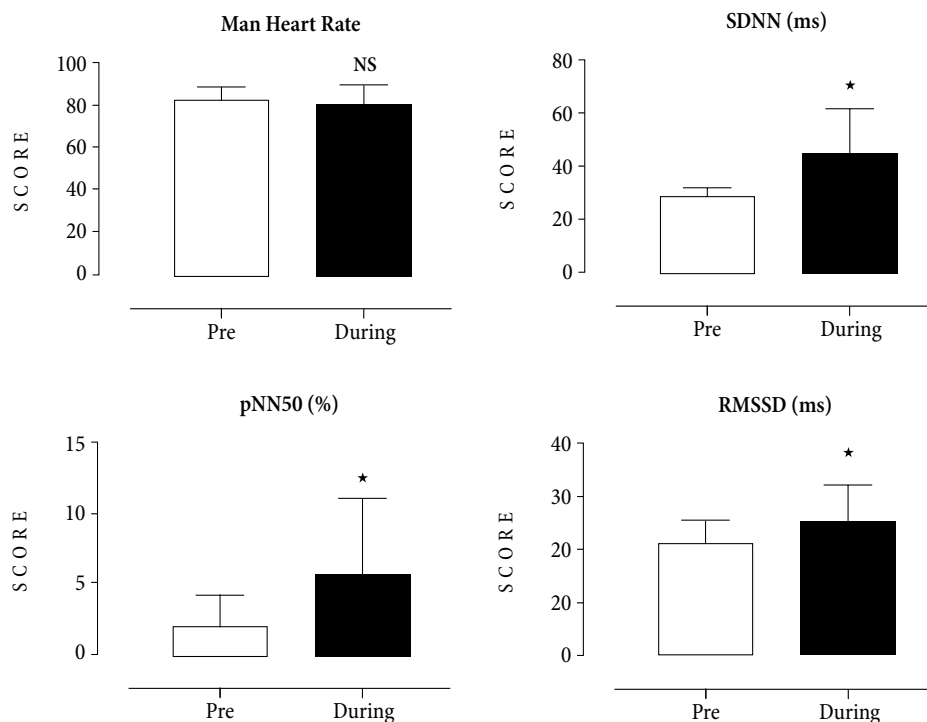
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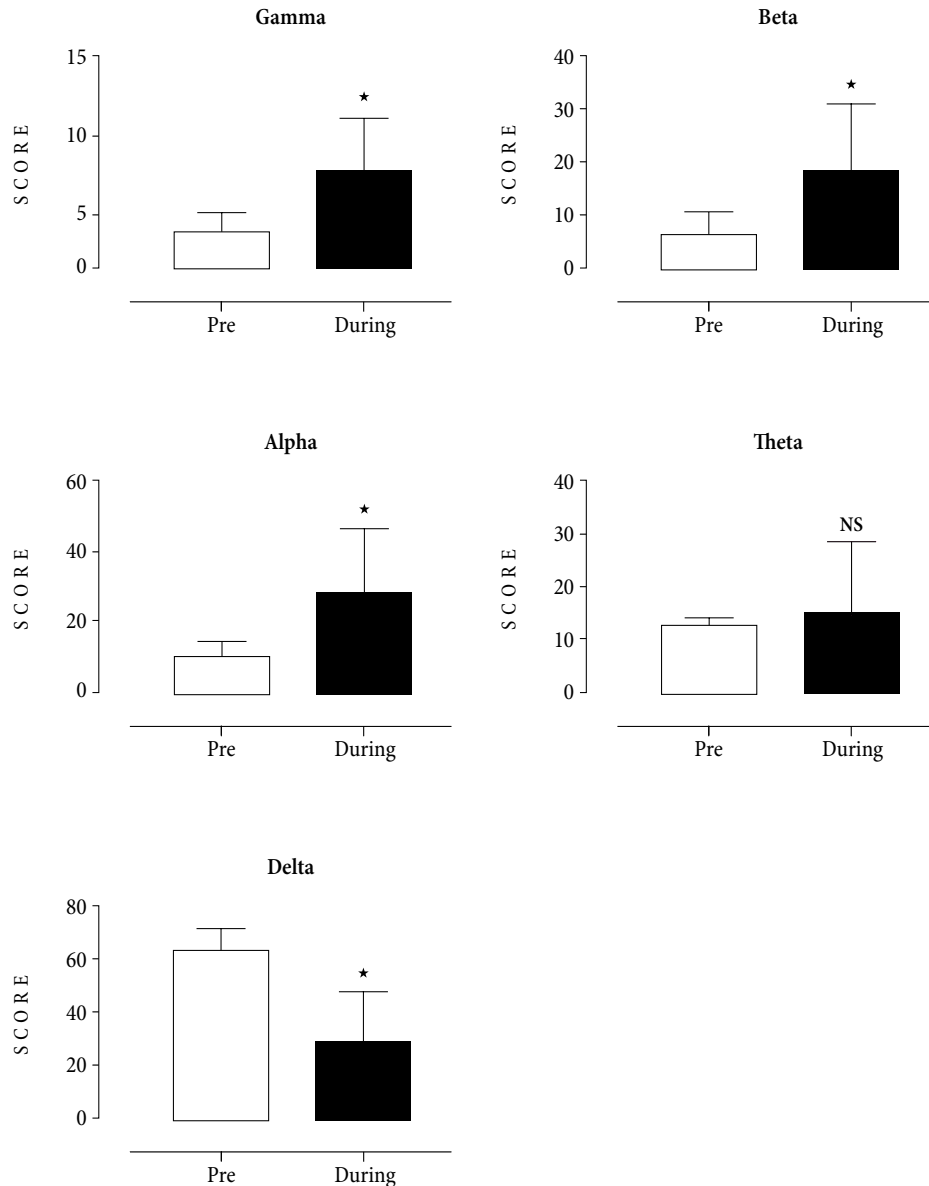
Figure 4 - Time domain HRV parameters. Audio BWE session was effective in changing HRV parameters.



