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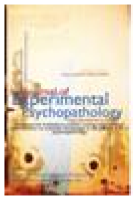

The Influence of Relaxation Training on Respiratory Variability and Self-Reported Relaxation

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Abstract

From a dynamic systems perspective, healthy breathing is characterized by complex variability. However, slow regular breathing instructions are often included in relaxation techniques. This study investigates the influence of relaxation training on natural breathing dynamics. Spontaneous breathing was measured in healthy persons during 5 sessions of relaxation training without breathing instructions (N = 29) vs. during quiet sitting resembling spontaneous relaxation (N = 29). Each session started with 10 minutes baseline recordings and ended with a mental stressor and a recovery phase. Results showed no differences between conditions in subjective relaxation and mean respiratory parameters. However, structured breath-to-breath variability increased across sessions during baseline and stress in the relaxation group only. In the control group, total variability increased across sessions during stress without concomitant changes in structured variability. The results point to the importance of respiratory variability in breathing regulation and suggest integration of a dynamic systems perspective in relaxation research.

Keywords: Respiration, Breathing regulation, Respiratory variability, Relaxation training, Dynamic Systems Perspective

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Table of Contents

Introduction

Because the respiratory system is unique in allowing voluntary control while being tied into processes of autonomic regulation, breathing instructions are typically included in clinically validated relaxation techniques and in popular stress management techniques to help people cope with everyday life stress and stress-related disorders.

In a traditional perspective, relaxation instructions often include slow regular breathing to induce a calm, pleasant mental state and to reduce stress-related physiological arousal (Cappo & Holmes, 1984). Slow regular breathing can be used as a convenient attentional object to distract attention from stress provoking content, while focusing on the waxing and waning of the respiratory movements. It has also been claimed that these breathing protocols increase autonomic balance (increased vagal tone) and homeostatic capacity and have positive health effects by increasing heart rate variability (HRV) (Lehrer, Vaschillo, & Vaschillo, 2000; Song & Lehrer, 2003), enhancing baroreflex efficiency, decreasing blood pressure (Bernardi, Gabutti, Porta, & Spicuzza, 2001; Bernardi, Porta, Gabutti, Spicuzza, & Sleight, 2001; Bernardi et al., 2002; Grossman, Grossman, Schein, Zimlichman, & Gavish, 2001; Grossman &

Taylor, 2007; Joseph et al., 2005), and improving asthma (Lehrer et al., 2004; Lehrer, 1998) and hypertension (Gavish, 2010; Joseph et al., 2005). These findings suggest that slow regular breathing (as measured by mean respiratory frequency and volume) can contribute toward subjective relaxation, well-being and health.

However, a different picture emerges from a dynamic systems regulation perspective. This view states that, in order to survive, a healthy physiological system should be able to appropriately respond to both internal and external environmental challenges and then return to a dynamic steady state. This suggests that variability is critical for adaptive physiological systems. From this perspective healthy breathing - in order to achieve a healthy state - should not be intentionally controlled for too long, should not be regular and should be characterized by complex variability consisting of a balanced combination of different variability components: correlated breath-to-breath variability and random variability (Vlemincx, Abelson et al., 2013). External stimuli (e.g. behavior, stressors) require the respiratory system to react sensitively to changing environmental demands causing random variability. Homeostatic control processes evoke correlated breath-to-breath variations in order to return the system to a dynamic steady state, and to increase homeostatic capacity and adaptability (Bruce & Daubenspeck, 1995; Donaldson, 1992; Wysocki, Fiamma, Straus, Poon, & Similowski, 2006). Deviations from the equilibrium reduce homeostatic capacity and system sensitivity: either too much randomness or a lack of structured variability can contribute to system irregularities. For example, mechanical ventilation with a variable pattern improves gas exchange and lung function compared to a fixed regular pattern (Mutch et al., 2000; Mutch, Graham, Girling, & Brewster, 2005; Pelosi, de Abreu, & Rocco, 2010). Also, sighing, which adds variability in breathing, has been related to relief from stress (Soltysik & Jelen, 2005; Vlemincx et al., 2009) and mindfulness induction has been shown to increase respiratory variability components (Vlemincx, Vigo, Vansteenwegen, Van den Bergh, & Van Diest, 2013). Furthermore, because a non-stressful sustained attention task has been shown to reduce respiratory variability (Vlemincx, Taelman, De Peuter, Van Diest, & Van den Bergh, 2010), the importance of sustained attention on internal (breathing) sensations or external stimuli for relaxation (Baer, 2003; Lazar et al., 2000; Shapiro, Schwartz, & Bonner, 1998) is also puzzling from a dynamic viewpoint. Moreover, continued voluntary breathing control can easily lead to dysregulation (e.g. hyperventilation) (Schwartz, 1981).

The traditional and dynamic systems perspectives each suggest conflicting strategies to induce relaxation and improve health (Abelson, Weg, Nesse, & Curtis, 2001; Blechert, Michael, Grossman, Lajtman, & Wilhelm, 2007; Tobin et al., 1983; Wilhelm, Trabert, & Roth, 2001). However, there is little research concerning the role of breathing during relaxation, its clinical effects and its mediating mechanisms. Existing research has focused on timing and volume parameters, whereas respiratory variability parameters have been mostly overlooked. In the present study, we compared a group receiving relaxation training on five consecutive days using imagery scripts as used in clinical practice, with a control group listening quietly to neutral texts about natural phenomena. We wanted to explore (1) how people actually breathe during a relaxed state induced by relaxation training as used in clinical practice without reference to breathing, and how this breathing pattern evolves over time, and (2) what the effect of relaxation training is on the response to and recovery from a stressor. A stressor was introduced after every script phase followed by a recovery period. If relaxation training increases homeostatic capacity, the relaxation group should show greater adaptive physiological and behavioral variability (Friedman & Thayer, 1998), and thus elevated responding to the stressor and faster recovery afterwards relative to control participants. Five sessions were conducted to assess training effects. Our main focus was respiratory variability parameters (i.e. random and structured variability components). Self-reported relaxation, respiratory (time, volume), cardiovascular and EMG responses were also measured.

