Respiratory hypoalgesia?

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Respiratory Hypoalgesia? The Effect of Slow Deep Breathing on Electrocutaneous, Thermal, and Mechanical Pain

Imke Courtois,^{*} Ali Gholamrezaei,^{*,†} Hassan Jafari,^{*} Stefan Lautenbacher,[§]
Ilse Van Diest,^{*} Lukas Van Oudenhove,[†] and Johan W.S. Vlaeyen^{*,‡}

^{*}Health Psychology, KU Leuven, Leuven, Belgium, [†]TARGID, KU Leuven, Leuven, Belgium, [‡]Experimental Health Psychology, Maastricht University, The Netherlands, [§]Physiological Psychology, University of Bamberg, Germany

Abstract: The aim of the study reported in this paper is to investigate the effect of slow-deep breathing (SDB) on self-reported pain, heart rate variability, and baroreflex sensitivity (BRS). These effects are examined in 3 separate experiments, each using a different phasic pain modality. For each experiment, different subjects were recruited. Eighty-three healthy female participants were instructed to breathe guided by a visual cue at a slow frequency (SDB: .1 Hz), and at a frequency close to the spontaneous breathing frequency (normal paced breathing, .2 Hz). Pain was induced during instructed breathing using electrocutaneous (experiment 1, n = 31), thermal (experiment 2, n = 28), or mechanical stimuli (experiment 3, n = 24). Participants were requested to rate the intensity of each painful stimulus (Numerical Rating Scale) and subjective level of pleasantness, arousal, and dominance (self-assessment manikin). During the experiment, R-R interval, blood pressure, tidal volume, and end-tidal CO₂ were continuously measured. Results for self-reported pain, self-assessment manikin, and physiological measurements were consistent across the 3 experiments. Although SDB significantly increased baroreflex sensitivity and heart rate variability, self-reported pain did not differ between breathing conditions, regardless of pain modality. Other potential mechanisms or components should be considered such as behavioral modulators including relaxation and treatment expectation.

Perspective: *Merely slowing down the breathing frequency to .1 Hz is not sufficient to induce hypoalgesia, despite the significant physiological effects associated with SDB compared to spontaneous breathing.*

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Key words: Pain, blood pressure, baroreflex sensitivity, heart rate variability, slow breathing.

Slow deep breathing (SDB) is commonly used as a self-management technique, and a therapeutic tool that often is implemented in relaxation and

meditation. In addition, the majority of the existing published clinical and experimental studies on the association of breathing and pain reported promising results (experimental studies: 4/6 or 67%, clinical studies: 6/8 or 75%).²⁷ However, some studies with similar methodologies of those that found a pain dampening effect, did not observe a clear association between breathing and pain. In addition, a number of underlying mechanisms that are proposed in the literature have not been systematically investigated yet. A deeper understanding of these mechanisms would increase our knowledge of the boundaries of when respiratory hypoalgesia might be expected to occur, and possibly enhance its effects by more systematically addressing the mechanism.

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Address reprint requests to Imke Courtois, MSc, Research group Health Psychology, Faculty of Psychology and Educational Sciences, KU Leuven, Tiensestraat 102, 3000 Leuven, Belgium. E-mail: imke.courtois@kuleuven.be

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The proposed psychological and physiological mediating mechanisms are baroreceptor stimulation⁵³ and increased vagal activity,^{12,32,36,64} attentional redirection away from pain,¹² expectancies,⁶⁴ and relaxation,¹¹ but no study so far has been able to provide sufficient evidence for these mechanisms. Because of the well-known functional interactions amongst respiration and cardiovascular activity,^{4,13,42,43} as well as cardiovascular activity and pain regulatory systems,^{9,10,48} a common physiological process may underlie the effects of respiration on pain. The baroreflex system represents an ideal candidate mechanism, fitting into both the “respiration—cardiovascular activity” and the “cardiovascular activity—pain” interactions. Slow and deep breathing increases the perturbation of intrathoracic pressure (more negative pressure during inspiration) and venous return,⁶³ which is associated with larger fluctuations in blood pressure (BP) and, consequently increasing stimulation of the baroreceptors located in the wall of the aortic arch, the carotid sinuses, and the chambers of the heart. As such, SDB enhances arterial baroreceptor activation.^{5,30,52}

When the arterial baroreceptors are stimulated, afferent information is transferred via the vagal and glossopharyngeal nerve to the nucleus of the solitary tract (NTS) located in the medulla.²⁰ The NTS subsequently projects to the parasympathetic (nucleus ambiguus and dorsal motor nucleus) and sympathetic (rostral ventrolateral medulla) brainstem nuclei, resulting in respectively decrease or increase in heart rate (HR) and vascular tone, depending on the afferent input. This negative feedback loop is described as the cardiovascular branch of the baroreflex system, and helps to rapidly restore or modulate changes in BP.^{57,58} The second branch of the baroreflex system is the central nervous branch, connecting the NTS with other regulatory centers in the brainstem and higher cerebral regions related to cognition, emotion, pain, and autonomic control such as the periaqueductal gray, nucleus raphe magnus, locus coeruleus, anterior cingulate cortex, hypothalamus, and thalamus.²⁰ Via these connections, afferent information on cardiovascular activity can influence activity in higher cerebral regions, which may in turn modulate efferent autonomic outflow and baroreflex function.⁵¹ This branch may allow baroreceptors to exert an influence on pain.¹⁹ However, Rau et al (1994) found that the pain reducing effect of artificial baroreceptor stimulation may depend on pain modality (eg, increase mechanical but not thermal pain thresholds).⁴⁹

We conducted 3 experiments in order to study 1) the effect of SDB on self-reported pain, 2) the (cardiovascular) physiological effects of SDB, and 3) the physiological mediators of the effect of SDB on self-reported pain. The same methodology was applied in each experiment, but with different pain modalities (electrocutaneous, thermal, and mechanical stimuli). In order to investigate the role of baroreceptor stimulation during SDB, these experiments were set up in way that other potential psychological mechanisms such as distraction and expectancy were kept constant as much as possible. The findings may have important methodological, and

clinical implications, indicating for which pain modality SDB is beneficial.

Methods

Subjects

Participants were recruited through the Experiment Management System website of the Faculty of Psychology and Educational Sciences at the University of Leuven, social media, and word of mouth. Participants read and signed an exclusion criteria checklist and the informed consent before the start of the experiment. Any kind of mental or physical disorders (including cardiovascular disorders), pain (acute and/or chronic), drug use, pregnancy, or electronic implants were considered exclusion criteria, and participants indicating their presence in the checklist were considered noneligible for the study (see Appendix 1).

