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Inclusion of a Rest Period in Diaphragmatic Breathing Increases High Frequency Heart Rate Variability: Implications for Behavioral Therapy

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Abstract

Heart rate variability (HRV) is associated with positive physiological and psychological effects. HRV is affected by breathing parameters, yet, debate remains regarding the best breathing interventions for strengthening HRV. The objective of the current study was to test whether the inclusion of a post-exhalation rest period was effective at increasing HRV, while controlling for breathing rate. A within-subjects crossover design was used with 40 participants who were assigned randomly to a breathing pattern including a post-exhalation rest period or a breathing pattern that omitted the post exhalation rest period. Participants completed training on each breathing pattern, practiced for six minutes, and sat quietly during a five-minute washout period between practices. Participants were given instructions for diaphragmatic breathing (DB) at a pace of six breaths/minute with or without a post-exhalation rest period. Recordings of heart rate, breathing rate, HF-HRV, RMSSD, LF-HRV, SDNN were collected before and during each of the breathing trials. HRV indices were derived from Lead one ECG recordings. Pairwise contrasts showed inclusion of a post-exhalation rest period significantly decreased heart rate ($p < .001$) and increased HF-HRV ($p < .05$). No differences were found for breathing rates ($p > .05$), RMSSD ($p > .05$), and SDNN ($p > .05$). Results indicted omission of the post-exhalation rest period resulted in higher LF-HRV ($p < .05$). A post-exhalation rest period improves HF-HRV, commonly associated with self-regulatory control, yet a post-exhalation rest period's importance requires further exploration.

Introduction

Slow-paced diaphragmatic breathing is an important component of behavioral interventions for a broad spectrum of symptoms including pain, anxiety, and motion sickness (Ferreira et al., 2013; Fried & Grimaldi, 1993; Lehrer & Gevirtz, 2014; Litchfield, 2003; Russell,

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Hoffman, Stromberg, & Carlson, 2014; Sauer, Burris, & Carlson, 2010; Zucker, Samuelson, Muench, Greenberg, & Gevirtz, 2009). Manipulation of breathing through behavioral interventions strengthens autonomic reflex efficiency and promotes appropriate behavioral or emotional responses (Appelhans & Luecken, 2006; Gevirtz, 2013; Porges, 2007). Additionally, breathing training increases self-regulation of physiological, affective, and cognitive processes associated with problematic behaviors such as impulsiveness or failure to plan (Brown, Gerbarg, & Muench, 2013; Litchfield, 2003; Porges, 2007; Thayer, Hansen, Saus-Rose, & Johnsen, 2009; Thayer & Lane, 2009). Taken together, these findings suggest breathing training is an important component of behavioral therapies.

The efficacy of breathing control in behavioral therapies may be partly due to its broad psychophysiological effects (Brown et al., 2013; Fried & Grimaldi, 1993; p. 193–227). When breathing treatments are employed, breathing frequency, respiration mechanics, tidal volume, and inhalation-exhalation ratios are important considerations (Porges, 2007). Each exerts significant effects on cardiac functioning quantified as high frequency heart rate variability (HF-HRV; 0.15-0.4 Hz). HF-HRV is a naturally occurring variation in the heartbeat-to-heartbeat time interval and is positively associated with cardiac health, autonomic balance, self-regulation, task performance, and negatively associated with chronic muscle pain, anxiety, depression, hypertension, and nausea (Lehrer et al., 2010; Lehrer & Gevirtz, 2014; Litchfield, 2003; Nazarewicz, Verdejo- Garcia, & Giummarra, 2015; Russell et al., 2014; Sargunraj et al., 1996). Cumulatively, these studies indicate slow-paced diaphragmatic breathing may improve psychological functioning by altering HF-HRV.

Despite the benefits of HF-HRV, how to maximize HF-HRV during breathing training is not well understood. A series of studies by Lehrer and colleagues indicated breathing at a rate of 5.5 breaths/minute (or ~0.092 Hz) with diaphragmatic breathing mechanics might be optimal for increasing HF-HRV (Lehrer et al., 2000; Vaschillo, Lehrer, Rishe, & Konstantinov, 2002). The work of Lehrer and colleagues suggested HRV biofeedback allows individuals to synchronize respiration with the naturally corresponding increases and decreases of heart rate through the baroreflex. The baroreflex rapidly modulates blood pressure through control of heart rate and contractility of blood vessels to maintain a balanced blood pressure and is thought to occur at a frequency of 0.1 Hz, which translates to approximately six breaths/minute (Hall & Guyton, 2011, p. 205–208). Thus, evidence suggests that paced breathing at 5.5 breaths/minute produces the maximum effect on HF-HRV (Lehrer & Gevirtz, 2013).