Method

Participants

Fifty-nine university undergraduates (six men, mean age 20.6 years, range 18-31) volunteered to participate. None of them suffered from cardiovascular or pulmonary disease, nor reported any other health problems. One subject was excluded from analysis because she became ill after the second session and could not subsequently attend. All participants were paid 10 euros per session or given course credits in exchange for participation. The experiment was approved by the Ethics Committee of the Faculty of Psychology and Educational Sciences of the University of Leuven.

Measures

Questionnaires.

Self-reported relaxation was assessed by an ad hoc Dutch translation of the Smith Relaxation States Survey (SRSI-3; Smith, 2001) and the Relaxation Inventory (RI) (Crist et al., 1989). The SRSI-3 is 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. It contains five subscales referring to different relaxation states (R-states: basic relaxation, mindfulness, positive energy, transcendence and stress). Cronbach's 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). The Relaxation Inventory (RI) is a 45-item questionnaire with three subscales: physiological tension, physical assessment, and cognitive tension. The higher the score, the more relaxation reported. Test-retest reliability for this measure is between 0.87 and 0.97 (Pearson *r* correlation). Alpha coefficients range from 0.81 to 0.95 for the three subscales.

Additionally, several control questionnaires were administered to evaluate trait affect: (1) *Positive and Negative Affect Schedule (PANAS)*. The Dutch trait version of the PANAS consists of 10 positive (positive affectivity; PA) and 10 negative adjectives (negative affectivity; NA). Participants have to indicate on a 5-point rating scale the extent to which they experience each of the emotions. The reliability and construct validity of the PANAS have been documented elsewhere (Engelen, De Peuter, Victoir, Van Diest, & Van den Bergh, 2006; Watson, Clark, & Tellegen, 1998). This questionnaire was added because aversive psychological states such as negative affect are associated with excessive random respiratory variability (McClernon, Westman, & Rose, 2004) and with different effects of sighing on respiratory variability (Wuyts et al., 2011).

(2) *Mindful Attention Awareness Scale (MAAS)*. The MAAS is a well-validated questionnaire that assesses individual differences in the frequency of mindful states over time using a 6-point Likert scale. The items focus on assessing the presence or absence of attention to and awareness of what is occurring in the present moment (Brown & Ryan, 2003; MacKillop & Anderson, 2007).

(3) *State – Trait Anxiety Inventory (STAI)*. A Dutch validated version of the trait version of the STAI was administered. This consists of 20 items in which individuals have to rate how they generally feel on a 4-point Likert scale (Van der Ploeg, 1980). The total score ranges from 20 to 80 points.

(4) *Anxiety Sensitivity Index (ASI)*. The ASI is a 16-item questionnaire that measures fear of anxious symptoms (illness, embarrassment or additional anxiety). The latter two measures (STAI and ASI) were added because research has shown that relaxation training can initially induce anxiety, especially in anxiety prone subjects (Heide & Borkovec, 1984).

Physiological measures.

Breathing behavior was measured continuously and non-invasively by means of respiratory inductive plethysmography (RIP) (LifeShirt System®, Vivometrics Inc., Ventura, CA). Two RIP transducers sewn into the LifeShirt garment at the level of the rib cage and the abdomen were connected to the LifeShirt recorder, a digital processing unit including a data storage card. This allowed assessment of both rate and volume of each breath. The LifeShirt contains three accelerometer sensors that detect body movement and changes in posture that might affect interpretations of respiratory volume. Electrical activity of the heart was measured by means of three Silvertrace ECG sensors attached to the chest that were connected with the LifeShirt System. Surface electromyography (sEMG) of the Frontalis pars medialis was measured using pre-gelled Ag/AgCI contact electrodes (Nikomed, Denmark), attached according to recommendations made by Fridlund and Cacioppo (1986) and wired to a digital-analog converter unit (National Instruments, Austin, TX), sampled at 1000 Hz and digitized (24 bits) before storing on a personal computer.

Procedure

Participants were contacted via email to take part in a study about the influence of active and passive mental strain on several physiological processes. Breathing and relaxation were not mentioned because breathing behavior is known to be altered when participants are aware that respiration is being measured (Han, Stegen, Cauberghs, & Van de Woestijne, 1997) and because the control group did not receive relaxation training. Participants were followed up for five consecutive days, each day at (approximately) the same hour to avoid circadian rhythms to influence the results. Upon arrival on the first day, participants were informed about the course of the experiment and all participants provided informed consent. Then they put on the LifeShirt garment, all electrodes were attached, a nose cannula was attached and all sensors were connected to the LifeShirt recorder. At the start of the first session, participants were instructed to lift their eyebrows twice for five seconds separated by a 30-seconds pause to extract the maximal voluntary contraction (MVC) of the Frontalis pars medialis. Apart from the first session, each session lasted an hour and consisted of the following phases: (1) Baseline phase: participants were positioned on a comfortable chair for 10 minutes whilst the equipment was calibrated. They were instructed to sit comfortably but motionless and in silence with their eyes open during the recording. (2) Manipulation phase: half of the participants received relaxation instructions as used in clinical practice without reference to breathing. Each day, a different relaxation script of 10 minutes was administered through headphones (See Appendix). The order of the four scripts was counterbalanced across participants. Each script contained a mixture of elements of muscle relaxation, imagery and autogenic training and were derived from scripts used in clinical treatment units of the hospital of the university, for in- and across-session relaxation training. The introduction and end of all relaxation scripts was identical. The control group received four neutral scripts describing natural phenomena that lasted for 10 minutes each, presented in counterbalanced order. These scripts were chosen because they resemble what people would spontaneously do when they want to ‘relax’ (i.e., sitting quietly, reading). This allowed us to assess the extra value of specific relaxation instructions as given in clinical practice compared to common sense relaxing. After listening to each script, participants were seated on a comfortable chair in front of a desk with a computer screen, where they completed the Dutch versions of the SSRI-3 and the RI. Electrodes were checked to ensure that all connections were in place. (3) Stress phase: Participants completed a visual search task (LARA software, Thum, Boucsein, Kuhmann, & Ray, 1995) in which they had to determine within a preset time window whether or not two predefined numbers were included in matrices of numbers presented on the screen. Answers could be made with a key press on keys ‘0’, ‘1’ or ‘2’, depending on how many predefined numbers the matrix contained (none, one or two). The difficulty of the task depended on the performance level: after one correct response the time window to answer became 0.5 seconds shorter and after two incorrect responses the time window increased again with 0.5 seconds. This procedure has been shown to induce an equal degree of mental strain for all subjects (Kuhmann, 1979; in Thum et al., 1995). Also in the present study, stress was maintained at approximately equal level as could be seen in the mean performance level and EMG data as a more objective indicator of stress. Although some improvement could be seen after the first sessions, it remained equal in the final three sessions (35% correct answers). The same was true for the EMG measure. Movement requirements were minimal: participants were seated at a distance that allowed them to place their arm in a natural position on the table with three fingers ready on the answer buttons. The task ended automatically after five minutes. (4) Recovery phase: before the stress task started participants were told that the task would stop automatically and they were instructed to maintain their posture (without moving) for another five minutes until the experimenter entered the room.