Since the effect of sex differences on pain reporting and cardiovascular reactivity are reported in previous studies, and in order to have a more homogeneous sample,^{45,46} only women were included.^{34,54} No formal a priori power analysis was conducted, but the sample size is based on a previous study conducted in our lab (Jafari, et al 2018, under revision). In this study, a medium to large effect size for the influence of breathing condition on pain was found (Cohen's *d* of .3–.8). In addition, we planned to perform multilevel modeling, which also maximizes the statistical power. Accordingly, we aimed at a sample size of 30 subjects for each experiment. In case of equipment failure during the experiment or when targeted pain level was not reached during calibration, the participant was classified as “exclusion during experiment”. Five participants (3 participants in study 2, and 2 participants in study 3) failed to complete the experiment because the targeted pain level was not reached during calibration (pain tolerance level exceeding the upper limit intensity of the built-in safety). Furthermore, data were not recorded for 3 participants in experiment 2 because of equipment malfunction. Finally, since the computer-controlled pneumatic pressure algometer (Mermaid Institute, Aalborg, Denmark) was available in our lab for a limited period of time, 26 participants were recruited for experiment 3. The final sample consisted of 31 participants in experiment 1 (electrocutaneous), 28 participants in experiment 2 (thermal), and 24 participants in experiment 3 (mechanical). The experiment was approved by the Social and Societal Medical Ethics Committee of the University of Leuven (reference number: G-2016 01 456).

Experimental Pain Induction Procedures

Three experiments were performed consecutively, each with a different pain modality. In all 3 studies, pain stimuli were presented with a total duration of 6 seconds, and consisted of 2 seconds ramp up until the desired intensity was reached (see calibration procedure), 2 seconds plateau, and 2 seconds ramp down to baseline. The inter-stimulus interval was presented in a

random order between minimum 10 and maximum 180 seconds during experimental blocks and at least 10 seconds during calibration. Furthermore, for thermal and mechanical pain stimuli, the location of the stimulator was changed in order to minimize habituation and/or local sensitization.^{3,41} For electrocutaneous pain stimuli, electrodes were not relocated since no local habituation and/or sensitization was expected.

Electrocutaneous Pain

In experiment 1, electrocutaneous pain stimuli were applied at the wrist (styloid process of the left ulnar bone). Reusable electrodes (Digitimer Ltd, Hertfordshire, UK) were filled with KY-gel (Johnson & Johnson, New Brunswick, NJ) and connected to the DS5 Isolated Bipolar Constant Current stimulator (Digitimer Ltd, Hertfordshire, UK). Each stimulus consisted of trains of rectangular 2 millisecond (1 millisecond up, 1 millisecond down) wave pulses at 500 Hz.

Thermal Pain

In experiment 2, the Medoc (Pathway model ATS; Medoc Ltd, Ramat Yishai, Israel) was used to deliver thermal pain stimuli. Baseline temperature was set at 32°C. In order to reduce habituation and/or local sensitization, the thermode was attached alternately to 2 different locations on the volar side of the left forearm (distal and more proximal part), and was moved from one to the other location during rest.

Mechanical Pain

In experiment 3, a computer-controlled pneumatic pressure algometer (Mermaid Institute, Aalborg, Denmark) was used to deliver mechanical pain stimuli.^{21,33} Stimuli were applied to the volar midphalanx of the left index or middle finger. Half of the participants started with the probe on the left index finger, while the other half started with the probe on the left middle finger. The location of the probe was moved to the other stimulation site during each block after receiving 50% (=7 stimuli) of the stimuli in order to reduce habituation and/or local sensitization.

Pain Calibration

The calibration procedure was carried out as follows, using the ascending method of limits: 1) the procedure was explained to the participant verbally, 2) the equipment was attached on the wrist area for electrocutaneous or thermal stimuli, or to the finger for mechanical stimuli, 3) a series of stimuli of increasing intensity was delivered (with at least 10 seconds interstimulus interval), whereby the participant rated the perceived pain intensity after each stimulus with a numerical rating scale (NRS; 0 = no pain; 10 = most intense pain imaginable), the endpoint being the intensity rated as 6. If rating 6 was skipped (ie, participants went from rating 5 to 7), the intensity corresponding to the previous highest rating was selected. After a short break, the procedure

was repeated for a second and third time to account for possible habituation effects. The average of the 3 calibration procedures was used to determine thresholds for mild (rating $\approx$ 1) and moderate pain (rating $\approx$ 6).

Pain Stimuli Delivery

In order to avoid habituation and to decrease predictability, 2 stimuli intensities were randomly delivered throughout the experimental block. Participants received 4 stimuli of mild intensity ($\approx$ 1 on NRS) and 10 stimuli of moderate intensity ($\approx$ 6 on NRS) during each block (a total of 14 stimuli per block). Only stimuli of moderate intensity are retained for analysis. Because of the potential influence of respiratory cycle phase on pain inhibition,^{1,26} 50% of the stimuli in this study started during inspiration and 50% during expiration (according to the visual cue used to pace respiration). There was sufficient time between each block to allow participants to relax.

Measures

Physiological Outcome Measurements

Electrocardiogram (ECG)—Three disposable electrodes (Kendall H66LG, round shape, 55 mm) were attached to the body of the participants, with 2 electrodes underneath each collarbone and 1 underneath the left ribs (lead II configuration). Cardiac electrical activity was continuously recorded at a sampling rate of 1000 Hz after analog amplification and filtering (gain: 1k, band-pass: 1–150 Hz, model V75-04, Coulbourn Instruments, Allentown, Pennsylvania). ECG and BP waveform data were stored simultaneously by the computer after digital conversion at the specified sampling rates using a National Instruments data acquisition system (16-bit PCI-6221 card with NI BNC-2111 connector block, National Instruments, Austin, Texas). During analysis, R-R interval was extracted in order to calculate HR, Heart Rate Variability (HRV) and—in combination with systolic BP—baroreflex sensitivity (BRS), using custom-made MATLAB scripts.

Blood Pressure (BP)—A finger cuff was attached to the middle phalange of the participant's middle finger of the left hand in experiment 1 and 2, and right hand for experiment 3. Beat-to-beat BP was recorded by the Portapres device (Finapres Medical Systems, Model-2, Amsterdam, The Netherlands) at a sampling rate of 1000 Hz. This is a non-invasive technique, based on the volume-clamp method. Automatic calibration was switched off during breathing blocks to avoid missing data, and switched on during rest to allow for recalibration (PhysioCal). Systolic and diastolic values were extracted for the analysis.

End-tidal CO₂ (ETCO₂) level—A sampling line was connected to a respiratory face mask (Hans Rudolph, model 7450 Series Silicone V2 Oro-Nasal Mask) in order to measure ETCO₂ concentration during each breathing cycle (capnography, model 17515, CO₂ Analyzer Gold

Edition). Sampling rate was set at 1000 Hz. Analog voltages correspond to %CO₂ and are converted to mmHg in data analysis. ETCO₂ was measured to ensure that participants were not hypo- or hyperventilating during the experimental breathing blocks.