Paced breathing of six or 5.5 breaths/minute, however, can be achieved through various methods. Inhalation for three seconds and exhalation for 2.5 seconds yields the same breathing frequency as an inhalation for two seconds and exhalation for 3.5 seconds. The optimal balance of inhalation and exhalation is the topic of scientific inquiry (Fried & Grimaldi, 1993, p. 129; Lin, Tai, & Fan, 2014; Strauss-Blasche, et al., 2000; Van Diest et al., 2014). Our data suggest a 4-2-4 paced diaphragmatic breathing cycle of inhale-exhale-rest, rather than a 5–5 (inhale-exhale), maximizes the effects of respiration on HF-HRV (Kniffin et al., 2014; Russell et al., 2014; Stromberg, Russell, & Carlson, 2015).

Although HF-HRV may be the most frequently examined quantification of HRV, there are at least three additional indices. The first is the root mean square of the successive differences between adjacent heartbeats (RMSSD; Task Force of the European Society of Cardiology, 1996). The second is low frequency HRV (LF-HRV; $\sim 0.04\text{--}0.15$ Hz). LF-HRV reflects the influence of the baroreflex on cardiac activity and is thought to reflect the balance between the sympathetic and parasympathetic systems (Berntson et al., 1997). LF-HRV becomes especially important if an individual's breathing rate falls out of the HF-HRV range (9–24 breaths/minute) and into the LF-HRV range (2.4 to 9 breaths/minute). The third is the standard deviation of normal R-R intervals (SDNN; Berntson et al., 1997). SDNN reflects all cyclical components of HRV and is used in 24-hour recordings (Task Force, 1996). Yet, little data exist directly comparing the effectiveness of different respiratory cycles on increasing HF-HRV or the impact on other HRV indices such as RMSSD, SDNN, LF-HRV, or LF/HF ratios.

Using a within-subjects crossover design, the current study examined the potential advantages of a 4-2-4 breathing cycle (inhale-exhale-*rest*) above the commonly practiced inhale-exhale approach to strengthen HF-HRV and RMSSD. It was expected that the 4-2-4 breathing cycle would exert a greater effect on cardiac parameter than the 5-5 breathing cycle. The impact of breathing cycle on HRV indices of LF-HRV, LF/HF ratios, and SDNN were also explored, as were associations with respiration rate, heart rate, the number of interval differences of successive N to N intervals or heartbeat intervals greater than 50ms divided by the total number of NN intervals yielding a percentage (pNN50), inter-beat-interval (IBI), and abdominal respiration amplitude measured in volts (ARA).

Method

Participants

Forty undergraduate students from the University of Kentucky were recruited. Participants were ruled out if they reported a history of asthma, cardiovascular disease such as hypertension, gastrointestinal disorders, panic disorder, or anxiety disorders on a study pre-screening questionnaire. Participants were self-selected volunteers who were screened for eligibility through an online screening questionnaire. They were provided course credit for their participation in the study. The University of Kentucky Institutional Review Board for the Protection of Human Participants approved this research protocol.

Procedures

Participants were provided with general information about the study and invited to sign up through the University of Kentucky's online system. Upon arrival at the lab, interested participants were scheduled and given informed consent. Participants were placed in a comfortable cushioned chair in an upright position for the duration of the experiment. Following consent, participants completed brief demographics questionnaires and baseline measurement of breathing rate and heart rate were taken. Electrodes to gather heart rate data were attached in a Lead One configuration with active leads on the right and left wrists and the ground lead on the right ankle. All HRV indices were calculated from heart rate recordings. Respiration was measured with a strain gauge placed around the abdomen just

above the navel. Five minutes of baseline physiological variables were collected as the participant was asked to sit quietly without talking and minimal movement.

A within-subjects crossover design was used (see Figure 1). Participants were randomly assigned to receive training on the 4-2-4 breathing cycle first or the 5-5 breathing cycle. Before beginning their breathing practice both conditions were provided approximately six minutes of audio directions on diaphragmatic breathing and the condition specific breathing pattern. Participants in the 4-2-4 condition received training on inhaling for 4 seconds, exhaling for 2 seconds, and resting for 4 seconds. Participants in the 5-5 condition were trained to inhale for 5 seconds and exhale for 5 seconds without a rest period. Prior to each trial, participants were given the opportunity to ask questions.

During both the 4-2-4 and 5-5 breathing tasks, participants were asked to follow a visual cue presented on a computer screen to aid with pacing their breathing rate. The visual cue consisted of an oval that expanded (inhale), contracted (exhale), or remained still (rest). The rest period was only presented in the 4-2-4 condition. The visual cue also included a soft tone corresponding with the initial moment of the inhalation orienting participants to the oval beginning to expand. Participants practiced the breathing cycle for six minutes as physiological recordings were collected. Following the six-minute practice period, they were asked to sit quietly without talking or moving for five minutes to serve as a washout period. Following the washout period, participants switched breathing cycles (4-2-4 to 5-5 or from 5-5 to 4-2-4).

Materials

Demographic information—Participants were asked age, year in school, and ethnicity.

Participant feedback—Participants were asked which of the two breathing cycles was easiest to follow and which of the two breathing methods made them feel the most relaxed. Items were dichotomous and forced choice.