In the first session no script phase was administered in order to have time to conduct the test of the MVC and to allow the administration of the control questionnaires. Moreover, it allowed participants to habituate to the experimental setting without the actual manipulation of interest already exerting any influence.

Data Analysis

Physiological data reduction.

Respiratory parameters.

Motion and posture were assessed by the LifeShirt accelerometers, which controlled for movement artifacts. All signals were plotted as a function of time and visually inspected to eliminate technical abnormalities, but none were found. Because participants were instructed through the headphones at the beginning of the script phases to sit comfortably, movements occasionally occurred at the start. Because scripts lasted slightly longer than 10 minutes, only the last 10 minutes without movement artifacts were analyzed. Raw respiratory data were edited by means of dedicated Vivologic software (Vivometrics Inc., Ventura, CA; for more details, see Vlemincx, Taelman, Van Diest, & Van den Bergh, 2010). Next, respiratory parameters (inspiratory volume (V'I), respiration rate (RR = 60/total breath time) and minute ventilation (V'E = V'I x RR)) were calculated breath by breath. The number of sighs was calculated for each phase. A sigh was defined as a breath with an inspiratory volume at least twice as large as the mean inspiratory volume during this phase. Total respiratory variability and correlated respiratory variability in V'I were quantified as the coefficient of variation (CV = SD/mean) and autocorrelation at one breath lag (AR) of V'I, respectively. Both measures of respiratory variability were calculated for each group and for each phase in every session. We only examined the first five minutes of the baseline and script phase to compare them to the five minutes of stress and recovery.

Cardiovascular parameters.

The Vivologic software was also used to derive the time between consecutive R-peaks (RR intervals) from the raw ECG signal. ARTiiFACT software (Kaufmann, Sütterlin, Schulz,& Vögele, 2011) was used to detect and linearly interpolate ectopic beats. Heart rate (HR) and the natural logarithm of the high frequency band of heart rate variability (ln(HF-HRV)) were calculated and averaged for each phase every session. The latter parameter was used because it is considered to be a good index of vagal activity (Task Force Guidelines, 1996).

EMG.

The sEMG signals were analyzed using MATLAB R2010a (Mathworks, NA). Means were calculated for the two MVC periods and per phase in each session. The sEMG data were rescaled relative to the MVC by taking the ratio of the mean EMG per phase and the highest mean MVC per participant, to allow inter- and intrasubject comparisons.

Statistical analysis.

Self-report measures.

A one way analysis of variance (ANOVA) was conducted on the control questionnaires with group as a between-subject measure. Self-report measures of relaxation were subjected to a mixed design analysis (ANOVA) with session (session 2, 3, 4, 5) as a within-subject variable and group (relaxation vs. control) as a between-subject variable.

Respiratory, cardiovascular and EMG measures.

Mixed design ANOVAs were conducted on the respiratory, cardiovascular and EMG measures with phase (baseline, script, stressor, recovery) as within-subject variable and group (relaxation vs. control) as a between-subject variable across session two to five together. Post hoc Tukey comparisons were tested in order to explore further differences between the different phases. Respiratory and cardiovascular measures were also subjected to a mixed design analysis (ANOVA) with session (1-5) as a within-subject variable and group (relaxation vs. control) as a between-subject variable, for each phase separately. Whenever the session by group interaction was significant, trend analysis was conducted to assess training effects across sessions.

Results

Control Questionnaires

The groups did not significantly differ on the MAAS, $F(1,57) < 1$, n.s., the STAI, $F(1,57) < 1$, n.s., and the ASI, $F(1,57) < 1$, n.s., nor did they show differences on the PA and NA scale of the PANAS, $F(1,57) = 1.43$, n.s., $F(1,57) < 1$, n.s. respectively.

Self-report Measures of Relaxation

None of the phase by group interactions with the scales of the RI and the SRSI3 reached significance. Groups did not differ across sessions in either of the phases on the physiological tension, physical assessment and cognitive tension scales of the RI, $F(3,168) < 1$, n.s.; $F(3,168) = 1.25$, n.s.; $F(3, 168) = 1.92$, n.s., respectively. They also did not differ on the basic relaxation, mindfulness, positive energy, transcendence and stress scales of the SRSI3 across sessions for each phase, $F(3,168) = 1.10$, n.s., $F(3,168) < 1$, n.s., $F(3,168) < 1$, n.s., $F(3,168) < 1$, n.s., $F(3,168) = 1.67$, n.s., respectively.

Respiratory Parameters

Mean respiratory parameters.

In the sample as a whole, only RR differed between phases, $F(3,147) = 10.34$, $p < 0.001$ (main effect of phase). Post hoc comparisons showed that participants breathed more rapidly during the stress phase than during the baseline, script and recovery phase, $p < .005$, $p < .01$, $p < 0.001$, respectively. None of the phase by group interactions were significant across sessions, nor were any of the session by group interactions separately per phase.

Table 1: Mean (SD) of total variability (CV(V'I)) and structured variability (AR(RR)) during the phases across session two to five for all participants.

	Phase			
	Baseline	**Script**	**Stress**	**Recovery**
CV(V'I)	33.18 (6.51) [a]	26.26 (4.38) [a]	42.28 (5.99) [b]	45.91 (6.70) [b]
AR(V'I)	0.16 (0.04) [a]	0.18 (0.04) [a]	-0.01 (0.02) [b]	0.07 (0.03) [b]

Note. Means with different subscripts are statistically different at $\alpha = .05$ using Tukey-corrected p values.

Respiratory variability.