Respiratory Flow—Respiratory flow was recorded at a sampling rate of 1000Hz by a pneumotach (Hans Rudolph, model 4813) attached to the respiratory face mask. For hygienic reasons, a new filter was placed between the face mask and pneumotach for each participant. The pneumotach was connected with a tube to the Pneumotach Amplifier 1 (Hans Rudolph, series 1110, Kasas). Analog output was converted in order to measure tidal volume (V_T) and calculate minute ventilation (V_E) (V_E = breathing rate $\times$ V_T). Respiration rate, V_T , and V_E were measured in order to verify whether participants were able to adapt their breathing rate to the desired frequency and to identify how pacing and/or control of breathing influences these variables.

Questionnaires

Questionnaires, used in all 3 experiments, were administered for descriptive purposes only. All questionnaires were completed after the experiment, except for the State Anxiety Inventory-State (STAI-S) and McGill Pain Questionnaire (MPQ). The STAI-S was completed twice, before and after the experiment, in order to explore if the experimental procedure influenced anxiety state. The MPQ was completed 4 times during the second run of the experiment. Dutch translated and validated versions were used for each questionnaire.^{15,31,44,56}

McGill Pain Questionnaire (MPQ)—The MPQ³⁹ uses pain-descriptive adjectives in order to measure subjective pain experience. Participants are requested to select an adjective that describes the pain received during the previous block. These words are subdivided in affective (5 groups of words), evaluative (3 groups of words), and sensory (12 groups of words) dimensions. Each adjective is assigned to an intensity value and added up to obtain a score for each dimension and a total score between minimum 20 and maximum 63 (pain rating index; PRI). Higher scores imply more intense pain.

State—Trait Anxiety Inventory (STAI-S and STAI-T)—The STAI is commonly used to measure state and trait anxiety levels.⁵⁹ Both inventories (state and trait) use 20 statements which participants have to rate on a 4-point-Likert scale ranging from 1 (not at all) to 4 (very much so) and 1 (almost never) to 4 (almost always) for STAI-S and STAI-T respectively.

Pain Catastrophizing Scale (PCS)—The PCS consists of 13 statements about how participants may feel and think when they are in pain.⁶¹ Participants rate each statement on a Likert scale ranging from 0 (=not at all) to 4 (=always).

Fear of Pain Questionnaire (FPQ)—The FPQ measures pain-related fear and consist of 30 items (eg, "Breaking your arm").³⁸ Each item is scored on a 5-point Likert Scale ranging from 1 (not at all) to 5 (extreme).

Rating Scales

Self-reported pain—Participants were requested to rate pain intensity after each stimulus according to a 0 to 10 numerical rating scale (NRS; 0 = no pain; 10 = most intense pain imaginable). The same NRS was also used during the calibration procedures.

Self-assessment manikin (SAM)—SAM is a nonverbal pictorial assessment technique that directly measures the pleasure, arousal, and dominance associated with a person's affective reaction to a wide variety of stimuli.⁸ The dominance dimension measures how much a person feels in control of the current situation. Participants completed the SAM after each breathing block during rest. Figures are converted to a 9-point rating scale for each dimension. Higher scores on "valence" and "dominance" are related to more feelings of happiness and more perceived control over the situation, respectively. Higher scores for arousal imply more aroused emotional state.

Comfort of paced breathing—After baseline measurements, participants were requested to complete a 1-minute practice phase of each breathing condition, SDB, and normal paced breathing (NPB). Subsequently, participants were asked to rate their level of (dis)comfort ("how comfortable was it to adjust your respiration according to the breathing bar") on a 10-point scale ranging from 1 (very comfortable) to 10 (not at all comfortable).

Perceived threat and avoidance—At the end of the experiment, both the level of threat and urge to avoid was measured retrospectively. Participants rated threat ("how threatening were the painful stimuli during the experiment?"), and urge to avoid ("to what degree did you want to avoid the painful stimuli during the experiment?") on a 11-point scale ranging from 0 (not at all) to 10 (very much).

Experimental Procedure

For each of the 3 pain modalities, a within-subject design was used to investigate pain and cardiovascular activity during the 2 breathing patterns (Fig 2). Each participant breathed either at a frequency close to the natural, spontaneous frequency (.2 Hz or 12 breaths/min), or at a slow frequency (.1 Hz or 6 breaths/min), with an inspiration/expiration (i/e) ratio of 1:2 (ie, prolonged expiration).^{17,28} A vertical moving bar was presented on a computer screen as a visual cue to pace the participant's respiration according to the desired breathing frequency. Participants were instructed to inhale during filling and exhale during emptying of the bar. Breathing at a (fixed) frequency in both breathing conditions was used to standardize the breathing frequency across subjects and in order to control the potentially confounding effects of mental effort or distraction induced by pacing. SDB at 6 breaths per minute is routinely used in clinical and experimental breathing technique interventions. No serious adverse effects are reported so far in experimental studies using this technique in healthy volunteers.^{1,7,12,65}

Participants were seated in a comfortable chair at a table in a quiet, temperature-controlled room. Prior to testing, demographics, and STAI-5 were administered on a tablet with Qualtrics software (Qualtrics, Provo, UT). Next, electrodes (ECG), finger cuff (BP), and face mask (respiratory measures) were attached to the participant. All further instructions were presented on a computer screen in front of the participant. In addition, this information was deliberately kept neutral regarding the effect of SDB on pain and physiology in order to reduce expectation bias. Baseline physiological activity including spontaneous respiration rate, V_T , $ETCO_2$, HR, and BP were recorded for 10 minutes. Afterwards, participants practiced both breathing frequencies for 1 minute and rated on a 0 to 10 NRS how comfortable it was to adjust their breathing rate to the visual cue/breathing frequency (0 = not comfortable at all; 10 = very comfortable).

Next, the experiment consisted of 2 experimental runs, consisting of 2 and 4 breathing blocks, respectively. During the first run, participants performed 7 minutes of SDB and 7 minutes of NPB without pain induction, followed by 10 minutes of rest. During the first 2 blocks, the equipment to induce painful stimuli was not attached to the participants in order to keep them as relaxed as possible. Next, individual pain threshold and tolerance were assessed with the ascending method of limits (see above) in order to estimate individually calibrated "mild" and "moderate" pain. During the second run, participants performed 2 blocks of 7 minutes SDB and 2 blocks of 7 minutes NPB with pain induction. The order of the breathing conditions (SDB or NPB) was counterbalanced (among participants). Each block was followed by 4 minutes of rest during which participants completed the SAM⁸ and MPQ.³¹ The complete experimental procedure was programmed in Affect Version 4.0.⁶⁰

Statistical Analyses

Data Processing and Parameter Extraction

Both ECG and BP were sampled at a sampling rate of 1000 Hz. Noise was reduced by using a low band pass filter for ECG (1–150 Hz) and a digital low pass filter for BP (30 Hz) signal. Analog output was A/D converted and imported in MATLAB (R2016b, Mathworks, Inc., Natick, MA), which allowed visual inspection. R-peak detection and R-peak interpolation (linear method) was performed using custom-made MATLAB scripts prior to further analysis. In case of more than 5% of artifacts per block, data from those trials were excluded from analysis. HRV was calculated using the square root of the successive RR interval differences (RMSSD), which is a time-domain measurement of short term, vagally mediated variations in R-R interval. Spectral analysis using the autoregressive model was performed, and results were averaged over subjects to visually demonstrate the influence of breathing frequency on HRV.