High frequency heart rate variability (HF-HRV) and heart rate—High frequency HRV (HF-HRV) was operationalized as spectral power occurring between 0.15-0.40 Hz based on the recommendations of the 1997 HRV Committee Report (Berntson et al., 1997). Although there is a significant debate regarding the need to covary the effects of respiration, “In general, the literature does not provide evidence that findings change when the amplitude of respiratory sinus arrhythmia is adjusted for respiration parameters” (Denver, Reed, & Porges, 2007, p. 288). Thus, the effects of respiration termed respiratory sinus arrhythmia were not covaried for HF-HRV (0.15-0.4 Hz; 9-24 breaths/minute) or LF-HRV (0.04-0.15 Hz; 2.4-9 breaths/minute). Heart function was recorded with three Ag/AgCl electrodes using shielded leads connected to BioPac ECG100C electrocardiogram amplifier module. Sampling rate for heart function was set to 2000 samples/second. Data were stored using Acq-knowledge software (Biopac, Santa Barbara, CA) and analyzed with the MindWare data analysis program version 3.1 (MindWare, Gahana, OH).

Root mean square of the successive differences (RMSSD)—When examining the effects of breathing on cardiac functioning it is important to consider the frequency of

breathing. As long as minute-by-minute breathing rates remain within the range of ~9 to 24 breaths, HF-HRV accurately assesses the effects of breathing on cardiac functioning. Within the range of 9 to 24 breaths/minute, HF-HRV values serve as an indicator of the parasympathetic tone within the body and index self-regulatory ability. However, if an individual's breathing rate falls below or above the range of 9 to 24 breaths/minute, the time domain measure of RMSSD should be considered as an assessment of parasympathetic tone within the body. According to the published standards (Berntson, et al., 1997; Task Force, 1996) RMSSD is an appropriate measure of parasympathetic tone if there are at least five minutes of heart rate data.

Low frequency heart rate variability (LF-HRV)—Low frequency HRV occurs in the range of 0.04-0.15 Hz and is often associated with influence of the baroreflex that regulates blood pressure given changes in heart rate (Berntson et al., 1997; Task Force et al., 1996). It is thought that LF-HRV is influenced by both autonomic nervous system branches of the making its interpretation difficult. Although the interpretation is complicated, LF-HRV is important to consider when respiration rates are between 2.4 to 9 breaths/minute (Moak et al., 2007).

Additional HRV indices—The standard deviation of NN intervals (**SDNN**) is considered a measure of overall HRV and is linked to general health (Berntson et al., 1997). SDNN takes into account the effects of both sympathetic tone and parasympathetic tone and indicates fluctuations of the two systems change over the course of 24 hours. Although SDNN is suggested for 24-hour heart recordings it provides an index of parasympathetic versus sympathetic balance and was therefore included. In order to provide a complete picture of the effects of the breathing cycle, we have also included: heart rate, the inter-beat interval between R-peaks in milliseconds, very low frequency that ranges from 0.0033 to 0.04 Hz and is not well defined, pNN50 which is the number of interval differences of successive N to N intervals or heartbeat intervals greater than 50ms divided by the total number of NN intervals yielding a percentage. Although these HRV indices vary in the level of empirical evidence clarifying their utility, each provides a unique vantage point for the effects of respiration on cardiac functioning. Therefore, we believe it advantageous to present a complete picture.

Breathing rate—Breathing rate was recorded using the BSL-SS5LB respiratory effort transducer and amplifier module for the BioPac MP100 system. The sensor was placed around the abdomen below the rib cage and an inch above the navel. Sampling rate for breathing rate was set to 2000 samples/second. Breathing rates were recorded as mean breaths/minute.

Abdominal respiration amplitude (ARA)—Abdominal movement was measured as change in tension on the abdominal pressure gauge and recorded as volts. ARA was measured with the same equipment as breathing rate and had a sampling rate set to 2000 samples/second. ARA serves as a mean measurement of abdominal displacement during each of the experiment's physiological recording stages. Therefore, ARA is used to indicate

mean diaphragm muscle engagement and estimate mean volume displacement during a breathing cycle.

HF-HRV Cleaning Procedures

ECG data were visually examined in 60-second tachograms without stage identifiers to avoid experimenter bias during cleaning. Missing or erroneous heartbeats were cleaned according to the recommendations made by the Task Force and then expanded by the 1997 Committee Report (Berntson et al., 1997, p. 631; Task Force, 1996). Specifically, movement or aberrant heartbeats were visually examined and adjusted for by either measuring the actual R-R interval or interpolating the missing heartbeat. Movement artifacts where the heartbeats were not clearly indicated were corrected with a mid-beat replacement. Double marked R-peaks and erroneous R-peaks were removed. Based on recommendation from Mindware Technologies, epochs with over 10% erroneous beats or that did not have at least 30 consecutive seconds of measurable data were not included in calculations. After initial visual inspection of files, three baseline files were excluded and one participant's breath cycle file were excluded from analysis due to the significant number of artifacts and the researcher's inability to visually identify R-peaks within the ECG recordings.