Each point represents the mean of 20 participants and vertical lines show the SEM. The Symbol * denotes a significant difference of $*P<0.05$ when compared to pre session evaluations. Statistical analysis was performed by t-test. NS – not statistically significant; BWE -Brainwave entrainment; HRV – Heart rate variability; SEM - Standard error of the mean.

BWE sessions indicated a shift in BFS towards alpha, beta and gamma predominance as observed in Figure 5. Suggesting that BWE session induced a relaxed (by increasing alpha waves) but awake and focused (increased gamma and beta activity) state. Interestingly, delta waves were reduced during BWE session and no statistically significant changes in theta waves were observed.

Figure 5 - Brainwave Frequency Spectrum. Audio BWE session affected brainwave frequencies.



Each point represents the mean of 20 participants and vertical lines show the SEM. The Symbol * denotes a significant difference of $*P<0.05$ when compared to pre session evaluations.

Statistical analysis was performed by t-test. NS – not statistically significant; BWE -Brainwave entrainment; HRV – Heart rate variability; SEM - Standard error of the mean.

DISCUSSION

Guided meditation in the BWE session included SDB. Calmness is essential for heart control. Normally, the heart pumps an average of 2.000 gallons or more of blood per day. If a person indulges in restlessness, worry, or other emotions, the heart beats faster to prepare the individual to face the threat. The heart of a mouse caught in a trap, e.g., beats at twice its normal rate because of intense fear. The hearts of the calm Napoleon and Duke of Wellington are said to beat only 50 times per minute¹⁸. Practice of pranayama naturally slows breathing, which in turn makes the heart calmer, as demonstrated by a statistically significant decrease in heart rate during five minutes of SDB^{19,20}.

The present study explored the acute effect of BWE on cardiac oscillations and observed that SDB guided meditation during BWE session with a frequency less than 6 breaths per minute (bpm) increased HRV, which is reflected in both the time domain and frequency domain parameters of HRV. SDNN is the most representative parameter of HRV. During the BWE session, SDNN increased to a high normal of 46 which may indicate that the autonomic nervous system (ANS) regulating function and stress-coping ability were good²¹. It indicates that total HRV increased, showing that simultaneously cardiac sympathetic and parasympathetic activation occurred.

RMSSD, an index of activity of the parasympathetic arm of autonomic regulation, increased at a high level of statistical significance. Although with resting spontaneous breathing, HF power represents the parasympathetic influence, if the breathing rate is below 8.5 bpm these can generate oscillations in the LF band, thereby increasing the LF power. Low Frequency is a band of the power spectrum range between 0.04 and 0.15 Hz. This parameter is a strong indicator of sympathetic activity but reflects both parasympathetic and sympathetic activities²². As the respiratory rate achieved was less than 5 bpm in all volunteers, we found LF power increased more than 10-fold indicating an increase in parasympathetic activity rather than an increase in sympathetic regulation.