Table 1 shows total variability (mean CV(V'I)) and structured variability (AR(V'I)) during the phases across session two to five for all participants. Both parameters differed between phases in the sample as a whole (main effect of phase), $F(3,150) = 21.08$, $p < 0.001$ for CV(V'I) and $F(3,150) = 48.68$, $p < 0.001$ for AR(V'I). Post-hoc Tukey tests showed that total variability was higher during the stressor and recovery phase than during the baseline and the script phase, whereas the opposite was true for structured variability. This indicates that there was higher random variability during the stress and recovery phases. However, in the recovery phase correlated variability was higher than during the stress phase, without concomitant changes in total variability, indicating that structured variability started to increase again after the stressor.

The phase by group interactions were not significant, $F(3,150) < 1$, n.s. However, session by group interactions per phase indicated differences between groups for structured variability for the baseline and stress phase measurements, $F(4,204) = 3.56$, $p < .01$ and $F(4,204) = 3.41$, $p < .01$ respectively. Figure 1 shows AR(V'I) during the baseline phase on consecutive sessions. Trend analysis showed a different pattern of results across baseline sessions for the two groups, $F(1,51) = 4.91$, $p < 0.05$. Structured variability during baseline measurements increased linearly across sessions in the relaxation group only, $F(1,51) = 7.98$, $p < .01$, whereas no such trend could be detected in the control group, $F(1,51) < 1$, n.s. Figure 2 shows AR(V'I) during the stress phase on consecutive sessions. Trend analysis also revealed a linear increase for the relaxation group only, $F(1,52) = 6.36$, $p < .05$. Again, the trends of the relaxation and control group differed significantly, $F(1,52) = 8.62$, $p < .005$.

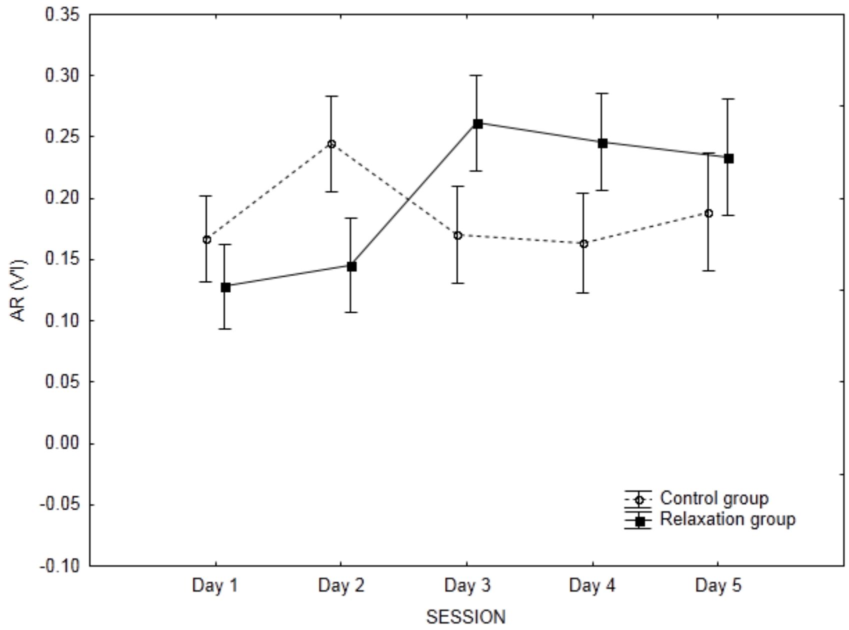

Figure 1: Structured variability (AR(V'I)) during the baseline phase on consecutive sessions. Vertical bars denote +/- standard errors

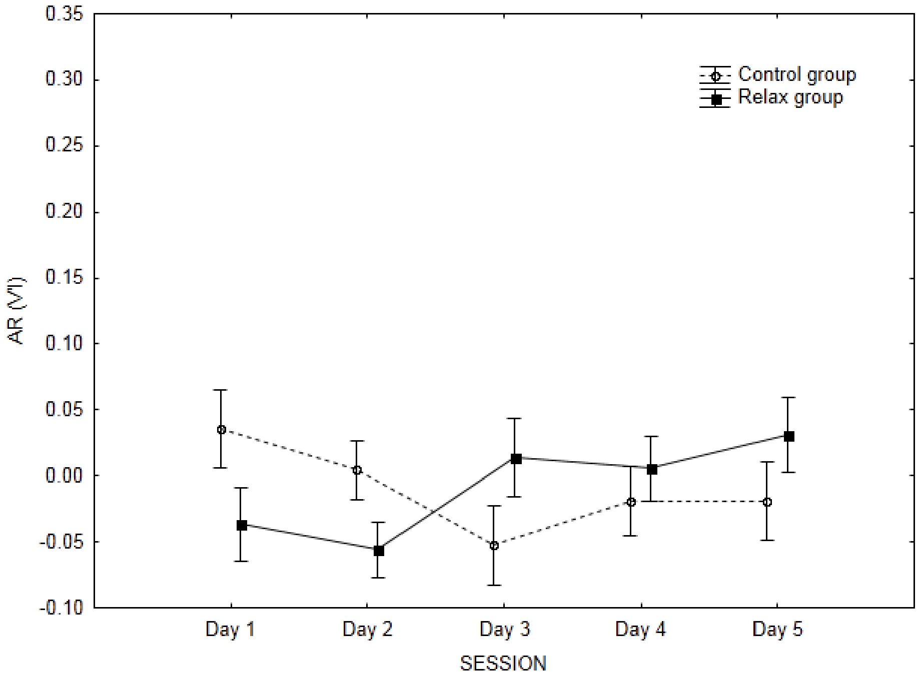

Figure 2: Structured variability (AR(V'I)) during the stress phase on consecutive sessions. Vertical bars denote +/- standard errors

Sigh rate.

Groups did not differ in their pattern of sigh rate within the sessions (phase by group interaction), $F(3,153) < 1$, n.s. Although the session by group interaction was not significant for each phase separately, a linear increase in sigh rate could be detected for the relaxation group only over sessions in the script phase, $F(3,53)$, $p < .05$. However, trends for both groups did not differ significantly, $F(3,153) = 1.62$, n.s.