Statistical Analyses Plan

In order to calculate the necessary sample size (Faul, Erdfelder, Buchner, & Lang, 2009), G*Power software was employed. Based upon previous research, it was determined that the effect size for this study was likely to be medium to large (Russell et al., 2014). It was determined that a sample size of 40 participants allowed for an 81% power with, $\alpha=.05$. All measures met assumptions of normality except abdominal respiration amplitude. Failing to adjust for non-normal distribution with transformations, seven participants were determined to be significant outliers (greater than 2 SD) and were excluded from analyses. In addition, three baseline and one 5-5 breath cycle physiological recording were excluded due to significant movement artifacts. No other data were excluded and no transformations were made.

Data were analyzed with SPSS version 22. First, bivariate correlations among HF-HRV, breaths/minute, heart rate, LF-HRV, RMSSD, SDNN, inter-beat interval, abdominal respiration amplitude, age, and sex at baseline were computed. Because of expected correlations between these outcomes, repeated measures multivariate analyses of variance (MANOVAs) were conducted to investigate within-subject differences between the three breathing stages (baseline, 4-2-4, 5-5). For these analyses, order was treated as a between-subject effect and breathing stage was the repeated measure, or within-subject effect. Within-subjects designs maximize power because each person effectively serves as her/his own control; that is, participants are compared to themselves in each stage. Greenhouse-Geisser correction was used to control for violation of sphericity in all MANOVA analyses. Pairwise contrasts were used to investigate significant omnibus tests. To control for the large number of comparisons, the Holm-Bonferroni method was used. This method is more precise than the overly-conservative Bonferroni method, and is considered the most appropriate method to use when dependent variables are correlated with each other. For details of the Holm-Bonferroni method, see Aickin and Gensler (1996).

Results

Demographics, Mean Differences, and Bivariate Correlations

The participant sample was 70% Caucasian, 12.5% African-American, 10% Hispanic, and 7.5% mixed race or other. The average age of the sample was 18.9 years ($SD = 1.00$) and 32 participants were female. Bivariate correlations among outcome measures at baseline are presented in Table 1. Means and SDs of study outcomes by breathing condition are presented in Table 2.

Training Feedback Ratings

Participants were asked which breathing pattern was easiest to follow and most relaxing. Sixty-eight percent of participants rated the 4-2-4 breath cycle as the easiest to follow. In addition, sixty-three percent of participants rated the 4-2-4 breath cycle as the most relaxing.

Effects of 4-2-4 vs. 5-5 Breathing Method on Physiological Functioning

A repeated measures MANOVA was used to test if breathing stage influenced physiological functioning; specifically, breaths/minute (BPM), heart rate (HR), HF-HRV, RMSSD, LF-HRV, SDNN, LF/HF ratio, VLF-HRV, pNN50, inter-beat interval (IBI), and abdominal respiration amplitude (ARA). Omnibus tests indicated no significant interactions between order (between subject) and breathing stage (within-subject) on any of the 11 physiological outcomes, $p's > .05$. Additionally, there were no significant order effects on any physiological variables. However, as hypothesized, omnibus tests revealed a significant main effect of breathing stage on BPM, HR, HF-HRV, RMSSD, SDNN, LF-HRV, LF/HF ratio, IBI, and ARA. Omnibus test values are presented in Table 3.

Pairwise contrasts further probed significant stage effects with results listed in Table 4. Multiple comparisons were controlled with the Holm-Bonferroni corrections. For breathing rate, no differences were found between the 4-2-4 or 5-5 stages but both stages breathing rates were lower than baseline. Heart rate decreased from baseline in both the 4-2-4 and 5-5 stages with the 4-2-4 stage being lower than the 5-5 stage. HF-HRV was higher for the 4-2-4 stage than the 5-5 stage, trending towards being higher than the baseline stage, and results indicated no difference between baseline and the 5-5 stage. RMSSD and SDNN results indicated increases from baseline for both stages but no differences between stages. Of note, RMSSD and SDNN were higher during the 5-5 stage than the 4-2-4 stage. LF-HRV increased from baseline to both stages with the 5-5 stage trending towards being significantly higher than the 4-2-4 stage. IBI increased from baseline to both stages with the 4-2-4 stage having higher IBI values than the 5-5 stage. Finally, pairwise contrasts indicated increases in ARA from baseline to both breathing stages with the 5-5 stage having significantly higher abdominal respiration amplitude values. For a summary of the primary results comparing the effects of each stage on HF-HRV, please see Figure 2.

Discussion

As predicted, results supported the inclusion of a post exhalation rest period for behavioral interventions targeting HF-HRV and improvement of self-regulatory ability. When including

a post inhalation rest period (4-2-4), participants achieved higher HF-HRV tone than without (5-5). In addition, the majority of participants reported the 4-2-4 breathing pace was easier to follow and more relaxing than the 5-5 breathing pace. For self-regulation protocols targeting HF-HRV, the present results highlight the potential importance for breathing entrainment programs to include a post exhalation *rest* period in the breathing cycle.

