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Author contact at KU Leuven	ilse.vandiest@ppw.kuleuven.be

Inhalation/exhalation Ratio modulates the Effect of Slow Breathing on Heart Rate Variability and Relaxation

Ilse Van Diest°, Karen Verstappen°, André E. Aubert⁺, Devy Widjaja§, Debora Vansteenwegen°, Elke Vlemincx°

°Faculty of Psychology and Educational Science, KULeuven, Tiensestraat 102, B-3000 Leuven, Belgium

⁺Department of Cardiology, KULeuven, UZ Gasthuisberg O/N1 704, Herestraat 49, B-3000 Leuven, Belgium

§Department of Electrical Engineering (ESAT) – STADIUS, KU Leuven, Kasteelpark Arenberg 10, box 2446, B-3001 Leuven, Belgium

§Future Health Department, iMinds, Kasteelpark Arenberg 10, box 2446, B-3001 Leuven, Belgium

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Abstract

Slow breathing is widely applied to improve symptoms of hyperarousal, but it is unknown whether its beneficial effects relate to the reduction in respiration rate per se, or, to a lower inhalation/exhalation (i/e) ratio. The present study examined the effects of four ventilatory patterns on heart rate variability and self-reported dimensions of relaxation. Thirty participants were instructed to breathe at 6 or 12 breaths/minute, and with an i/e ratio of 0.42 or 2.33. Participants reported increased relaxation, stress reduction, mindfulness and positive energy when breathing with the low compared to the high i/e ratio. A lower compared to a higher respiration rate was associated only with an increased score on positive energy. A low i/e ratio was also associated with more power in the high frequency component of heart rate variability (HF-HRV), but only for the slow breathing pattern. Our results show that i/e ratio is an important modulator for the autonomic and subjective effects of instructed ventilatory patterns.

Keywords: relaxation, breathing, respiration, heart rate variability, RSA

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Throughout history and in very different cultures, people have searched for behavioral strategies that can be helpful in reducing symptoms associated with a variety of stress-related disorders and conditions, such as panic and anxiety, asthma, chronic pain, hypertension and cardiovascular complaints. One widely applied strategy, used both as a stand-alone or as a component of a more encompassing intervention, involves the voluntary modification of the breathing pattern (Cappo & Holmes, 1984). The cardiac and respiratory changes elicited by breathing instructions are often assumed to establish a relaxation response. Psychologically, breathing exercises typically induce an increased focus on internal sensations and a relative neglect of external stimuli (Vlemincx, Van Diest, De Peuter, & Van den Bergh, 2007). Physiologically, most breathing exercises aim to reduce sympathetic activity and to increase parasympathetic (or vagal) activity (Benson et al., in van Dixhoorn, 1998).

Vagal efferent outflow to the heart is commonly studied non-invasively by means of heart rate variability (HRV) analysis. The use of HRV measures is often inspired by the polyvagal theory of Porges (2007). From an evolutionary perspective, this author links emotional and stress-related disorders to a decreased vagal activity to the heart, which could be indexed by a reduced respiratory sinus arrhythmia (RSA, the rising heartbeat during inhalation and the decrease in heart rate that follows during exhalation; Grossman & Taylor, 2007). Both peripheral and central mechanisms as well as their interaction likely contribute to the phenomenon of RSA. Eckberg (2003) refers to a central oscillator that gates autonomic outflow at respiratory rhythms. A peripheral contribution of respiratory gating of vagal outflow to the heart comes from the phasic increases in intra-thoracic pressures caused by breathing, thereby phasically stimulating baroreceptors and concomitant autonomic outflow to the heart, leading to cardiac acceleration during inspiration and deceleration during expiration (Berntson, Cacciopo, & Quigley, 1993).

Some authors consider both the amplitude of the high frequency band (HF; 0.15-0.40 Hz) of heart rate variability (in the frequency domain) and RSA (in the time domain) to be valid indicators of vagal tone and of a good prefrontal inhibitory capacity allowing for the inhibition of subcortical sympatho-excitatory stress responses (Thayer & Brosschot, 2005; Thayer & Lane, 2009; Thayer et al., 2012). Higher HF power or RSA would correlate with a better capacity to adapt to the environment and induces a calm, but alert state (Brown & Gerbarg, 2005a). Heart rate variability is also a marker of cardiovascular health and autonomic homeostatic control (Lehrer, Sasaki, & Saito, 1999; Thayer & Brosschot, 2005). Since both HF-HRV and RSA are strongly dependent on the respiratory rate and the tidal volume (Ritz, Thöns, & Dahme, 2001*;* Sakakibara & Hayano, 1996), it is indeed conceivable that voluntary changes in the breathing pattern may alter efferent autonomic activity. As such, respiration may be an interesting interface to behaviorally change autonomic activity and stress responses.