The sequence method was used to measure BRS. This method allows analyzing the relationship between increasing and decreasing ramps of BP and related changes in R-R interval through linear regression. The

lag between BP and R-R interval was set at 0. Also, a minimum of 3 consecutive changes of BP (minimum 1 mm Hg) and R-R intervals (minimum 5 milliseconds) was required to be considered a sequence.¹⁶ Sequences required a minimal correlation coefficient of $>.8$ between BP and R-R interval changes. The average value of the slopes was calculated. Every BP ramp (ie, sequence) with corresponding changes in R-R interval was considered an effective baroreflex response.

Data Analysis

After data processing, statistical analyses were performed using SAS University Edition (SAS Institute Inc., Cary, NC). Since baseline was not randomized with other breathing conditions during the first run, data only serve as exploratory measurements in the analysis. The experimental procedure was set up in a way that it allowed us to pool physiological and behavioral data measured during the first run (without pain induction) across the 3 experiments. One-way ANOVAs were used to determine whether groups (study 1, 2, 3) are similar in terms of anxiety levels (STAI), fear of pain (FPQ), pain catastrophizing (PCS), threat of induced stimuli, and the urge to avoid. Pain, physiological, and behavioral measurements during the second run of the experiments were analyzed and presented for each experiment separately. In addition, the 2 blocks of the same breathing condition were averaged in order to reduce measurement error and simplify the statistical model. Logarithmic or Box-Cox transformations were used if the residuals of the statistical model were not normally distributed. If transformations did not solve the violation of normality, nonparametric tests were used. Linear mixed models were performed to analyze self-reported pain, BRS, HRV, $ETCO_2$, SAM, V_T , and V_E with breathing condition as categorical independent variable. This model is considered to be robust for missing data. To test whether breathing frequency influenced self-reported pain upon repeated stimulation, stimulus number was used in the model as categorical independent variable, including a stimulus repetition X breathing condition interaction term. Results are expressed as beta coefficients $\pm$ standard error and significance level was set at .05. Finally, in case of significant interaction effects, post hoc t-tests with Tukey (all pairwise differences) or stepdown Bonferroni (planned contrasts) multiple testing correction were performed. Mediation was only considered if breathing condition (independent variable) showed clear (direct) effects on self-reported pain (dependent variable).² Wilcoxon signed rank test was applied to compare the effect of SDB and NPB on MPQ ratings. Effect sizes were calculated with Cohen's *d*, based on the estimates of the model. We also added the "SDB-effect" by calculating the difference (SDB–NPB) divided by the value under NPB.

Results

Sampling started in April 2016 and continued until at least 30 participants in each study completed the entire

study and were not excluded before or during the experiment (ended in February 2017). A total of 83 female participants were included in the analyses ([experiment 1: $n = 31$], [experiment 2: $n = 28$], [experiment 3: $n = 24$]). Participants characteristics are presented in Table 1 and an overview of participant flowchart is presented in Fig 1. Participants in the 3 experiments were similar in terms of anxiety levels, fear of pain, pain catastrophizing, threat of induced stimuli, and the level of urge to avoid the delivered painful stimuli (see Table 1). Participants were able to breathe according to the desired breathing rate, regardless of pain induction (see Table 2).

First, the effects of SDB and NPB on physiological and behavioral outcomes without pain induction are presented (see Table 3). Then, results regarding the effect of SDB and NPB on physiological, behavioral, and self-reported pain are presented for each experiment separately (see Table 4a, b, and c and Table 5). Effects of SDB and NPB on physiological outcome measurements are presented in Fig 3. There was no order effect of breathing pattern (SDB vs NPB) in neither of the experiments ($P > .2$).

The Effect of SDB Versus NPB on Physiological and Self-Report Outcomes, Without Pain Induction

Across experiments, breathing at .1 Hz (SDB) was perceived as more comfortable as compared to NPB ($P < .05$) during the practice phase (see Table 3). Furthermore, respiration rate during NPB was on average lower (spontaneous: $13.28 \pm .36$; NPB: $11.66 \pm .05$; $P < .0001$) compared to baseline. Spectral analysis shows how paced breathing at different frequencies influences

RMSSD (Fig 4). During the first experimental run, SDB significantly increased BRS and RMSSD (both $P < .0001$) compared to NPB. As a result of slowing down the breathing rate, V_T was significantly higher during SDB compared with NPB ($P < .0001$). V_E was lower during SDB ($P < .0001$), and a higher level of $ETCO_2$ ($P < .05$) was found compared to NPB. In order to explore the effect of pacing, we compared NPB with baseline and found that BRS was decreased ($\beta_{NPB \text{ vs Baseline}} = -.27 \pm .05$, $t(81) = -5.21$, $P < .0001$) while RMSSD was slightly increased ($\beta_{NPB \text{ vs Baseline}} = .06 \pm .03$, $t(77) = 2.19$, $P = .031$) during NPB compared to baseline. Finally, despite the effects of SDB on physiological outcome measurements, SDB did not significantly reduce the subjective level of arousal ($P = .935$). Moreover, SDB was perceived as less pleasant and controllable compared to NPB (valence: $P < .05$; dominance: $P < .05$) (see Table 5).