EMG

Muscle tension differed between phases across session two to five in the sample as a whole (main effect of phase), $F(3,168) = 18.31$, $p < .001$. Table 2 shows mean (SD) EMG during the phases across session two to five for all participants. Muscle tension was higher in the baseline phase than during the script phase and the recovery phase. EMG was also higher during the stress phase than during the script phase, and the recovery phase (post hoc Tukey comparisons). Groups did not differ across sessions (group by phase interaction), $F(3,168) = 1.75$, n.s. However, looking at each session separately, the phase by group interaction did reach significance in session five, $F(3,168) = 3.44$, $p < .05$. Only in the relaxation group was muscle tension higher during the stress phase than during the script phase, $p < .005$, or during recovery, $p < .01$. In the control group EMG during the stress phase did not differ from the other phases.

Muscle tension also changed in the consecutive baseline phases, $F(4,224) = 3.90$, $p < .005$, stress phases, $F(4,224) = 3.95$, $p < .005$, and recovery phases, $F(4,224) = 3.53$, $p < .01$ (main effects of session per phase). For the baseline, stress and recovery phases, decreasing linear trends of muscle tension could be detected across sessions for the sample as a whole, $F(1,56) = 4.09$, $p < .05$, $F(1,56) = 12.04$, $p < .005$, and $F(1,56) = 9.89$, $p < .005$ respectively. None of the session by group interactions were significant.

Table 2: Mean (SD) EMG during the phases across all sessions for all participants.

	Phase			
	Baseline	Script	Stress	Recovery
EMG	0.11 (0.12) [a]	0.09 (0.01) [b]	0.13 (0.02) [a]	0.09 (0.01) [b]

Note. Means with different subscripts are statistically different at α = .05 using Tukey-corrected p values.

Cardiovascular Parameters

Heart rate.

A main effect of phase could be detected for HR, $F(3,147) = 2.91$, $p < 0.05$. HR was lower in the script phase than in the recovery phase, $p < 0.05$, as shown in post hoc Tukey tests. During both baseline and script phase, HR increased across sessions, $F(4,208) = 3.49$, $p < 0.01$ and $F(3,159) = 4.\ 10$, $p < 0.01$, respectively. Trend analysis showed that for both phases the linear trend was significant, $F(1,52) = 11.37$, $p < 0.005$ and $F(1,53) = 7.\ 54$, $p < 0.01$, respectively. None of the group interactions reached significance.

Heart rate variability.

ln(HF-HRV) differed between phases across all sessions in the sample as a whole (main effect of phase), $F(3,165) = 8.19$, $p < .001$. The post hoc Tukey test showed that ln(HF-HRV) was higher during baseline and script measurements than during the stress phase, $p < .05$ and $p < .001$, respectively. For all participants together, a main effect could be detected for the baseline measurements, $F(4,220) = 5.13$, $p < .001$. ln(HF-HRV) decreased on consecutive sessions during the baseline measurements, as was reflected in a significant linear trend, $F(1,55) = 12.96$, $p < 0.001$. ln(HF-HRV) was also higher during the script phase than during the recovery period, $p < .05$. The phase by group interaction was not significant, $F(3,165) = 2.04$, n.s., neither were any of the of the session by group interactions per phase.

Discussion

The main aim of the present study was to explore the influence of specific relaxation training on natural breathing dynamics and to see whether it influenced the response to and recovery from a stressor. For this purpose, common clinical relaxation instructions that do not reference breathing were compared with quiet sitting while listening to neutral texts in five consecutive sessions, in an analog sample of students. In every session a stressor was introduced. Three important results were found.

Summary and Discussion

First, no differences in subjective relaxation were observed between the relaxation and control group after every script phase. Apparently, specific relaxation training did not induce more intense subjective relaxation when compared to listening to neutral material. This lack of difference in subjective relaxation is in line with what was found by Conrad et al. (2007): Subjective relaxation did not differ after eight minute baseline measurements in which participants were asked to simply 'relax' and sit motionless in a comfortable position, and after a period of four minutes breathing at a slower pace than usual, which is normally associated with an increase in subjective relaxation. Perhaps a more extended training period would have produced differences in subjective relaxation. However, Watson, Tuorila, Vickers, Gearhart, and Mendez (1997) also did not find an additional effect of deep breathing and thermal biofeedback on posttraumatic stress disorder symptoms above simple instructions to sit in a comfortable chair and relax for 10 sessions.

Second, mean respiratory parameters did not differ between both groups. Moreover, in contrast with what is commonly found (Arambula, Peper, Kawakami, & Gibney, 2001; Peng et al., 2004, Travis, 2001; Lehrer, Sasaki, & Saito, 1999), neither the relaxation scripts nor the control manipulation decreased respiratory rate (RR) below baseline levels. Breathing mainly tends to react to arousal state (Van Diest et al., 2001), hence the lack of difference in RR between the relaxation phase and the control manipulation may not be that surprising since both phases were rated as equally relaxing. Previous research by our group also failed to find a decrease in RR from baseline following a mindfulness induction, despite higher arousal reported during baseline measurements. However, both phases were rated as equally pleasant (Vlemincx, Vigo et al., 2013). Perhaps paying attention to breathing is crucial for respiratory rate to decrease. It is a component of many forms of meditation, which has been shown to lower breathing rate (Hölzel et al., 2011). Conrad et al. (2007) also found that, of many breathing instructions, only paying attention to breathing significantly reduced respiratory rate.

Finally and interestingly, variability parameters did reveal a different effect of both relaxation training manipulations. For the control group, total variability increased linearly across sessions during stress without concomitant changes in structured variability, suggesting an increase in random variability that might lead to respiratory irregularities. The group who received specific relaxation instructions showed a linear increase in structured variability across sessions during baseline and stress, indicating an increased homeostatic capacity and adaptability. In line with these findings, the relaxation group showed a greater EMG response to the stressor and a greater recovery to baseline levels thereafter in the last session. This increased physiological flexibility can be interpreted as a higher homeostatic capacity, because a reduction in the complexity of physiological responding is associated with a lack of adaptive variability in behavioral and cognitive functioning and poor health outcomes (Friedman & Thayer, 1998). Further research is needed to examine which specific components of the relaxation training are responsible for these effects and to explore the role of an increase in structured respiratory variability.