Previous studies validated the use of slow-paced breathing to increase HF-HRV tone (Courtney, Cohen, & van Dixhoorn, 2011; Henriques, Keffer, Abrahamson, & Horst, 2011; Lehrer et al. 2000; Patron et al., 2013; Prinsloo, Derman, Lambert, & Rauch, 2013; Song & Lehrer, 2003; Strauss-Blasche, et al., 2000; Wang et al., 2010; Whited, 2014). Each program, however, uses a rhythmic breathing cycle limited to inhalation and exhalation. Yet, the present data suggest the gains in HF-HRV through manipulation of breathing can be exploited to a greater degree through inclusion of a *rest* period in the breathing cycle. The 4-2-4 pattern (inhalation-exhalation-*rest*) increased the length between inhalations associated with increased vagal influence on cardiac activity and greater parasympathetic tone without adverse effects. This is consistent with previous work demonstrating the current protocol is a reliable method to strengthen HF-HRV, parasympathetic tone, and improve performance on a variety of in lab stressor tasks (Kniffin et al., 2014; Russell et al., 2014; Stromberg et al., 2015). Furthermore, the use of rest periods avoids unnecessary strain on muscles of respiration accompanying breathing protocols advocating prolonged exhalations or potential hyperventilation from protocols advocating prolonged inhalations.

Although significant differences were found in HF-HRV between the 5-5 and the 4-2-4 conditions, no differences were found between breathing cycles for RMSSD, SDNN, and LF-HRV. Despite a within-subjects crossover design, interpretation is potentially complicated by the potential influence of age, physical conditioning, depth of respiration, possible cardiac medication use, and variable respiratory frequencies (Berntson, Lozano, & Chen, 2005). The impact of variable respiratory frequencies was explored by limiting analyses to participants whose breaths/minute remained within the LF range of 2.4-9 breaths/minute. No differences in results were found. Interpretation may be further complicated by participants' depth of respiration, which was not controlled. Although participants with cardiovascular diseases were excluded from participation and potential effects are controlled for with the within-subjects design, it may be important to record the use of cardiovascular. In addition, as participants' breathing slowed to 0.1 Hz or ~6 during both breathing cycles, LF-HRV interpretations become conflated by the combined influence of vagal and sympathetic systems (Berntson et al., 1997). Also, as RMSSD estimates can be biased by differences in heart rate and appreciable contributions from LF sympathetic cardiac control, especially when comparing across conditions, delineating the impact of breathing cycles becomes challenging (Berntson, Lozano, & Chen, 2005).

Of additional interest is the non-significant change in HF-HRV values from baseline to the 5-5 breathing cycle despite a significant drop in breathing rate from approximately 15 breaths/minute to approximately seven breaths/minute. The data were reviewed carefully to insure these results were accurately interpreted. Given the healthy nature of the participants, the 5-5 breathing cycle intervention in this study perhaps did not exert enough influence on short-term measurements of HF-HRV for this sample. Moreover, participants within the 5-5

breathing cycle had significantly higher abdominal respiration amplitude values than in the 4-2-4 breathing cycle; such an outcome may have altered arterial CO² levels enough to stimulate sympathetic activity diminishing HF-HRV for that group. Without the use of a *rest* period in the breathing cycle, participants may have experienced slight over-breathing due to the 5-second inhalation period triggering an increase in sympathetic tone and a subsequent reduction in HF-HRV. However, without measurement of end tidal CO² values to index arterial CO² levels the outcomes observed are not fully explainable. Future studies should look at this issue more carefully.

Although the diaphragm is considered important when teaching slow-paced breathing, the influences of thoracic (chest) versus diaphragmatic (abdominal) muscles should be further explored. Because breathing data were collected from a single pressure belt placed around the abdomen, the use of thoracic muscles versus abdominal muscles and the importance of tidal volume can only be hypothesized in the present study. Notably, results indicated that participants found the 4-2-4 breathing cycle to be easier to follow and more relaxing than the 5-5 breathing cycle. These data suggest individuals may be more motivated to use the 4-2-4 breathing cycle because the physiological effects are more noticeable. The importance of “diaphragmatic” breathing remains speculative until validated within a more clinical population.

To date, the training protocol has only demonstrated effectiveness in predominately healthy samples of young female adults. Although the training protocol has significantly increased HF-HRV tone for over 300 participants in other published projects, future studies should test the training protocol with a wider array of individuals and in clinical settings characterized by low resting HF-HRV tone (Hallman & Lyskov, 2012; Kang, Chen, Chen, & Jaw, 2012; Solberg Nes, Carlson, Crofford, de Leeuw, & Segerstrom, 2010; Schmidt & Carlson, 2009; Zucker et al., 2009). Given that higher HF-HRV is considered a protective factor for a variety of health conditions including chronic pain, continued exploration of breathing entrainment programs for self-management of such symptoms is warranted. In addition, the long-term effectiveness of the training protocol on baseline levels of HF-HRV has yet to be established.