The Effect of SDB Versus NPB on Physiological and Behavioral Outcomes, and Self-Reported Pain

Experiment 1: Electrocutaneous Pain

Thirty-one women participated in this experiment ($M_{age} = 22.45$ years, $SD_{age} = 3.10$). Compared with NPB, SDB increased BRS ($P < .0001$) and RMSSD ($P = .0008$). Both $ETCO_2$ and V_T were also higher during SDB ($ETCO_2$: $P < .05$; V_T : $P < .0001$) and V_E lower during SDB as compared to NPB ($P < .0001$). However, compared to NPB, SDB neither reduced the subjective level of arousal, nor increased the subjective level of pleasantness or perceived control (all dimensions $P > .05$, see Table 5). There was no main effect of breathing condition ($F(1,30) = .28$,

Table 1. Participants Characteristics

	BETWEEN GROUP COMPARISONS			
	ELECTROCUTANEOUS	THERMAL	MECHANICAL	P
n	31	28	24	-
age	22.45 ± 3.10	20.25 ± 2.50	22.55 ± 3.16	-
Right-handed, n (%)	27 (87%)	26 (93%)	22 (92%)	-
BMI (kg/m^2)	23 ± 4.10	22 ± 3	23 ± 4.10	-
Non-smokers, n (%)	31 (100%)	28 (100%)	19 (79%)	-
Anti-conception, n (%)	16 (52%)	13 (46%)	15 (63%)	-
Menstrual phase, n (%)	2 (6%)	6 (21%)	5 (21%)	-
Trait-Anxiety (STAI-T)	38.16 ± 9.07	38.25 ± 9.10	36.21 ± 10.13	$F = .39$
State-Anxiety (STAI-S)	36.32 ± 9.60	34.36 ± 10	35.79 ± 9.21	$X^2 = .85$
Fear Of Pain (FPQ)	71.8 ± 2.9	71.8 ± 2.5	67.6 ± 2.6	$F = .75$
Pain Catastrophizing (PCS)	18.4 ± 1.8	18.2 ± 1.5	15.8 ± 2	$F = .64$
threat (NRS)	$2.26 \pm .45$	$2.50 \pm .47$	$2.57 \pm .49$	$X^2 = .41$
avoidance (NRS)	$4.94 \pm .53$	$5.64 \pm .51$	$4.71 \pm .63$	$X^2 = 1.75$
pain threshold	$1.3 \text{ mA} \pm .9$	$40.4^\circ\text{C} \pm 2$	$117.6 \text{ kPa} \pm 68.9$	-
supra-threshold (6 on NRS)	$2.9 \text{ mA} \pm 1.9$	$44.7^\circ\text{C} \pm 1.7$	$254.4 \text{ kPa} \pm 104.4$	-
Systolic BP (mmHg)	114 ± 13.8	115 ± 14.8	114 ± 11.1	-
Diastolic BP (mmHg)	78 ± 13.2	76 ± 8.3	78 ± 10.1	-
rest HR (bpm)	81 ± 13	76 ± 8.2	78 ± 10.9	-

Abbreviations: BMI, body mass index; bpm, beats per minute; NRS, numeric rating scale (0–10); BP, blood pressure; HR, heart rate.

Data are presented as mean $\pm$ standard deviation unless otherwise indicated. Inferential statistics: one-way ANOVA or Kruskal-Wallis test if not normally distributed.

Figure 1. Participant Flowchart.

$P = .600$) nor stimulus number ($F(9,270) = 1.70$, $P = .088$) on self-reported pain (Fig 5 and Table 4a). In other words, there was no difference in pain ratings between SDB and NPB. No breathing condition X stimulus repetition interaction effect was observed ($F(9,264) = 1.17$, $P = .312$). Considering the possible association between BP and pain,⁹ we tested if excluding subjects with a high systolic BP (>130 mm Hg) confounded the results ($n = 4$). However, results for self-reported pain were not

different ($F(1,26) = .40$, $P = .531$). Finally, there was no effect of breathing condition on PRI, measured with the MPQ (all dimensions $P > .05$, see Table 5).

Experiment 2: Thermal Pain

Twenty-eight women participated in this experiment ($M_{age} = 20.33$ years, $SD_{age} = 2.51$). Results were in line with experiment 1 (see Table 4b), in that SDB increased

Figure 2. Experimental procedure.

Table 2. Manipulation Check of Breathing Intervention

Breathing Condition	Experiment 1: Electrocutaneous		Experiment 2: Thermal		Experiment 3: Mechanical	
	M	SD	M	SD	M	SD
SDB (6 breaths/min)						
RR without pain	5.94	.15	5.93	.16	5.98	.30
RR during pain	5.90	.13	5.91	.13	5.92	.11
NPB (12 breaths/min)						
RR without pain	11.88	.34	12.05	.53	11.88	.30
RR during pain	11.80	.28	11.90	.25	11.84	.27

Abbreviations: MD, mean; SD, standard deviation; RR, respiration rate; SDB, slow deep breathing; NPB, normal paced breathing.

BRS ($\beta_{\text{SDB vs NPB}} = .75 \pm .10$, $t(27) = 7.76$, $P < .0001$) and RMSSD ($\beta_{\text{SDB vs NPB}} = .26 \pm .05$, $t(27) = 5.16$, $P < .0001$). ETCO_2 and V_T were increased (ETCO_2 : $P < .0001$; V_T : $P < .0001$) and V_E was decreased during SDB as compared to NPB ($P < .0001$). Further, as in experiment 1, there was no difference between the effects of SDB and NPB on subjective level of arousal, pleasantness and perceived control measured with the SAM (all dimensions $P > .05$, see Table 5). There was no main effect of breathing condition on pain ($F(1,27) = .14$, $P = .711$) (Fig 5) even when subjects ($n = 2$) with high systolic BP ($>130\text{mm Hg}$) were excluded from the dataset ($F(1,25) = .15$; $P = .703$). However, self-reported pain significantly changed with stimulus repetition ($F(9,243) = 12.54$, $P < .0001$), regardless of the breathing condition (no interaction effect: $F(9,234) = 1.56$, $P = .128$). Post hoc contrasts demonstrated that self-reported pain significantly decreased for only the first 4 thermal stimuli ($[\beta_{\text{stim1 vs stim2}} = -.55 \pm .07$, $t(243) = -8.14$, $P < .0001$], $[\beta_{\text{stim2 vs stim3}} = -.29 \pm .07$, $t(243) = -4.31$, $P < .0001$], $[\beta_{\text{stim3 vs stim4}} = -.17 \pm .07$, $t(243) = -2.55$, $P < .05$], $[\beta_{\text{stim4 vs stim5}} = -.09 \pm .07$, $t(243) = -1.33$, $P = .184]$). Only the affective dimension (PRI-Affective) of pain measured with the MPQ was significantly different between breathing conditions ($P = .047$), while other dimensions and the total score were not (see Table 5).