Perhaps other mechanisms operate in the effects of relaxation training on psychophysiological functioning, when applying it to different populations. In the present study, only healthy subjects participated while being compared to a conservative healthy control group (performing spontaneous efforts to relax). In these participants, relaxation training affected physiological flexibility as evidenced in the baseline and recovery phases, possibly inducing more respiratory health effects on the long term. These effects seemed uncorrelated to subjective relaxation and cardiovascular parameters. Other studies using more clinical populations and less restrictive control groups report more direct physiological effects of relaxation, as well as self-reported relaxation. For example, Delgado et al. (2010) examined psychological and physiological indices of emotional regulation in high worriers, after a mindfulness-based training program of 10 sessions. The participants showed an improvement of somatic and autonomic regulation, as demonstrated by a reduced breathing rate and increased vagal reactivity during evocation of cardiac defense. Urech et al. (2010) found that, after a 30 min accommodation phase, even a 10 minute passive relaxation phase in which pregnant women had to sit quietly in a semi-recumbent position could reduce endocrine activity. However, only a 10 minute session of guided imagery and progressive muscle relaxation succeeded in decreasing heart rate below baseline levels in these participants.

In their review of studies on the physiological effects of progressive relaxation, Borkovec and Sides (1979) concluded that the likelihood of progressive relaxation to produce significant physiological changes is greater when multi-session, subject-controlled training is conducted with subjects for whom physiological activity contributes to a presenting clinical problem. These findings make the present results even more encouraging to investigate the effects of extended training periods on respiratory variability in different clinical populations. For example, the present findings may be of particular relevance for panic disorder patients, which are characterized by persistent respiratory irregularity and elevated sigh rate (Abelson et al., 2001; Wilhelm et al., 2001). The trend towards an increasing sigh rate in our relaxation group is interesting in this respect. Sighing has been shown to act as a psychophysiological "resetter" restoring structured respiratory variability (Vlemincx, Van Diest, Lehrer, Aubert, & Van den Bergh, 2010; Wuyts, Vlemincx, Van Diest, & Van den Bergh, 2011) and is related to relief from stress (Soltysik & Jelen, 2005; Vlemincx et al., 2009). If sighing induces relief, it might be negatively reinforced and thus lead to excessive sighing in these patients who often experience stress, which may lead in itself to physiological dysregulation (Wilhelm et al., 2001). Increasing their sigh rate by certain relaxation techniques thus warrants caution.

The decrease in HF-HRV in the consecutive baseline measurements is interesting and may be related to increasing boredom across repeating sessions caused by the quiet sitting episode. The other phases showed no such changes however.

Limitations and Future Studies

Some limitations to the present study have to be considered. First, the effects found were most pronounced for the variability measures of V'I only, whereas relaxation instructions usually tend to influence respiratory rate. However, Tobin, Mador, Guenther, Lodato, and Sackner (1988) found that, when looking at breath-to-breath variability in the breathing pattern of healthy subjects, indexes of respiratory volume and drive are more variable than those of respiratory timing. Second, no respiration-based correction or other control procedure for breathing on HRV-measurements was used. Statistical control for breathing is a frequently used approach (e.g. Burleson et al., 2003; Hughes & Stoney, 2000), but discussion remains about the importance of such correction. Lewis, Furman, McCool, and Porges (2012) recently investigated this issue and concluded that, for the majority of HRV-related measures derived in normal breathing conditions, correction for breathing leads to equal results compared to no correction. Others also have shown that uncorrected measures of HRV are at least as accurate as corrected measures (Houtveen, Rietveld, & de Geus, 2002; Thayer, Friedman, & Borkovec, 1996). Given these conclusions and because HRV was not the main dependent measure in the present study, no respiration-based correction or other control procedures for breathing were used. Third, our conclusions are based on a restricted sample with regard to age and gender. All participants were healthy psychology undergraduates approximately aged 21, most of them female, so the results cannot be generalized to older or clinical samples, which may have higher rates of cardiopulmonary disorders. Future studies should target these populations to increase clinical validity. To extend knowledge regarding the influence of relaxation on breathing regulation further, it would also be interesting to investigate the influence of yet another type of relaxation training on breathing dynamics, that does not necessarily include breathing instructions nor relaxation induction directly, such as mindfulness training. This training does teaches subjects the skill to flexibly focus the mind and regulate attention, often leading to relaxation, in turn going beyond merely listening to scripted recordings and thus mimicking clinical reality. Finally, exploration of the data was limited to the analysis of the autocorrelation at one breath lag, which holds only one level of correlated variability and thus reflects only a fraction of all structured variability. In the future, chaos and complexity measures of variability could be taken into account.

Conclusions and Clinical Implications

In sum, the findings of the present study showed no differences between conditions in subjective relaxation or in mean respiratory parameters. However, structured variability increased linearly across sessions during baseline and stress phase measurements in the relaxation group only. In the control group, total variability increased linearly across sessions during the stress phase without concomitant changes in structured variability, suggesting an increase in random variability. The results point to the importance of respiratory variability in breathing regulation and suggest a dynamic systems perspective should be integrated into future relaxation research and its applications. Whereas most relaxation techniques and breathing instructions focus on slow and regular breathing, studies show that this might compromise the responsiveness and adaptability of the respiratory system. Moreover, this study suggests that a commonly used relaxation technique without breathing instructions naturally leads people to a more complex breathing pattern characterized by structured respiratory variability, instead of more regular and slow breathing. Because a large amount of patients using relaxation therapies show anxiety- and stress-related disorders with related respiratory problems (excessive respiratory randomness, too much respiratory rigidity), caution is warranted and more research needed on the effects of already used relaxation and breathing instructions on the respiratory system. The dynamic systems perspective might prove a useful framework to conduct such research and to develop new breathing techniques that can be incorporated in relaxation therapies, maintaining already established effects without compromising healthy respiratory functioning.

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Appendix

Relaxation scripts, translated from Dutch

Script 1

We start with a relaxation exercise. Start the exercise by taking a position on the chair on which you are sitting now, that is as comfortable as possible for you. Comfortable does not mean slumped, but in a position that allows you to relax your muscles as good as possible. Therefore, it is important to have sufficient support in the chair. So, put your back against the back of your chair, with your feet flat on the floor next to each other, and your arms loosely on the arms of the chair or on your thighs.

When you sit comfortable, we can start the actual exercise. It is the easiest to do the exercise with your eyes closed. Close your eyes, so you can concentrate yourself better.

This is the beginning of a deeper relaxation, where your mind comes to rest. I count back from 10 to 1, and while I say every number, you relax deeper and deeper. I start now.