In the present study, the guided SDB during BWE involved breath holding after deep inspiration, which can have a mechanical effect on the heart via a direct influence on sinoatrial (SA) node activity. In addition, breath-holding may have increased end-tidal CO₂ levels, thereby influencing the central command to the heart. In five patients, the resting HF value was very high and dropped during SDB. The possible underlying mechanism could be, first, decreased vagal tone with increased heart rate, and sympatho-vagal balance during SDB due to conscious effort to maintain the same breathing count during inhalation, holding, and exhalation²³. During yoga, upregulation of both autonomic activities may occur. This notion is supported by the view that sympathetic and parasympathetic branches of the ANS do not always act reciprocally but may act synergistically and complementarily²⁴.

Slow breathing during BWE increases baroreceptor sensitivity^{25,26}. When blood pressure tends to increase, the baroreceptor reflex increases vagal and decreases sympathetic outflow to the SA and atrioventricular node. This helps to decrease the heart rate, as seen in our study from 85 bpm to 82 bpm. The increased LF power during SDB may be due to the mechanical effect of breathing rate, which gives an additional or false rise in sympathetic tone²⁷. Various studies have found that increase in amplitudes of blood pressure oscillations and HRV during slow breathing and that this is particularly significant at a respiration rate of 6 bpm (0.1 Hz).

McCraty and Shaffer²⁸ (2015) have advocated that below 0.1 Hz rhythm (6 breaths in 60 seconds or 6/60 = 0.1 Hz), the sympathetic branch of the ANS does not appear to be involved, and that heart rhythms are affected mainly by the parasympathetic branch. During slow breathing below 8.5 bpm, vagal activity can generate oscillations in the LF band. This is related to the link between respiration and HRV²⁸. Breathing is slowed to a point where resonance occurs between respiratory-induced oscillations and heart rate oscillations that naturally occur at this rate. It has been shown that any changes in breathing frequency that almost coincide with spon-

taneous Mayer wave frequency (6 bpm), such as regulated slow breathing or chanting of Ave Maria or yoga mantra, enhance HRV and baroreflex sensitivity by synchronizing inherent cardiovascular rhythms²⁹.

This may explain the changes in the decrease in heart rate and increase in HRV components, that occurred during the guided SDB when the rate of breathing was slower. Deep breathing helps convert the majority of venous deoxygenated blood to become oxygenated blood. A large increase in the waste carbon dioxide elimination from the lungs during exhalation facilitates this purifying process. As a result, very little waste tissue is present in the blood. There is less need to breath, whit that extraordinary rest is given to the heart and lungs, then heart rate decreases. In the present study, the LF/HF ratio increased significantly during SDB, LF, and HF power, and their ratios were significantly different during-SDB versus pre-SDB, indicating the lasting effect of the slow yogic breathing maneuver on the cardiovascular autonomic system³⁰.

Changes in heart rate in relation to the respiratory cycle are referred to as Sinus Arrhythmia. Under physiological conditions, during inspiration, there is an increase in the heart rate, and during expiration, there is a fall in the heart rate. Several mechanisms have been postulated to explain this phenomenon. The neuronal activity of inspiratory neurons in the medulla, besides initiating inspiration, also discharges to the Nucleus Tractus Solitarius (NTS) and Nucleus Ambiguous (NA). It inhibits both relays of the baroreceptor-NTS-NA pathway, thus inhibiting cardiac vagal discharge. Therefore, during inspiration, sympathetic activity is enhanced, which in turn leads to an increase in heart rate and a decrease during expiration. This aids in increasing the HRV which we observed in our study during BWE SDB guided meditation.

In 1915, Bainbridge noticed that if the initial heart rate of dogs was low, a rapid infusion of saline induced an increase in heart rate. This reflex is referred to as Bainbridge reflex. There are tachycardia producing receptors in the atria at the venoatrial

junction which get activated due to increase in right atrial filling. The same mechanism was observed during inspiration. The increase in negative intrathoracic pressure draws more blood, increasing the venous return, which in turn stretches the right atrium and tachycardia-producing receptors, leading to an increase in heart rate. Additionally, a stretch of the right atria also brings about stretching of the SA node which also contributes to the tachycardia³¹.

Various BWE techniques include isochronic tones, binaural and monaural beats, and amplitude modulation^{32,33}. The most used frequencies include delta frequencies for insomnia, alpha frequencies for relaxation, and theta frequencies for guided meditation. Alpha waves are direct indicators of alertness while being relaxed and are probably the most receptive state of the mind for enhancing creativity. Naturally, these states are found just before entering sleep at night and just after waking from sleep in the morning. Statistically significant difference between the baseline states (11.25, S.D. 4.37) and BWE (29.5, S.D. 18.59) suggests that the induction of the alpha state is responsible for a heightened sense of relaxed alertness in 20 individuals at a p value of 0.000124. The rhythmic fluctuations produced by BWE could open a new possibility of increasing the awareness required by an individual while performing a task. We also detected an increase in attention to follow the instructions of the SDB guided meditation.