Although most breathing techniques described in the literature involve a slowing down of the breathing pattern, there is quite some variation in the specifics of the applied techniques, as well as in the underlying rationale and the complaints they are thought to improve. Many breathing exercises originate from the yoga tradition. Often, the instruction of yoga breathing comprises a diaphragmatic breathing or “tanden breathing” (Lehrer et al., 1999), as well as a nasal inhalation combined with an exhalation through the mouth, which is slow and involves a pause between the inspiration and the expiration (Miles, 1964). According to yoga, the ideal breath rate is situated around six breaths per minute, with an exhalation that is twice as long as the inhalation (ratio 1:2). General yogic breathing is believed to stimulate a good mental health, as well as a state of calm alertness, mental focus and stress tolerance, by means of several mechanisms.

Interestingly, with a respiratory rate of about six breaths a minute, variations in heart rate that follow respiration shift from the HF to the low frequency band (LF; 0.04 - 15 Hz; Berntson et al., 1997; Grossman & Kollai, 1993). Also, RSA amplitude seems to reach a maximum at a respiration rate of about 6 breaths minute, likely because at that rate breathing occurs at a resonance frequency of the cardiovascular system, caused by the rhythm in heart rate produced by the baroreflex (spontaneous oscillations in blood pressure that are reflexively compensated for by changes in heart rate) in that individual (Giardino, Glenny, Borson, and Chan, 2003; Song and Lehrer, 2003). At resonance frequency, RSA and baroreflex effects mutually stimulate each other, causing very high oscillations in heart rate, thereby stimulating modulatory effects of both processes, and apparently increasing the power of modulatory processes in the cardiorespiratory system (Lehrer, Vaschillo and Vaschillo, 2000). Based on this phenomenon, Lehrer and colleagues have developed a particular form of HRV biofeedback training, called ‘Resonance frequency training’, in which people regularly exercise breathing at their particularly resonance frequency (Lehrer et al., 2000). Growing evidence indicates that the intervention is associated with beneficial clinical outcomes in a variety of disorders (hypertension, asthma, depression and chronic pain) and with an increased sensitivity of the baroreflex (e.g., Nolan et al., 2010; Wheat & Larkin, 2010: Lehrer et al., 2003; Lehrer et al., 2004).

Breathing retraining is also often applied to reduce symptoms of panic or anxiety. Typically, it implies adopting a diaphragmatic, slow, regular and shallow breathing pattern (Meuret, Wilhelm, & Roth, 2004). Meuret, Wilhelm, Ritz, and Roth (2008) introduced a new, brief, capnometry-assisted breathing retraining therapy (BRT), aimed at increasing self-monitored end-tidal pCO_2 (to reach or maintain a pCO_2 range higher than 35mmHg) and reducing respiration rate by means of breathing exercises. Such breathing retraining was generally found to reduce panic frequency and to normalise pCO_2 levels (Meuret et al., 2008; Salkovskis, Jones, & Clark, 1986). Han, Stegen, De Valck, Clément, and Van de Woestijne (1996) reported a significant change in the breathing pattern of patients with hyperventilation and/or anxiety disorders following a similar breathing retraining. Mean values of inspiration time (Ti), expiration time (Te) and tidal volume (Vt) were found to be increased (Vt/Ti decreased as a result), indicating that a prolonged breath was compensated for by an increase in tidal volume. A further analysis showed that the relief of the symptoms correlated mainly with a slowing down of the breathing rate.

Although breathing retraining is a popular and relatively simple stress intervention, a theoretical concern is that the therapeutic results may be achieved primarily by non-respiratory mechanisms (Meuret, Wilhelm, Ritz, & Roth, 2003). Hibbert and Chan (1989) proposed that placebo mechanisms may explain the beneficial effects of breathing retraining (Garssen, de Ruiter, & van Dyck, 1992). However, recent evidence does not support the idea that the reduction in panic symptoms in patients receiving respiratory training is mediated by non-specific factors, such as perceived control (Meuret, Rosenvield, Seidel, Sbaskara, & Hofmann, 2010).