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Focus all your attention on the feeling of relaxation. The more you focus your attention on that feeling, the stronger that feeling gets. Stronger and stronger, so you become more and more relaxed, deeper and deeper.

And as you become deeper and deeper relaxed, you will also notice that the feeling that comes with this relaxation, expands itself further and further throughout your body. Further and further. Feel that feeling of relaxation in the muscles of the legs, the feet, the calves, the knees, the thighs. Relax all those muscles and notice that feeling of warmth and heaviness in these muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on the feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

And while you are so relaxed, you can gradually direct your attention to a pleasurable idea. A situation in which you feel yourself comfortable. Imagine yourself how you find yourself alone on a tropical island. Next to a pond that is being filled by a waterfall. Around you, you see trees, you can hear the wind rustling, you can hear the birds chirping. And you smell the exotic scent. Direct all your attention to that image. Focus yourself, on what you see and hear. What you can smell and feel. The water of the pond looks delightful and inviting. It gets a little heated by the volcanic rocks, and small clouds of steam rise from the surface. The water is so clear that you can see the bottom of the pond entirely. It has exactly the right temperature. And slowly, you feel the water on your body, while you enjoy every second of that experience. Feel the warmth coming up. From your feet, to your knees, and further on up. Feel how the warmth spreads over your entire body, while you slowly drift on the rhythm of the water, slowly to the waterfall.

And directly under the waterfall is a large, flat rock, which is big enough to lie on. The stone feels wonderfully warm, and you feel the warm water of the waterfall flowing over your body. You feel the water, and it feels as if it slowly massages you. Take your time to enjoy that experience, and feel the relaxation, the tranquility and serenity that flows through every muscle and every fiber from your body. It feels as if you are totally massaged by thousands of hands. Enjoy the experience. The fresh air, the warm sun on your skin, the warm ground underneath you, they give you such a wonderful sensation that it makes you drowsy, peaceful and calm. Let yourself go on quietly with deeper and deeper relaxation, and feel how you soak up the peace and tranquility like a sponge, while you relax yourself more and more, deeper and deeper.

When you are ready, focus your attention back on your body, and say to yourself: relax. And feel how relaxed you feel. Feel that sensation of relaxation in the muscles of your legs, your feet, your calves, your knees and thighs. Relax all those muscles and notice that feeling of warmth and heaviness in your muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on that feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

After that, become aware again, step by step, of the sounds from outside, the chair in which you sit. And come back to here.

We are going to end the exercise now gradually. We do this by counting from 1 to 10. On the count of 10 you open your eyes, you are wide awake and alert. More rested and relaxed, and with a good feeling inside you. I now start counting.

12345678910

Eyes open, and wide awake

Script 2

We start with a relaxation exercise. Start the exercise by taking a position on the chair on which you are sitting now, that is as comfortable as possible for you. Comfortable does not mean slumped, but in a position that allows you to relax your muscles as good as possible. Therefore, it is important to have sufficient support in the chair. So, put your back against the back of your chair, with your feet flat on the floor next to each other, and your arms loosely on the arms of the chair or on your thighs.

When you sit comfortable, we can start the actual exercise. It is the easiest to do the exercise with your eyes closed. Close your eyes, so you can concentrate yourself better.

This is the beginning of a deeper relaxation, where your mind comes to rest. I count back from 10 to 1, and while I say every number, you relax deeper and deeper. I start now.

10987654321

Focus all your attention on the feeling of relaxation. The more you focus your attention on that feeling, the stronger that feeling gets. Stronger and stronger, so you become more and more relaxed, deeper and deeper.

And as you become deeper and deeper relaxed, you will also notice that the feeling that comes with this relaxation, expands itself further and further throughout your body. Further and further. Feel that feeling of relaxation in the muscles of the legs, the feet, the calves, the knees, the thighs. Relax all those muscles and notice that feeling of warmth and heaviness in these muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on the feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

And while you are so relaxed, you can gradually direct your attention to a pleasurable idea. A situation in which you feel yourself comfortable. Imagine yourself how you find yourself alone on a beach, far from here. Edgewise to the dunes. You lie down, wonderfully relaxed, in the warm and soft sand. The sun is shining, and you feel warm and relaxed. A gentle sea breeze occasionally ensures some cooling. You feel very pleasant and you rest comfortably with your eyes closed. You know that the sky is above you, fresh and bright. The sky is blue, the clear cool see is in front of you. Focus all your attention on that image. Direct yourself to what you see and hear, what you can smell and feel. You hear the gentle sound of the sea, the recurrence of the waves. The beach is white and is stretched out before you. The water sparkles in the sun. The sand feels soft and warm under your bare feet. Closer to the sea the sand gets a bit wetter and firmer. It feels enjoyable.

Small waves flow over your feet, and pull themselves back. Again and again. The water now comes up to your knees. It has a perfect temperature. Cool, but not cold. Feel that coolness coming up. From your feet to your knees, and further on up. Feel how the coolness spreads over your entire body. While you enjoy this experience, you see a long lounge chair with cushions on the beach. You lie on it and enjoy the sunshine, that dries up your legs slowly and warms you up. Take your time to enjoy that experience, and feel the relaxation, the tranquility and serenity that flows through every muscle and every fiber from your body. Let yourself go on quietly with deeper and deeper relaxation, and feel how you soak up the peace and tranquility like a sponge, while you relax yourself more and more, deeper and deeper.

When you are ready, focus your attention back on your body, and say to yourself: relax. And feel how relaxed you feel. Feel that sensation of relaxation in the muscles of your legs, your feet, your calves, your knees and thighs. Relax all those muscles and notice that feeling of warmth and heaviness in your muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on that feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

After that, become aware again, step by step, of the sounds from outside, the chair in which you sit. And come back to here.

We are going to end the exercise now gradually. We do this by counting from 1 to 10. On the count of 10 you open your eyes, you are wide awake and alert. More rested and relaxed, and with a good feeling inside you. I now start counting.

12345678910

Eyes open, and wide awake

Script 3

We start with a relaxation exercise. Start the exercise by taking a position on the chair on which you are sitting now, that is as comfortable as possible for you. Comfortable does not mean slumped, but in a position that allows you to relax your muscles as good as possible. Therefore, it is important to have sufficient support in the chair. So, put your back against the back of your chair, with your feet flat on the floor next to each other, and your arms loosely on the arms of the chair or on your thighs.