Experiment 3: Mechanical Pain

Twenty-four females participated in this experiment ($M_{\text{age}} = 22.55$ years, $SD_{\text{age}} = 3.16$). Also, in this study, SDB increased BRS ($P < .0001$) and RMSSD ($P < .05$) as compared to NPB (see Table 4c). Although experiment 1 and 2 demonstrated that ETCO_2 was higher during SDB as compared to NPB, there was no difference between breathing conditions on this variable in this study. ETCO_2 was somewhat higher during SDB, but the difference did not reach statistical significance ($P = .234$). As expected, V_T was increased and V_E decreased during SDB (V_T : $P < .0001$; V_E : $P < .0001$) as compared to NPB. In line with the results of the previous 2 experiments, there were no effects of breathing condition on subjective level of arousal, pleasantness and perceived control as measured with the SAM (all dimensions $P > .05$, see Table 5). There was neither a main effect of breathing condition ($F(1,23) = .30$, $P = .589$) nor stimulus repetition ($F(9,207) = 1.36$, $P = .209$), nor a breathing condition X stimulus repetition interaction effect ($F(9,204) = .34$, $P = .962$) for self-reported pain (Fig 5). In other words, there was no difference in pain ratings between SDB and NPB ($P = .589$). Only 1 subject was detected with a resting systolic BP higher than 130mm Hg. Excluding this subject did not affect the results ($F(1,22) = .31$; $P = .583$). Finally, there was no effect of breathing

Table 3. Results—Untransformed Data and Mixed Model Estimates of SDB Compared to NPB During the First Experimental Run (No Pain)

First Experimental Run (Pooled Data)							
Variables	SDB	NPB	SDB-EFFECT	ES	Mixed Models		
	MEAN $\pm$ SD (UNTRANSFORMED DATA)	MEAN $\pm$ SD (UNTRANSFORMED DATA)	%	D_z	$\beta_{\text{SDB vs NPB}} \pm \text{SE}$	T VALUE (DF)	P
Comfort (NRS 0–10)	6.46 $\pm$ 2.10	5.80 $\pm$ 1.90	11.38	.30	.66 $\pm$.25*	2.62 (79)	<.05
BRS (ms/mmHg)	20.72 $\pm$ 10.66	10.48 $\pm$ 5.53	97.71	1.06	.80 $\pm$.07	11.47 (80)	<.0001
RMSSD (ms)	73.27 $\pm$ 39.18	55.24 $\pm$ 28.01	32.64	.71	.28 $\pm$.03	8.43 (82)	<.0001
ETCO_2 (%)	6 $\pm$ 1.22	5.86 $\pm$ 1.06	2.39	.28	1.19 $\pm$.39	3.09 (80)	<.05
V_T (mL)	1414.45 $\pm$ 513.17	1017.30 $\pm$ 362.63	39.04	.94	417.96 $\pm$ 48*	8.71 (75)	<.0001
V_E (L/min)	8.04 $\pm$ 2.97	11.86 $\pm$ 4.26	–32.21	1.09	–3.55 $\pm$.41*	–8.59 (75)	<.0001

Abbreviations: SDB, slow deep breathing; NPB, normal paced breathing; ES, effect size; NRS, numeric rating scale; BRS, baroreflex sensitivity; RMSSD, root mean square of successive differences; ETCO_2 , end-tidal CO_2 ; V_T , tidal volume; V_E , minute ventilation; β , beta coefficient; SE, standard error; SD, standard deviation. Mean $\pm$ standard deviation of untransformed data are presented in this table. However, P values are based on the model with the best fit of the data. In case of (*), the best fit was with untransformed data. Cohen's d for paired data (d_z) is reported for ES. Mixed models are expressed as beta coefficients ($\beta_{\text{SDB vs NPB}} \pm \text{SE}$ and t value (Degrees of freedom (DF)). SDB-effect is the difference between SDB – NPB expressed in percentage (%).

Table 4a. Results—Untransformed Data and Mixed Model Estimates of SDB Compared to NPB During Electrocutaneous Pain Induction

EXPERIMENT 1: ELECTROCUTANEOUS (n = 31)							
VARIABLES	SDB	NPB	SDB-EFFECT	STATISTICS	MIXED MODEL		
	MEAN ± SD (UNTRANSFORMED DATA)	MEAN ± SD (UNTRANSFORMED DATA)	%	D _z	β _{SDB vs NPB} ± SE	T VALUE (DF)	P
Pain (NRS 0–10)	5.12 ± 2	5.01 ± 1.81	2.20	.12	.40 ± .75	.53 (30)	.600
BRS (ms/mmHg)	21.84 ± 11.28	11.98 ± 6.46	82.30	1.21	.88 ± .12	7.25 (28)	<.0001
RMSSD (ms)	87.39 ± 44.52	75.43 ± 41.88	15.86	.68	.20 ± .05	3.71 (30)	.0008
ETCO ₂ (%)	5.87 ± 1.16	5.74 ± 1.11	2.26	.36	3.97 ± 1.74	2.28 (29)	<.05
V _T (mL)	1397.42 ± 458.46	947.43 ± 323.88	47.50	1.29	.43 ± .05	8.26 (29)	<.0001
V _E (L/min)	8.03 ± 2.64	11.05 ± 3.85	−27.33	1	−.60 ± .11	−5.39 (28)	<.0001

Abbreviations: SDB, slow deep breathing; NPB, normal paced breathing; NRS, numeric rating scale; BRS, baroreflex sensitivity; RMSSD, root mean square of successive differences; ETCO₂, end-tidal CO₂; V_T, tidal volume; V_E, minute ventilation; β, beta coefficient; SE, standard error; SD, standard deviation. Mean ± standard deviation of untransformed data are presented in this table. However, P values are based on the model with the best fit of the data. In case of (*), the best fit was with untransformed data. Cohen's d for paired data (d_z) is reported for effect size. Mixed models are expressed as beta coefficients (β_{SDB vs NPB}) ± SE and t value (Degrees of freedom (DF)). SDB-effect is the difference between SDB—NPB expressed in percentage (%).

condition on PRI measured with the MPQ (all dimensions $P > .05$, see Table 5).

Discussion

Three experiments were conducted in order to investigate the effect of SDB on self-reported pain, physiological

outcomes and the potential pain-dampening role of baroreceptor stimulation induced by SDB. A within-subject design was used to investigate pain and cardiovascular activity during 2 paced breathing conditions: SDB (.1 Hz) and NPB (.2 Hz). Compared to NPB, breathing was deeper (greater V_T) during SDB, whereas V_E was reduced and ETCO₂ increased. Three major findings emerged from the

Table 4b. Results—Untransformed Data and Mixed Model Estimates of SDB Compared to NPB During Thermal Pain Induction

EXPERIMENT 2: THERMAL PAIN (n = 28)							
VARIABLES	SDB	NPB	SDB-EFFECT	ES	MIXED MODEL		
	MEAN ± SD (UNTRANSFORMED DATA)	MEAN ± SD (UNTRANSFORMED DATA)	%	D _z	β _{SDB vs NPB} ± SE	T VALUE (DF)	P
Pain (NRS 0–10)	4.66 ± 2.14	4.75 ± 2.07	−1.89	.10	−.05 ± .13	−.37 (27)	.711
BRS (ms/mmHg)	21.63 ± 11.25	11.46 ± 5.99	88.74	1.08	.75 ± .10	7.76 (27)	<.0001
RMSSD (ms)	78.18 ± 32.38	65.89 ± 35.61	18.65	.79	.26 ± .05	5.16 (27)	<.0001
ETCO ₂ (%)	6.23 ± 0.87	6.04 ± 0.81	3.15	1.07	.20 ± .04*	4.77 (26)	<.0001
V _T (ml)	1161.17 ± 437.20	831.57 ± 242.14	39.64	.93	329.6 ± 72.54*	4.54 (23)	<.0001
V _E (l/min)	6.69 ± 2.61	9.75 ± 2.84	−31.38	1.19	−3.06 ± .52*	−5.86 (23)	<.0001