Breathing techniques are also applied to treat cardiovascular complaints (Grossman, Grossman, Schein, Zimlichman, & Gavish, 2001; Pitzalis et al., 1998). E.g., a fda-approved intervention in reducing high blood pressure in hypertensives involves device-guided breathing exercises (see http:// www.resperate.com). With differentiated inspiration and expiration "sounds" the user is guided to lower the respiratory frequency to less than 10 breaths/min with prolonged expiration. Studies on the efficacy of this treatment (Grossman et al., 2001; Logtenberg, Kleefstra, Houweling, Groenier, & Bilo, 2007; Rosenthal, Alter, Peleg, & Gavish, 2001; Schein et al., 2009; Viskoper et al., 2003) reported significant reductions in systolic blood pressure. Van Dixhoorn (1998) instructed myocardial infarction patients to actively regulate respiration by making the passage of air audible for 5-6 breaths/min. This was achieved by breathing through slightly pursed lips and by employing slight movements of the body that facilitated the respiration. Again, it was found that the slower breathing pattern implemented in this intervention was associated with a significant decrease in heart rate and an increase in RSA (Lehrer et al., 1999; van Dixhoorn, 1998).

Thus, different breathing techniques are described in literature and applied to cope with a variety of stress-related conditions. The most common feature of these breathing exercises is a reduction in respiratory rate, which is assumed to increase parasympathetic activity. However, despite the wide application and likely effectiveness of such breathing techniques, some rather basic questions remain unsolved.

First, in contrast with the cardiovascular effects, the subjective effects of instructed slower breathing have been described rather poorly. Whereas some studies apply some sort of self-report on anxiety, avoidance, depression intensity (Clark, Salkovskis, & Chalkley, 1985; Hibbert & Chan, 1989) or on panic, asthma or hypertension complaints, they lack a measure specifically related to relaxation. Interestingly, Smith (2001) has listed different affective states of relaxation and published inventories to asses 19 relaxation states (R-States) that can be divided in four categories based on factor analytic research (Smith et al., 2000): basic relaxation, mindfulness, positive energy transcendence and stress. It is largely unknown how instructed breathing patterns affect these R-States and categories.

Second, it is unclear to which extent features of the breathing pattern other than respiratory rate contribute to the effects of slow breathing. In particular, the ratio between inspiration and expiration (i/e ratio) may be a relevant breathing parameter to look at. Several studies have documented that states of relaxation and stress induce decreases and increases in i/e ratio's, respectively (e.g., Boiten, 1998; Gomez, Stahel, & Danuser, 2004; Van Diest et al., 2001). In addition, the literature suggests that HF-HRV and RSA are greater when breathing with a low compared to a high i/e ratio (Porges, 2007; Strauss-Blasche et al., 2000). Although the latter phenomenon does not necessarily reflect an increased vagal tone (see Song & Lehrer, 2003 for an explanation in terms of a more complete hydrolization of acetylcholine at the SA-node), findings from Cappo and Holmes (1984) corroborate the idea that arousal is smaller with low compared to high i/e ratio's. In the latter study, participants breathed at 6 breaths/min during three different conditions: one condition with quick inhalation (2 sec) and slow exhalation (8 sec), one with slow inhalation (8 sec) and quick exhalation (2 sec) and a third condition with equal inspiratory and expiratory times (5 sec). The authors reported that “fast/slow breathing” reduced electrodermal activity and subjective arousal during anticipation of an electric shock (Cappo & Holmes, 1984). Together, the above mentioned findings suggest that the arousal reducing effect of slow breathing may be partly explained by the low i/e ratio's that typically accompany slow breathing patterns.

Therefore, the present study aimed to examine and disentangle the effects of respiratory rate and i/e ratios on self-reported relaxation states, self-reported affect, and on RSA and HF-HRV. Participants were instructed to adopt a slow or normal respiration rate, either with a high or a low i/e ratio. Heart rate and respiration were continuously measured and subjective dimensions of relaxation and affect were assessed after each instructed breathing exercise.

Method

Participants

Participants were recruited through the Experiment Management System (EMS) website of the Faculty of Psychology and Educational Sciences at the University of Leuven. The sample consisted of 30 undergraduate students (age range: 18-22 years; 2 men), who participated in return for course credit. The sample size was based on that of similar studies in the field (e.g., Song & Lehrer, 2003).

Exclusion criteria were: any self-reported cardiac or respiratory disorder, use of psychopharmaca or presence of a psychiatric disorder in the past 2 years, pregnancy, and practicing yoga/meditation/relaxation/mindfulness on a regular basis. Five participants were excluded from analysis because they were positive on at least one criterion: one participant with experience in relaxation and breathing techniques, two participants taking selective serotonin reuptake inhibitors and/or beta-blockers and two participants reporting asthma. Two other participants were excluded from analysis because they did not comply with the breathing instructions. As a result, N was 23 in our final dataset, which was subjected to statistical analysis.

All participants provided their informed consent and the study was approved by the local ethical committee.