The role of breathing training in health interventions and the ability of behavioral health specialists to alter breathing is important. An individual’s ability to volitionally alter breathing parameters and within minutes also alter HF-HRV tone is a powerful tool for the behavioral health specialist to exploit. With breathing interventions being relatively rapid interventions to implement and also demonstrating a wide range of positive clinical outcomes, breathing interventions warrant closer consideration from healthcare professionals. For breathing interventions focused on increasing HF-HRV tone, the present results demonstrated the importance of including a *rest* period into the traditional breath cycle.

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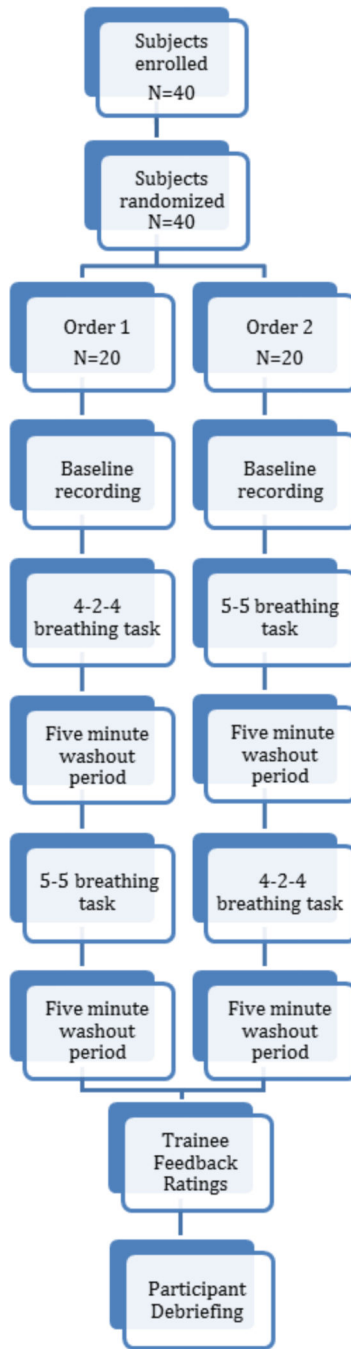


Figure 1.
Study Flow Chart.