When you sit comfortable, we can start the actual exercise. It is the easiest to do the exercise with your eyes closed. Close your eyes, so you can concentrate yourself better.

This is the beginning of a deeper relaxation, where your mind comes to rest. I count back from 10 to 1, and while I say every number, you relax deeper and deeper. I start now.

10987654321

Focus all your attention on the feeling of relaxation. The more you focus your attention on that feeling, the stronger that feeling gets. Stronger and stronger, so you become more and more relaxed, deeper and deeper.

And as you become deeper and deeper relaxed, you will also notice that the feeling that comes with this relaxation, expands itself further and further throughout your body. Further and further. Feel that feeling of relaxation in the muscles of the legs, the feet, the calves, the knees, the thighs. Relax all those muscles and notice that feeling of warmth and heaviness in these muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on the feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

And while you are so relaxed, you can gradually direct your attention to a pleasurable idea. A situation in which you feel yourself comfortable. Imagine yourself a stone terrace, of a beautiful house, which overlooks a beautiful garden. See the wide stone staircase in front of you, leading from the terrace to the garden. From the bottom step, a lush green lawn stretches out around a decorative pond. Focus all your attention on that image. Focus yourself on what you see and hear, what you can smell and feel. It is a windless, warm summer day. The warmth of the sun is calming. The brightness of the sun lights every detail of the garden. See how the garden is surrounded by trees and shrubs, that shelter and protect the garden. In the lawn, flowerbeds are filled with beautiful, fragrant flowers. They smell delicious. You feel great and deeply relaxed. Calmed and soothed by the warmth of the sunlight.

The grass feels delightful on your bare feet. It is soft and supple. Enjoy that experience, enjoy every second of that feeling. The grass ensures a little refreshment. A pleasant temperature rises up from your feet to your knees, and further on up. Feel how the coolness spreads over your entire body. Not far away from you, you see a garden bench with big fluffy pillows, surrounded by fragrant flowers. A quiet place. And the smell from the flowers flows through you. You nestle yourself down in the pillows. And while you lay down in the pillows you relax yourself, more and more, deeper and deeper.

When you are ready, focus your attention back on your body, and say to yourself: relax. And feel how relaxed you feel. Feel that sensation of relaxation in the muscles of your legs, your feet, your calves, your knees and thighs. Relax all those muscles and notice that feeling of warmth and heaviness in your muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on that feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

After that, become aware again, step by step, of the sounds from outside, the chair in which you sit. And come back to here.

We are going to end the exercise now gradually. We do this by counting from 1 to 10. On the count of 10 you open your eyes, you are wide awake and alert. More rested and relaxed, and with a good feeling inside you. I now start counting.

12345678910

Eyes open, and wide awake

Script 4

We start with a relaxation exercise. Start the exercise by taking a position on the chair on which you are sitting now, that is as comfortable as possible for you. Comfortable does not mean slumped, but in a position that allows you to relax your muscles as good as possible. Therefore, it is important to have sufficient support in the chair. So, put your back against the back of your chair, with your feet flat on the floor next to each other, and your arms loosely on the arms of the chair or on your thighs.

When you sit comfortable, we can start the actual exercise. It is the easiest to do the exercise with your eyes closed. Close your eyes, so you can concentrate yourself better.

This is the beginning of a deeper relaxation, where your mind comes to rest. I count back from 10 to 1, and while I say every number, you relax deeper and deeper. I start now.

10987654321

Focus all your attention on the feeling of relaxation. The more you focus your attention on that feeling, the stronger that feeling gets. Stronger and stronger, so you become more and more relaxed, deeper and deeper.

And as you become deeper and deeper relaxed, you will also notice that the feeling that comes with this relaxation, expands itself further and further throughout your body. Further and further. Feel that feeling of relaxation in the muscles of the legs, the feet, the calves, the knees, the thighs. Relax all those muscles and notice that feeling of warmth and heaviness in these muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on the feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

And while you are so relaxed, you can gradually direct your attention to a pleasurable idea. A situation in which you feel yourself comfortable. Imagine yourself, that you are traveling to a quiet town, in a beautiful warm country, far away from here. A city, where everything is relaxed. A city, with a quiet and slow pace. Not so slow, that it is annoying. Simply pleasant slow. Imagine yourself, that you float above the rooftops, that you look down on everyone and everything from the sky. Quiet, protected and at ease. You let yourself glide along gently and easily, and you become more and more relaxed, deeper and deeper. Focus all your attention on that image. Focus yourself on what you see and hear, what you can smell and feel. Slowly you float away from the city, in the direction of the sea and the coast.

A beach kite sways along, on the gentle rhythm of the wind, just like you. The sky is clear blue, the sunshine warms you up. It is exactly how it should be. The sea breeze provides some cooling. Feel how that coolness spreads, from your feet up to your knees, and further on up. Feel, how that feeling spreads itself over your entire body. You feel safe, and outlawed. The wind calmly carries you in the direction of the country. The rolling farmland sparkles in the sun. Under a tree, in the middle of the fields, you see a bench. The bench is very comfortable. You enjoy the view, the warmth of the sun, the shadow of the trees, the landscape around you. You become deeper and deeper relaxed.

Take your time to enjoy that experience, and feel the relaxation, the tranquility and serenity that flows through every muscle and every fiber from your body. Let yourself go on, quietly, with deeper and deeper relaxation, and feel how you soak up the peace and tranquility like a sponge, while you relax yourself more and more, deeper and deeper.

When you are ready, focus your attention back on your body, and say to yourself: relax. And feel how relaxed you feel. Feel that sensation of relaxation in the muscles of your legs, your feet, your calves, your knees and thighs. Relax all those muscles and notice that feeling of warmth and heaviness in your muscles. Also in your back and abdomen, chest and shoulders. Concentrate yourself on that feeling of relaxation. Make it deeper and deeper, warm and heavy. Further and further relaxed. The muscles of your arms, upper arms, forearms, hands and fingers. Deeper and deeper relaxed.

After that, become aware again, step by step, of the sounds from outside, the chair in which you sit. And come back to here.

We are going to end the exercise now gradually. We do this by counting from 1 to 10. On the count of 10 you open your eyes, you are wide awake and alert. More rested and relaxed, and with a good feeling inside you. I now start counting.

12345678910

Eyes open, and wide awake