Stimuli

Four different breathing videos of 5 minutes each were presented to assist the participant in altering his or her breathing pattern. Each displayed a vertical bar (4 cm x 19,5 cm) filling up while inspiration was expected and emptying while expiration was expected, along with a tone varying in pitch (with a high frequency of approximately 630 Hz for inspiration, and a low frequency of approximately 330 Hz for expiration).

The videos were presented on a Flat Panel Monitor computer screen in front of the participant, who was wearing headphones (Philips-SPH2500).

A first breathing video (“1.5_3.5”) reflected a breathing rate of 12 breaths/min and an i/e ratio of 0.42 (an inhalation of 1.5 s to an exhalation of 3.5 s). A second video (“3.5_1.5”) was created to display a breathing rate of 12 breaths/min, with an i/e ratio of 2.33 (a 3.5 s inhalation to a 1.5 s exhalation). The third (“3_7”) consisted of a breathing rate of 6 breaths/min with a 3 s inhalation and a 7 s exhalation (i/e ratio of 0.42). A fourth and final video ("7_3”) contained a slow breathing rate of 6 breaths/min with an i/e ratio of 2.33 (a 7 s inhalation to a 3 s exhalation).

Measures

Heart rate and breathing pattern. Respiratory and ECG signals were obtained with the

LifeShirt System® (Vivometrics Inc., Ventura, CA); a non-invasive, ambulatory monitoring system. Data were sampled at 200 Hz for the ECG and at 50 Hz for the respiratory signals. Respiratory data were continuously collected by means of respiratory inductive plethysmography (RIP). Two shielded electrical wires that were sewn into an elastic LifeShirt garment at the level of the rib cage and abdomen served as RIP transducers. Tidal volume was defined as the sum of the rib cage and the abdominal deflections (Konno & Mead, 1967). A Qualitative Diagnostic Calibration of the Vivologic software was used. This procedure automatically selects a 5 min recording of breaths that are averaged to a default value of 400 ml. Thereafter, the software calculates and applies a calibration factor, generating ml values for all breaths in the respiratory trace. We did not calibrate the RIP signal with spirometry, so the reported volumes should not be used as absolute quantitative values. They merely reflect changes in breathing patterns within an individual, which is appropriate and sufficient given the within-subject design of the present study.

Electrical activity of the heart was registered by means of three Silvertrace ECG sensors, connected with the LifeShirt system. The RIP transducers and ECG sensors were connected with the LifeShirt recording system, which contained a compact flash memory card on which data were stored.

Self-reports. A short, custom-made health questionnaire was used to assess the participants' medical history and experience in relaxation and breathing techniques.

Furthermore, the participants were asked to rate their expected emotional state if they would maintain breathing for a few minutes in accordance with the displayed breathing video. Pleasantness, arousal and feeling of control were rated on three custom-made scales, each ranging from -50 to +50, with -50 referring to the highest unpleasantness, the lowest arousal and lowest level of control.

Following the actual performance of adopting a particular breathing pattern for 5 minutes, participants rated their emotional state on the affective dimensions of valence, arousal and dominance using the pencil-and-paper-version of the nine-point Self-Assessment Manikin (SAM) (Bradley & Lang, 1994).

They also completed a Dutch translation of the Smith Relaxation States Survey (SRSI-3) (Smith, 2001); a 38-item relaxation questionnaire with a 6-point likert scale (1 = *not at all*, 6 = *the maximum*) used to measure the experiences people have when practicing different kinds of relaxation.

Because no Dutch version of the SRSI-3 is available, we made and used an ad-hoc translation. The data of the SRSI-3 were scored according to the inventory manual and mean values of the different relaxation states (R-states) subscales, i.e. basic relaxation, mindfulness, positive energy, transcendence and stress were calculated. Chronbach alpha reliabilities for the R-state scales (of previous versions of the inventory; excluding three new mindfulness items) range from .60 to .88 (Smith, 2001).

In a custom-made post-experimental questionnaire, the participants rated the extent to which they had complied with the breathing instructions on a 7-point likert scale, ranging from 1 = *absolutely true* to 7 = *absolutely untrue*. Also, this post-experimental questionnaire asked them to write down what they thought to be the hypotheses of the study.

Procedure

Phase 1: baseline measurements and practice period. Participants were tested individually in a single experimental session that lasted approximately one hour. After arrival, the female experimenter (KV) provided a short outline of the experimental procedure. The participants were told that their heartbeat would be recorded during the induction of four different breathing patterns, that they would receive rating scales and questionnaires to be filled out at different times during the experiment and that they were to put on the Life Shirt after the attachment of three electrodes (on either side of the participant’s chest and right beneath the left rib cage). The participants filled out an informed consent and a short questionnaire referring to their health background. Next, the electrodes were attached and they put on the Life Shirt. Following this, the experimenter asked them to sit quietly for a 7-minute baseline assessment.