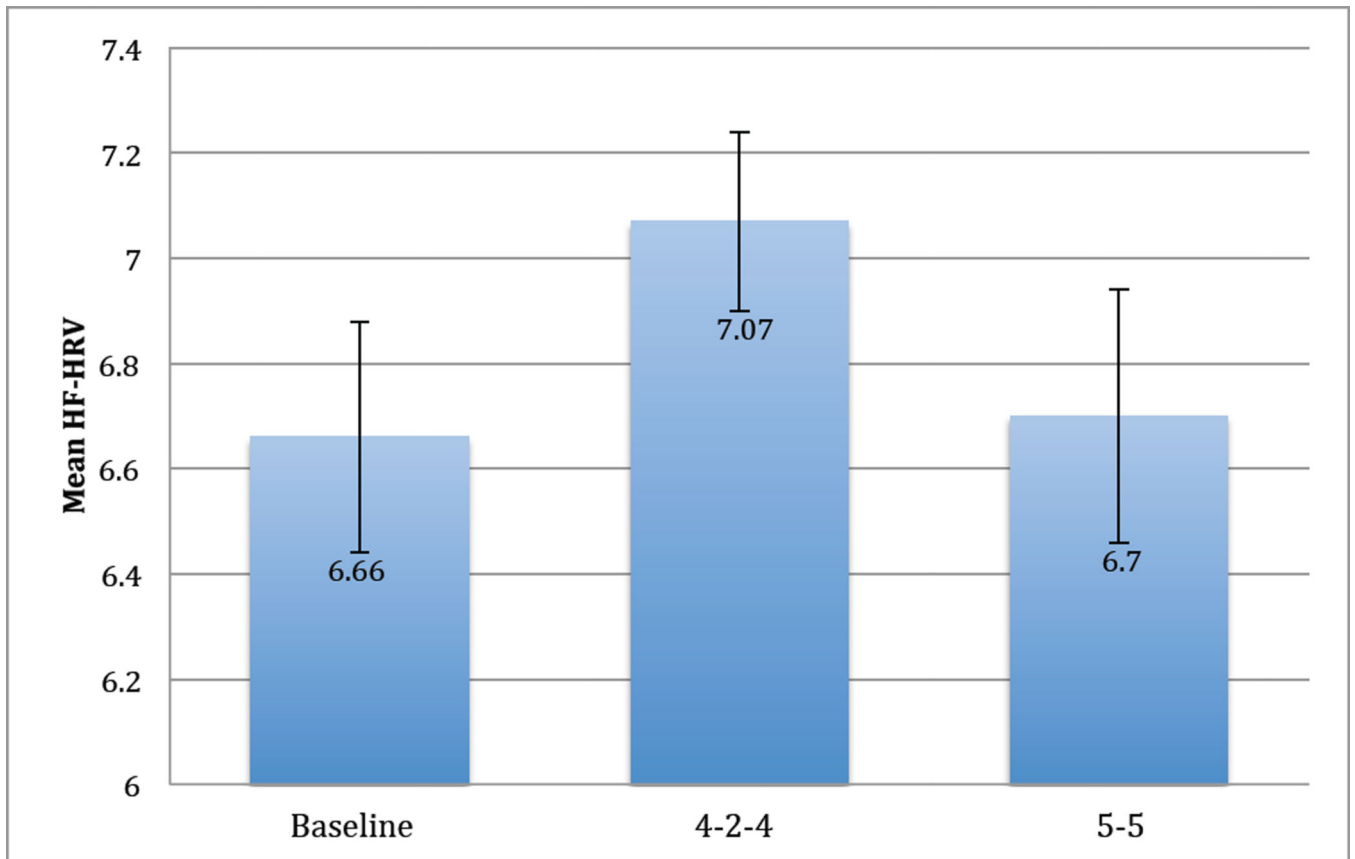


Figure 2.

Repeated Measures MANOVA Test of HF-HRV Differences. High frequency heart rate variability (HF-HRV)*. Cohen's d value represents the difference between the 4-2-4 training and 5-5 training, $d = .32$

* HF-HRV stage means presented in Figure 2 are not consistent with HF-HRV stage means presented in Table 2 due to missing data for the Repeated Measures MANOVA analyses. For further detail, please see Statistical Analyses Plan.

Table 1

Bivariate Correlations among Outcomes at Baseline

Variable	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
1. HF-HRV	-	-.53**	-.70**	.83**	.86**	.66**	-.72**	.62**	.84**	.67**	.40	.01	-.15
2. BPM		-	.30	-.18	-.29	-.09	.61**	-.10	-.06	-.29	.16	-.39*	.11
3. HR			-	-.66**	-.64**	-.47**	.38*	-.42*	-.58**	-.97**	-.22	.00	-.05
4. RMSSD				-	.93**	.78**	-.50**	.70**	.93**	.70**	.02	-.10	.00
5. SDNN					-	.83**	-.45**	.83**	.86**	.66**	.07	-.05	.00
6. LF-HRV						-	-.10	.71**	.63**	.49**	.04	-.09	.02
7. LF/HF Ratio							-	-.26	-.47**	-.38*	-.04	-.03	.18
8. VLF-HRV								-	.62**	.43**	.12	.08	.13
9. pNN50									-	.60**	-.06	-.14	.00
10. IBI										-	.22	.03	.19
11. ARA											-	-.12	.30
12. Age												-	.11
13. Sex													-

Note: High frequency heart rate variability (HF-HRV; 0.15 Hz to 0.4 Hz). Respiration rate was measured in breaths-per-minute (BPM). Heart rate measured as the number of beats in 60 seconds (HR). Root mean squared of the successive differences between adjacent N-to-N peaks measured in ms (RMSSD). Standard deviation in N-to-N interval measured in ms (SDNN). Low frequency heart rate variability measured in ms² (LF-HRV). Low frequency to high frequency ratio measured in ms² (LF/HF Ratio). Very low frequency heart rate variability measured in ms² (VLF-HRV). The number of interval differences of successive N to N intervals or heartbeat intervals greater than 50ms divided by the total number of NN intervals yielding a percentage (pNN50). Inter-beat-interval measured in ms (IBI). Abdominal Respiration Amplitude measured in volts (ARA).

** p<.01 two-tailed.

* p<.05 two-tailed.

Table 2

Assessment of Physiological Measures

Variable	Baseline		4-2-4 Stage		5-5 Stage	
	Mean	SD	Mean	SD	Mean	SD
HF-HRV	6.68	1.19	6.98	0.99	6.67	1.29
BPM	15.25	3.19	6.61	1.61	6.57	1.44
HR	75.15	11.91	71.26	10.24	72.60	10.08
RMSSD	52.16	30.39	65.35	34.57	66.23	38.36
SDNN	61.10	26.70	94.07	38.76	99.40	45.44
LF-HRV	981.47	666.42	5573.39	4605.47	6593.45	5618.72
LF/HF Ratio	1.27	1.00	4.47	3.13	7.51	5.35
VLF-HRV	223.08	240.13	139.79	95.51	191.95	176.12
pNN50	30.32	18.91	30.83	14.27	30.05	16.87
IBI	816.92	120.29	858.04	113.64	841.03	104.85
ARA	7.02	7.56	16.02	21.01	27.95	31.59

Note: High frequency heart rate variability (HF-HRV). Respiration rate was measured in breaths-per-minute (BPM). Heart rate measured as the number of beats in 60 seconds (HR). Root mean squared of the successive differences between adjacent N-to-N peaks measured in ms (RMSSD). Standard deviation in N-to-N interval measured in ms (SDNN). Low frequency heart rate variability measured in ms² (LF-HRV). Low frequency to high frequency ratio measured in ms² (LF/HF Ratio). Very low frequency heart rate variability measured in ms² (VLF-HRV). The number of interval differences of successive N to N intervals or heartbeat intervals greater than 50ms divided by the total number of NN intervals yielding a percentage (pNN50). Inter-beat interval measured in ms (IBI). Abdominal Respiration Amplitude measured in volts (ARA).