Abbreviations: SDB, slow deep breathing; NPB, normal paced breathing; ES, effect size; NRS, numeric rating scale; BRS, baroreflex sensitivity; RMSSD, root mean square of successive differences; ETCO₂, end-tidal CO₂; V_T, tidal volume; V_E, minute ventilation; β, beta coefficient; SE, standard error; SD, standard deviation. Mean ± standard deviation of untransformed data are presented in this table. However, P values are based on the model with the best fit of the data. In case of (*), the best fit was with untransformed data. Cohen's d for paired data (d_z) is reported for ES. Mixed models are expressed as beta coefficients (β_{SDB vs NPB}) ± SE and t value (Degrees of freedom (DF)). SDB-effect is the difference between SDB—NPB expressed in percentage (%).

Table 4c. Results—Untransformed Data and Mixed Model Estimates of SDB Compared to NPB During Mechanical Pain Induction

EXPERIMENT 3: MECHANICAL (n = 24)							
VARIABLES	SDB	NPB	SDB EFFECT	STATISTICS	MIXED MODEL		
	MEAN ± SD (UNTRANSFORMED DATA)	MEAN ± SD (UNTRANSFORMED DATA)	%	D _z	β _{SDB vs NPB} ± SE	T VALUE (DF)	P
Pain (NRS 0–10)	5.84 ± 1.54	5.73 ± 1.43	1.92	.21	.46 ± .84	.55 (23)	.589
BRS (ms/mmHg)	19.50 ± 7.69	11.54 ± 5.65	68.98	1.15	7.96 ± 1.48*	5.41(21)	<.0001
RMSSD (ms)	69.82 ± 27.48	61.48 ± 27.90	13.57	.53	.15 ± .04	3.64(22)	<.01
ETCO ₂ (%)	6.19 ± 1.51	6.14 ± 1.48	.81	.17	.08 ± .07*	1.22 (22)	.234
V _T (mL)	1471.81 ± 514.96	1070.74 ± 254.43	37.46	1.02	.53 ± .09	5.69 (22)	<.0001
V _E (L/min)	8.49 ± 3.04	12.45 ± 2.94	−31.81	1.58	−3.82 ± .53*	−7.24 (22)	<.0001

Abbreviations: SDB, slow deep breathing; NPB, normal paced breathing; NRS, numeric rating scale; BRS, baroreflex sensitivity; RMSSD, root mean square of successive differences; ETCO₂, End-Tidal CO₂; V_T, tidal volume; V_E, minute ventilation; β, Beta coefficient; SE, Standard error; SD, standard deviation. Mean ± standard deviation of untransformed data are presented in this table. However, P values are based on the model with the best fit of the data. In case of (*), the best fit was with untransformed data. Cohen's d for paired data (d_p) is reported for effect size. Mixed models are expressed as beta coefficients (β_{SDB vs NPB}) ± SE and t value (Degrees of freedom (DF)). SDB-effect is the difference between SDB – NPB expressed in percentage (%).

breathing manipulations and will be discussed in separate paragraphs.

In line with our hypotheses, SDB significantly enhanced BRS and HRV compared to NPB in the present study. This is consistent with findings from other studies^{5,30,37,40,47} as well as with the idea that respiration is major modulator of cardiovascular activity.⁴ Both direct and indirect mechanisms are thought to potentially contribute to respiratory modulation of cardiovascular activity, including alterations in autonomic nervous system activity,⁶ mechanical influences of respiration on cardiac filling,¹⁸ and central processes (respiratory and cardiovascular control in the brain stem).¹⁴ Since the present study found no major change in average HR (ie, no change in cardiac vagal tone), the large influence of respiration on HRV is a cardiac vagal *phasic* response to changes in respiration frequency.²² BRS seems to depend more on BP variability, which in the present experiments was likely induced by deeper breathing and concomitant greater fluctuations in intrathoracic pressure.²⁵ Thus, although we did not measure intrathoracic pressure directly, but we assume that the fluctuations in intra-thoracic pressure were strongly increased during SDB and mildly during NPB because of the increased depth of respiration (and thus V_T). As such, we would expect enhanced BP variability induced by deep breathing and consequently enhanced baroreceptor activity.

In contrast with the advanced hypotheses, self-reported pain was not influenced by breathing condition in any of the 3 tested pain modalities. As such, findings from the present study support the conclusion drawn from a recent literature review, that the effects of SDB on pain remain far from established in

experimental research.²⁷ As we found enhancing effect of SDB on HRV and BRS, but not on pain, the findings from the 3 present experiments strongly suggest that that elevated BRS/HRV activity is not a sufficient condition for pain reduction to occur during SDB. Interestingly, also previous experimental studies, regardless of whether or not a pain dampening effect was found, concluded that there is no evidence for a clear relationship between increased respiratory-induced vagal activity and pain modulation.^{1,36} Although this can point towards the lack of appropriate measures of true changes in autonomic nervous system function during manipulation of respiration,⁵⁵ it is important to recognize that underlying mechanisms other than parasympathetic activation may be involved in instances where respiratory hypoalgesia is observed. Studies that did find a pain dampening effect of SDB on pain have proposed different mechanisms,^{7,11,12,36,50,64} and no consensus currently exists about which modulatory factors could be critical. One important factor may be relaxation, as suggested by findings of Busch et al (2012). In their study, they compared 2 breathing interventions, separated by a wash-out period of 6 months. During relaxing SDB, participants were requested to relax and to follow the verbal breathing instructions given by the experimenter, without any visual control or feedback. During attentive SDB, participants were requested to fit their own breathing curve to the ideal breathing curve presented on a monitor. Their results demonstrated that SDB effectively reduced pain only during *relaxing* SDB but not during attentive SDB. Thus, the influence of SDB on pain perception may critically depend on the way SDB is instructed and applied, potentially regardless of its cardiovascular effects. The present study did not