Afterwards, the participants practiced each of the four breathing patterns (i.e., 1.5_3.5, followed by 3.5_1.5; 3_7; and 7_3). Each pattern was practiced for approximately 45 seconds. The experimenter remained with the participant to guide him or her throughout this practice session. The participants were asked to try to follow the instructed rhythm as closely as possible and were told to control their breathing rate without forcing themselves to breathe in deeply. After each short period of practicing a different breathing pattern, the participants rated on a -50 to +50 scale how they expected to feel in terms of pleasantness, arousal and control if they would adopt the practiced breathing pattern for a couple of minutes.

Phase 2: breathing instructions. Subsequently, in order to obtain a baseline measure of the self-reports, the participants completed the SAM scales and the SRSI-3. Then, the experimenter instructed the participants to adjust their breathing by following the instructions of the breathing video that would be shown. Next, the experimenter left the room and the breathing video was started. After this 5 min breathing exercise, the SAM-scales and the SRSI-3 were administered. This sequence was repeated for each of the four breathing patterns. Participants were counterbalanced across the 24 possible presentation orders.

Phase 3. After the last breathing video, the electrodes were detached and participants would change back into their own clothes. Next, they filled out the short Social Desirability questionnaire and the post-experimental questionnaire. Following this, the participants received a written debriefing and had the opportunity to leave their e-mail address to receive more clarification on the findings of the study.

Data Analyses

Physiological data reduction.

Respiration. Dedicated Vivologic software (Vivometric Inc., Ventura, CA) was used to edit raw respiratory data. Respiratory parameters – inspiration to expiration time (i/e ratio = Ti/Te), respiration rate (RR), and tidal volume (Vt) were calculated breath-by-breath and then averaged for each period of interest (the baseline period and each of the four instructed breathing periods).

ECG. The Vivologic software was also used to derive the time between consecutive R-peaks (RR intervals) from the raw ECG signal, and to detect and linearly interpolate ectopic beats. Finally, the data were visually inspected for additional artefacts, but none were found. Heart rate (HR), RSA (difference between maximum and minimum cardiac interbeat interval per breath) and power in the high (HF-HRV) and the low frequency band (LF-HRV) of heart rate variability were calculated and averaged for each of the 5-minute breathing instructions and the baseline measurement. To reduce skewness, HF-HRV and LF-HRV were log-transformed before entering the statistical analyses.

Statistical analyses. A two-way repeated measures ANOVA analysis was performed using the SPSS General Linear Model procedure on cardiovascular and respiratory parameters and on each of the self-reports. "RR" (12 and 6) and "i/e ratio" (low and high) served as two within-subject variables (each with two levels). Bonferonni corrections were applied for multiple follow-up comparisons of significant interactions. We will also report partial êta-squared effect sizes.

Results

Respiratory parameters

Table 1 lists the mean values for the respiratory parameters. The main effects of i/e ratio on Ti/Te ($F(1, 22) = 355.34, p < .05, \eta^2_p = .94$, and on respiratory rate ($F(1, 22) = 184.12, p < .05, \eta^2_p = .89$) indicated that participants changed their breathing pattern according to the instructions. Participants breathed with larger volumes when breathing at 6 as compared to 12 breaths a minute (main effect of RR on Vt ($F(1, 22) = 142.06, p < .05, \eta^2_p = .87$). This effect interacted with the inhalation to exhalation ratio of the breathing (i/e ratio x RR interaction: $F(1, 22) = 4.59, p < .05, \eta^2_p = .17$). The difference in tidal volume when adopting a respiration rate of 6 versus 12 was more pronounced when breathing at a low i/e ratio, compared to breathing at a high i/e ratio.

Self-reported relaxation and affect

Prior to the actual performance of the breathing exercises, participants expected higher pleasantness and marginally significantly also less arousal when breathing at 6 compared to 12 breaths a minute (main effects of RR on valence, $F(1, 22) = 6.17, p < .05, \eta^2_p = .22$; on arousal, $F(1, 22) = 4.03, p < .06, \eta^2_p = .15$). In addition, they tended to anticipate higher feelings of control with a low compared to a high i/e ratio (main effects of i/e ratio, $F(1, 22) = 4.17, p = .05, \eta^2_p = .16$).