Table 3

Omnibus Tests of Within Subject Effects

Dependent Measures	<i>F</i> (df)	<i>P</i>	<i>n</i> ²	Post-hoc Power
HF-HRV	3.51 (2, 68)	< .05	.09	0.57
BPM	218.23 (2, 68)	< .001	.87	1.00
HR	21.66 (2, 68)	< .001	.39	1.00
RMSSD	10.21 (2, 68)	< .001	.23	0.98
SDNN	50.80 (2, 68)	< .001	.60	1.00
LF-HRV	39.72 (2, 68)	< .001	.54	1.00
LF/HF Ratio	36.87 (2, 68)	< .001	.52	1.00
VLF-HRV	3.12 (2, 68)	> .05	.08	0.53
pNN50	0.69 (2, 64)	> .05	.008	0.08
IBI	17.25 (2, 68)	< .001	.34	1.00
ARA	13.02 (2, 54)	< .001	.33	1.00

Note: Omnibus tests indicated no significant order effects, all p 's > .05. Omnibus tests indicated significant stage (baseline, 4-2-4, and 5-5) effects and are represented here. High frequency heart rate variability (HF-HRV). Respiration rate was measured in breaths-per-minute (BPM). Heart rate measured as the number of beats in 60 seconds (HR). Root mean squared of the successive differences between adjacent N-to-N peaks measured in ms (RMSSD). Standard deviation in N-to-N interval measured in ms (SDNN). Low frequency heart rate variability measured in ms² (LF-HRV). Low frequency to high frequency ratio measured in ms² (LF/HF Ratio). Very low frequency heart rate variability measured in ms² (VLF-HRV). The number of interval differences of successive N to N intervals or heartbeat intervals greater than 50ms divided by the total number of NN intervals yielding a percentage (pNN50). Inter-beat-interval measured in ms (IBI). Abdominal Respiration Amplitude measured in volts (ARA).

Table 4

Pairwise Contrasts Between Baseline, 4-2-4, and 5-5 Breathing Stages

Variable	Stage	Comparison Stage	Mean Difference (Stage -Comparison)	S.E.	<i>p</i> [±]
HF-HRV	Baseline	4-2-4	-0.33	0.13	.016
	Baseline	5-5	0.04	0.18	.843
	4-2-4	5-5	0.36	0.12	.006
BPM	Baseline	4-2-4	3.79	0.64	< .001
	Baseline	5-5	8.78	0.55	< .001
	4-2-4	5-5	0.08	0.17	.64
HR	Baseline	4-2-4	3.79	0.64	< .001
	Baseline	5-5	2.14	0.67	< .001
	4-2-4	5-5	-1.65	0.28	< .001
RMSSD	Baseline	4-2-4	-12.90	2.91	< .001
	Baseline	5-5	-12.70	4.02	.004
	4-2-4	5-5	0.20	2.87	.945
SDNN	Baseline	4-2-4	-33.58	4.35	< .001
	Baseline	5-5	-37.00	5.08	< .001
	4-2-4	5-5	-3.42	2.47	.175
LF-HRV	Baseline	4-2-4	-4773.52	823.16	< .001
	Baseline	5-5	-5547.25	1005.39	< .001
	4-2-4	5-5	-773.73	385.10	.03
IBI	Baseline	4-2-4	-40.60	7.89	< .001
	Baseline	5-5	-19.47	8.14	.023
	4-2-4	5-5	21.13	3.73	< .001
ARA	Baseline	4-2-4	-9.10	3.31	.03
	Baseline	5-5	-20.98	5.16	< .001
	4-2-4	5-5	-11.88	2.68	< .001

Note:

[±]Using the Holm-Bonferroni correction for multiple comparisons, only *p*-values smaller than .011 are considered significant and are **bolded**. Pairwise contrasts were used to investigate between stage differences. High frequency heart rate variability (HF-HRV). Respiration rate was measured in breaths-per-minute (BPM). Heart rate measured as the number of beats in 60 seconds (HR). Root mean squared of the successive differences between adjacent N-to-N peaks measured in ms (RMSSD). Standard deviation in N-to-N interval measured in ms (SDNN). Low frequency heart rate variability

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measured in ms^2 (LF-HRV). Low frequency to high frequency ratio measured in ms^2 (LF/HF Ratio). Very low frequency heart rate variability measured in ms^2 (VLF-HRV). The number of interval differences of successive N to N intervals or heartbeat intervals greater than 50ms divided by the total number of NN intervals yielding a percentage (pNN50). Inter-beat-interval measured in ms (IBI). Abdominal Respiration Amplitude measured in volts (ARA).