Theta frequency is known to be directly associated with brain activity at the time of stress relief, the benefits of which may include improved concentration and memory. These cognitive parameters have also been associated with low blood pressure, respiratory rate, and heart rate. This state is particularly responsible for incorporating suggestions and executing them and is often utilized in hypnosis. Although there was an increase in Theta BFS during the session, there were no statistically significant differences (p value < 0.4) in theta BFS between baseline and during BWE.

Delta waves are predominant during dreamless sleep and are the deepest state of conscious-

ness that can be achieved in deep sleep or meditation/spiritual experiences. Delta-frequency BWE is also known to induce sleep. A statistically significant difference between the baseline state (65.5, S.D. 8.03) and during BWE (30.4, S.D.19.03) suggest that reduction of the delta state at p value of <0.00001, is responsible for meditation-like experiences among 20 individuals³⁴.

Gamma waves are very high frequency, known for their reflection of the information processing of the brain during focused activity and application of intelligence in daily life, and complicated mental processing. A statistically significant difference between the baseline state (2.75, S.D. 1.33) and during BWE (7.15, S.D.3.69) at p value of 0.000013 suggests the induction of higher engagement in mental processing at the time of the experiment³⁵.

The similar phenomenon of binaural beats resembles music related brain activity and has been studied in association with Indian 'Rasas' and the brain of Musicians³⁴. In a recent study, EEG signals from 13 healthy subjects showed an increase in theta and alpha bands and a decrease in beta band during delta and alpha stimulations³⁶; adversely, in the present study we saw an increase in beta frequency spectrum. One hypothesis is that during the BWE session, even though the participants relaxed (increased alpha and small increase in theta, although not statistically significant), the conscious effort to follow the instructions of the SDB guided meditation directly influenced the BFS, which led to an increase in beta activity.

There are a series of limitations present in our study since this was a first pilot study to investigate the effects of BWE with a guided SDB meditation on BFS and HRV. Firstly, the sample size was small (only 20 participants), which may limit the generalizability and validity of the results. Secondly, the study did not have a control group and was a pre-post intervention design, which makes it difficult to isolate the effects of BWE from other factors such as placebo, expectation, or learning effects. Thirdly, a single BWE session with a fixed frequency range and duration was used, which may not reflect the

optimal or individualized parameters for BWE. Different frequencies, durations, and modalities of BWE may have different effects on HRV and BFS. Also, we used an indirect measure of BFS based on a mathematical algorithm derived from ECG signals, which may not accurately capture the brain-wave activity or coherence. A direct measure of EEG would be more reliable and valid for assessing BFS. Additionally, increased attention or conscious effort to follow the instructions of the guided SDB may have directly influenced the outcome of the BFS leading to an increase in beta activity. At last, our study did not measure any behavioral or psychological outcomes such as sleep quality, mood, cognition, or stress levels, which are relevant for evaluating the benefits of BWE. HRV and BFS are only physiological indicators that may not reflect the subjective experiences or functional outcomes of BWE. Additional studies will be conducted to address all these limitations.

FINAL CONSIDERATIONS

In healthy individuals, cardiac oscillations are synchronous with respiratory frequency. The present study showed an increased LF power of HRV during guided SDB in BWE, which might be indicative of the mechanical effect of respiration. Simultaneously, the increased time-domain parameters and decreased HF power generate transient (in 5 participants), rapid excitation of cardiovascular autonomic centers due to respiratory modulation. These may be vagally mediated and predominantly caused by central non-baroreflex mechanisms. Transient and rapid excitation of the cardiovascular system during BWE suggest SDB may serve as a physiological method to draw upon cardio-vagal reserve. This shows that this may have beneficial effects on cardiovascular autonomic regulation in health and in various cardiovascular diseases.

In Sum, SDB during BWE induced a relaxed (increased alpha waves) and focused (increased gamma activity) state. The subjects were more alert, awake, aroused but relaxed (increase alpha waves), but attentive.

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CONFLICTS OF INTEREST

The authors declare no competing interests and received no funding for the purpose of carrying out this project.

AUTHOR CONTRIBUTION

V. Malhotra, D. Javed, A. Sampath: The contributions of the first three authors were equal and can be considered co-first authors.

Francisco José Cidral-Filho, Nathalia Nahas Donatello, Patrick Porter: helped to write this manuscript.

Roshan Sutar, Santosh Wakode, Brijesh Kumar, Priyanka Kashyap, Rekha Jivne: conducted the trial.

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