Following the performance of each instructed breathing pattern (see Table 2), participants reported higher pleasantness for the low compared to the high i/e ratio, $F(1, 22) = 7.41, p < .05, \eta^2_p = .25$, and with a respiration rate of 6 compared to 12 breaths a minute, $F(1, 22)= 4.43, p < .05, \eta^2_p = .17$. Also, they reported lower arousal with a respiration rate of 6 compared to 12 breaths a minute, $F(1, 22) = 8.17, p < .05, \eta^2_p = .27$) and higher feelings of control for the low compared to the high i/e ratio, $F(1, 22)= 6.89, p < .05, \eta^2_p = .24$.

Mean values on the relaxation dimensions are displayed in Table 2. The participants scored higher on basic relaxation, mindfulness, positive energy and stress reduction after adjustment to the breathing patterns with a low as compared to a high i/e ratio (significant effect of i/e ratio on basic relaxation ($F(1, 22) = 13.06$, $p < .05$, $\eta^2_p = .37$), mindfulness ($F(1, 22) = 5.93$, $p < .05$, $\eta^2_p = .22$), positive energy ($F(1, 22) = 5.77$, $p < .05$, , $\eta^2_p = .21$) and stress reduction ($F(1, 22) = 13.40$, $p < .05$, $\eta^2_p = .38$). Breathing at 6 as compared to 12 breaths a minute resulted only in higher self-reported levels of positive energy, ($F(1, 22) = 8.70$, $p < .05$, $\eta^2_p = .28$). The participants' feelings of transcendence (as sensing the deep mystery of things and accepting this) did not differ according to the breathing patterns.

Cardiovascular parameters

The mean values of cardiovascular parameters are listed in Table 1.

Heart rate. A lower i/e ratio (as compared to a high i/e ratio) resulted in a higher heart rate (significant main effect of i/e ratio on HR ($F(1, 22) = 4.77$, $p < .05$, $\eta^2_p = 18$). There was no effect of RR on heart rate ($F(1, 22) = 0.44$, $p = .52$).

RSA. In addition to the main effects of RR, $F(1, 22) = 211.14$, $p < .05$, $\eta^2_p = .91$, and of i/e ratio, $F(1, 22) = 7.77$, $p < .05$, $\eta^2_p = .26$, also the RR x i/e ratio interaction was significant for RSA, $F(1, 22) = 7.24$, $p < .05$, $\eta^2_p = .25$. As expected, RSA was higher when breathing at 6 compared to 12 breaths a minute. Also, follow up comparisons of the interaction showed that the low i/e ratio was associated with a significantly higher RSA when breathing at 6 breaths a minute, $F(1, 22) = 9.30$, $p < .025$, but not when breathing at 12 breaths a minute, $F(1, 22) = 0.64$, n.s..

HF-HRV. HF-HRV was significantly lower at 6 than at 12 breaths a minute, $F(1, 22) = 39.64$, $p < .05$, $\eta^2_p = .64$, and with the high compared to the low i/e ratio minute, $F(1, 22) = 25.46$, $p < .05$, $\eta^2_p = .54$). However, following up on the RR x i/e ratio interaction, $F(1, 22) = 18.58$, $p < .05$, $\eta^2_p = .46$, our findings showed that the low i/e ratio was associated with a significantly higher HF-HRV when breathing at 6 breaths a minute, $F(1, 22) = 36.12$, $p < .025$, but not when breathing at 12 breaths a minute, $F(1, 22) = 0.59$, n.s..

LF-HRV. LF-HRV was higher when breathing at 6 compared to 12 breaths a minute, $F(1, 22) = 862{,}48$, $p < .05$, $\eta^2_p = .98$). No other effects were present for LF-HRV.

Discussion

Instructed breathing patterns are widely applied to treat a variety of complaints and conditions. Most often, they involve a voluntary slowing down of the breathing frequency. Although voluntary reductions in respiration rate typically induce a relative shift towards expiration, or, a lower i/e ratio, the literature remains unclear about whether the beneficial effects of adopting a slow breathing pattern may be explained by a concomitant reduction in i/e ratio. Moreover, the subjective effects of the instructed breathing patterns have been described rather poorly. Therefore, the present study aimed to investigate the effects of altering both respiration rate and i/e ratio on self-reported affect and relaxation, and on RSA and HF-HRV.

Generally, our findings show that i/e ratio is the more important determinant for self-reported effects of relaxation as obtained by instructed breathing. Although participants did not expect such effect prior to performing the breathing exercises, they reported higher pleasantness and more feelings of control for the breathing patterns with a low compared to a high i/e ratio. In addition, participants reported more relaxation, more positive energy, less stress, and higher mindfulness when adopting a breathing pattern with a low i/e ratio as compared to a high i/e ratio. In contrast, effects of respiration rate were observed only for positive energy. A possible explanation for the association between i/e ratio and self-reported relaxation is that inspiration is an active process requiring inspiratory muscle activity, whereas expiration is a passive process characterized by relaxation of the inspiratory muscles. An alternative explanation may be that the high i/e ratio applied in the present study is a rather artificial breathing pattern that is unlikely to occur during spontaneous breathing in natural circumstances. Participants may have experienced more difficulties with complying with the breathing patterns with a high i/e ratio, resulting in more stress and lower relaxation. Future research may want to investigate whether variations in a more natural range of i/e ratios would yield similar effects to the ones presently observed.

In line with several other studies (e.g., Giardino et al., 2003; Song & Lehrer, 2003), we observed a higher RSA when the participants breathed at a lower as compared to a higher respiratory rate. HF-HRV showed a reversed pattern of results: it was greater when adopting a respiratory rate of 12 as compared to 6 breaths/min. This is due to the fact that participants breathed at a rate outside the HF band (0.15-0.40 Hz) when breathing at 6 breaths/min (Berntson et al., 1997; Grossman & Kollai, 1993). Our finding of an increased HRV in the LF when breathing at 6 compared to 12 breaths/min is consistent with this and is likely due to the fact that RSA at a respiratory rate of 6 breaths/min resonates with the cardiac loop of the baroreflex (Vaschillo, Vaschillo, & Lehrer, 2006). Nonetheless, the fact that there is still a considerable power in the HF-HRV when breathing at 6 breaths/min is very intriguing. Visual inspection of each participant's power density spectrum of HRV (Fourier Analysis) in the 6 breaths/min condition revealed clear harmonics of the 0.1 Hz peak in most participants. That is, there was not only a clear peak at the (instructed) respiratory frequency (0.1 Hz), but also a smaller one at 0.2 Hz and sometimes also yet a smaller one at 0.4 Hz. Interestingly, the power spectrum of the respiratory volume signal showed similar harmonics, which may be due to the fact that the respiratory signal was not a true sinus signal and/or to participants not following the imposed breathing perfectly. As a consequence, the resulting tachogram is not perfectly sinusoidal either, and fourier analysis applied to such tachogram may easily produce power spectra that consist of the fundamental frequency component, or first harmonic, together with its harmonics (Beauchamp, 1973; Ramirez, 1985). As the present study did not include beat-to-beat blood pressure recordings, it remains unknown whether the harmonics in the power spectra of heart rate variability and the respiratory signal co-occur with harmonics in the baroreflex. Also, whether the harmonics in heart rate variability reflect vagal activity or not, is unclear and subject to further research.

The present findings confirm the relatively scarce reports in the literature on an increase in RSA and HF-HRV when breathing with a low i/e ratio (Porges, 2007; Strauss-Blasche et al., 2000). However, this effect of i/e ratio on RSA and on HF-HRV interacted with respiration rate in our study. A low compared to a high i/e ratio resulted in a significantly higher HF-HRV when participants were breathing at 6, but not at 12 breaths/minute. As of now, we can only speculate on why a lower i/e ratio yielded more power in the HF band when breathing at 6 breaths/minute. One explanation may be that it is related to the higher tidal volumes in the low compared to the high i/e ratio condition (at 6 breaths/min, see Table 1).

The respiratory findings indicate that the participants generally managed to change their inspiratory and expiratory times according to the instructed pacing. As can be expected (e.g., Han et al., 1996), a stronger reduction in breathing frequency (6 as compared to 12 breaths/min) was compensated for by an increase in tidal volume. Although we instructed persons not to breathe very deeply when following the paced breathing patterns, minute ventilation (respiration rate x tidal volume) during instructed breathing was high compared to spontaneous breathing during baseline. This is a common phenomenon, rather typical for untrained persons. Our participants may have been hyperventilating during instructed breathing, which may explain the rather small effects observed on self-reported relaxation and changes in affect for each of the breathing patterns. It is recommended that future research takes special care to avoid hyperventilation during instructed breathing, e.g., by implementing a more rigorous training phase, and/or by implementing ETCO2 biofeedback.

Contrary to what one would expect, heart rate was increased when breathing at a low i/e ratio as compared to a high i/e ratio. This finding is new and was not observed by Cappo and Holmes (1984) in their study. Visual inspection of the respiratory signals in our study indicated that when breathing with a high i/e ratio, most participants approached their inspiratory volume relatively early on in the inspiratory cycle. As such, inspiratory flow returns to zero relatively long before expiration is actually initiated, producing breathing patterns with rather 'flattened' peaks - sometimes similar to what one would observe with a post-inspiratory pause. The opposit pattern (sharp peaks and a tendency to post-expiratory pauses) was typical for the breathing patterns with a low i/e ratio. Therefore, the former breathing pattern (high i/e ratio) may have been associated with relatively longer episodes of high intrathoracic pressure, thereby increasing stroke volume and mean arterial blood pressure, which in turn may decrease heart rate via the baroreflex.

No significant differences in heart rate were found between the two different respiration rates of 6 and 12 breaths/min; which is in accordance with the results of Song and Lehrer (2003) and other studies (Grossman, Karemaker, & Wieling, 1991; Hayano et al., 1994; Pitzalis et al., 1998).

In summary, the present results strongly suggest that voluntary changes in i/e ratio are an important determinant of self-reported states of relaxation, and of RSA and power in the HF-band when breathing at 6 breaths/min. Our results suggest that beneficial effects of slow breathing described in the literature may be primarily due to concomitant changes in i/e ratio. Inconsistencies in clinical or physiological outcomes of slow breathing or HRV biofeedback likely result from a lack of control of i/e ratios. Breathing retraining and respiratory biofeedback-treatments may benefit from a more careful consideration of the i/e ratios they apply.

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Table 1

Means and (SDs) of Cardiac and Respiratory Parameters during Baseline and each of the 5-Minute Breathing Exercises

	Baseline	12 breaths/minute		6 breaths/minute	
		Low i/e ratio	High i/e ratio	Low i/e ratio	High i/e ratio
RR	14.88 (4.22)	12.27(0.90)	12.48(0.64)	7.32(1.90)	7.69(2.08)
Ti/Te	0.67 (0.11)	0.53(0.07)	1.42(0.19)	0.49(0.06)	1.44(0.26)
Vt	532 (216)	854 (325)	874 (269)	1383 (424)	1262 (372)
HR	79.11 (11.10)	75.73 (10.72)	74.66 (9.25)	77.90 (12.37)	73.64 (10.26)
RSA	94 (53)	137 (58)	131 (55)	275 (92)	234 (76)
HF-HRV	6.60 (0.82)	7.50 (0.91)	7.42 (0.81)	7.13 (0.82)	6.31 (0.77)
LF-HRV	6.66 (0.93)	6.28 (0.70)	6.37 (0.67)	8.93 (0.69)	9.08 (0.49)

Note. RR = Respiration rate (in breaths/minute); Ti/Te = ratio between duration of inhalation and exnalation (i/e ratio); Vt = tidal volume (in ml); HR = Heart rate (in beats/minute); RSA = Respiratory sinus arrhythmia (difference between maximum and minimum cardiac interbeat interval, in ms); HF-HRV = Log-transformed power in the high frequency band of heart rate variability; LF-HRV = Log-transformed power in the low frequency band of heart rate variability

Table 2

Means and SDs of Valence, Arousal, Control and five Dimensions of Relaxation Dimensions (SRSI-3 Questionnaire) to Baseline and each of the 5-Minute Breathing Exercises

	Baseline	12 breaths/min		6 breaths/min	
		Low i/e ratio	High i/e ratio	Low i/e ratio	High i/e ratio
SAM					
Arousal	3.43 (1.85)	3.09 (1.54)	4.17 (1.88)	2.52 (1.90)	3.52 (1.53)
Control	5.52 (1.78)	5.91 (1.62)	4.87 (1.74)	5.96 (1.85)	4.91 (1.98)
Valence	6.91 (1.24)	5.96 (1.15)	5.22 (1.31)	6.52 (1.56)	5.39 (1.70)
SRSI-3					
Basic relaxation	3.00 (0.80)	3.51 (0.98)	3.06 (0.98)	3.76 (0.91)	3.08 (0.70)
Mindfulness	3.22 (0.65)	2.93 (0.59)	2.76 (0.55)	3.09 (0.61)	2.89 (0.49)
Positive energy	2.48 (0.73)	2.49 (0.79)	2.34 (0.69)	2.66 (0.72)	2.48 (0.72)
Transcendence	2.01 (0.62)	2.01 (0.62)	2.01 (0.62)	2.01 (0.62)	2.01 (0.62)
Stress	1.63 (0.74)	1.58 (0.70)	2.12 (0.92)	1.55 (0.58)	1.92 (0.81)

Note. Higher scores on arousal, control and valence indicate more arousal, more control and higher pleasantness, respectively; the Self-Assessment Manikin (SAM Scale) is from Bradley and Lang (1994).the Smith Relaxation States Survey (SRSI-3) is from Smith (2001).