Table 5. Results Secondary Outcome Measurements During Pain Induction

FIRST EXPERIMENTAL RUN (WITHOUT PAIN INDUCTION)						
POOLED DATA (n = 83)						
SAM [†]	$\beta_{\text{SDB vs NPB}} \pm \text{SE}$ [T VALUE (DF)]		P			
Arousal	.01 ± .15 [.08 (79)]		.935			
Valence	−.4 ± .18 [−2.24 (79)]		<.05			
Control	−.4 ± .19 [−2.22 (79)]		<.05			
SECOND EXPERIMENTAL RUN (WITH PAIN INDUCTION)						
MPQ [*]	EXPERIMENT 1: ELECTROCUTANEOUS (n = 31)		EXPERIMENT 2: THERMAL (n = 28)		EXPERIMENT 3: MECHANICAL (n = 24)	
	MD±SEM	P	MD±SEM	P	MD±SEM	P
PRI-affective	−.196 ± .186	.419	−.304 ± .144	.047	.043 ± .175	.643
PRI-sensory	−1.339 ± .578	.052	−.125 ± .344	.753	−.109 ± .608	.687
PRI-evaluative	−.036 ± .148	.816	−.107 ± .119	.119	.109 ± .241	.798
PRI-total	−1.5 ± .741	.087	−.536 ± .461	.273	−.043±.840	.628
SAM [†]	$\beta_{\text{SDB vs NPB}} \pm \text{SE}$ [t VALUE (DF)]		$\beta_{\text{SDB vs NPB}} \pm \text{SE}$ [t VALUE (DF)]		$\beta_{\text{SDB vs NPB}} \pm \text{SE}$ [t VALUE (DF)]	
Arousal	< .01 ± .21 [<.01 (30)]	1	−.05 ± .21 [−.25 (27)]	.804	−.28 ± .22 [−1.77 (20)]	.092
Valence	.06 ± .22 [−.30 (30)]	.770	−.16 ± .2 [−.79 (27)]	.435	.19 ± .23 [.83 (20)]	.418
Control	.21 ± .21 [.99 (30)]	.328	−.25 ± .23 [−1.07 (20)]	.294	−.07 ± .13 [−.57 (20)]	.576

Abbreviations: MD, mean difference MPQ, McGill pain questionnaire; SAM, self-assessment manikin.

*Wilcoxon signed rank test: expressed as mean difference (SDB – NPB) $\pm$ Standard Error (SE).

†Linear mixed models: expressed as beta coefficients ($\beta_{\text{SDB vs NPB}}$) $\pm$ SE [(Degrees of freedom (DF)) = t value].

aim for a maximal relaxing state during neither of the breathing conditions. As a consequence, our findings on self-reported arousal and pleasantness strongly suggest that participants did not experience SDB as more relaxing compared to NPB. The absence of relaxation during SDB may therefore explain the findings in the current study. Other potentially critical factors that relate to how SDB is applied are distraction¹² and treatment expectation.⁶² Chalaye et al (2009) investigated the effect of paced breathing on pain and autonomic cardiac activity, but also controlled for distraction effects by adding a condition in which participants played a videogame.¹² Their results demonstrated that both SDB and the distraction condition increased thermal pain threshold, but pain tolerance was only affected by SDB. However, not all conditions were systematically randomized in their study. In addition, it remains unclear if the cardiac effects which were present during SDB and not during distraction in their study could explain the analgesic effects, since these are more likely to be a consequence of the manipulation of breathing rate rather than mediating respiratory-induced analgesia. However, it is clear that future studies should consider behavioral factors during paced breathing, experimentally control for them or evaluate their relative contribution in pain reduction during SDB. In the present study, both SDB and NPB were visually paced in

order to keep the level of attention and distraction comparable and neutral information about the study procedure was delivered via a computer screen. In summary, it can be expected that major insights can be gained from future studies that carefully manipulate and measure levels of relaxation, distraction, and expectation in relation to SDB.

An explorative finding in the present study was that reported pain did not change over time (ie, stimulus number) for electrocutaneous and mechanical pain. For thermal pain, however, there was an effect of stimulus number on self-reported pain levels. Post hoc analysis revealed a significant decrease of self-reported pain for both breathing conditions for the first 4 thermal stimuli, which stagnates at stimuli 5 and is no longer statistically different for the remaining stimuli. It is known that pain modalities differ in terms of the quality, temporal, and spatial characteristics and elicit a variety of responses to the painful stimuli.^{23,29} As such, it is not surprising that pain modalities show a different course over time with repeated administration. The nature of these changes is beyond the scope of this study, but nonetheless the finding highlights the importance of considering all dimensions of a pain modality when setting-up an experiment, replicate studies, and investigate the effect of any intervention on pain.

Figure 3. The effects of normal paced breathing (NPB) and slow deep breathing (SDB) on physiological outcome measurements during the first (no pain) and second (pain) run of the experiments. Results for the 'no pain' condition are based on the total number of participants, across three studies ($n = 83$) whereas results during pain induction are presented for each study separately. Abbreviation: RMSSD, root mean square of the successive differences.

Finally, it is worth mentioning possible limitations of this study. First, the present results cannot be generalized to men nor to individuals experiencing pain. The present study specifically aimed at investigating the relationship among SDB, BRS, and pain in a homogeneous group of healthy female individuals. The

relationship between BP and pain may be disturbed, reversed, or even absent in individuals with chronic pain.¹⁰ In addition, although the impact of chronic pain on respiration remains unclear in the literature, previous studies demonstrated that acute pain alters respiratory patterns (flow, volume, frequency).²⁷ As such, it is

Figure 4. Spectral analysis using the autoregressive model demonstrates the influence of breathing frequency on heart rate variability. Variations are concentrated at low frequency during slow deep breathing (SDB) and are greater compared to normal paced breathing (NPB) indicating influence of breathing on cardiac vagal activity. PSD = power spectral density.

likely that respiratory patterns are also disturbed in chronic pain, and as a consequence should be considered in studying the functional interactions between breathing, cardiovascular parameters, and pain. Because this study did not aim for exploring if gender moderates the relation between breathing condition and pain, only 1 gender was included. Since chronic pain is more prevalent in females,²⁴ only this gender was included. However, it is possible that the hormonal status (ie, menstrual cycle phase), determined by self-report, might have biased the experimental pain sensitivity.³⁵ Second, although 3 different pain modalities were used in the

present study, experimental stimuli often fail to mimic clinical pain and might therefore not be able to support the respiratory hypoalgesic effects found in clinical studies. Third, the present study aimed at investigating the role of the baroreflex system in respiratory hypoalgesia. However, since SDB significantly affected BRS but not self-reported pain it was not suitable to run a mediational model.² Future studies should explore other potential behavioural modulators such as treatment expectation, distraction, and relaxation and address these potential mechanisms by experimentally manipulating them.

Figure 5. Self-reported pain over time. Per breathing block, 10 stimuli of moderate pain (rating ≈ 6 on numeric rating scale; NRS) were delivered. Self-reported pain did not differ between slow deep breathing (SDB) and normal paced breathing (NPB), in all three experimental studies. Post hoc analysis demonstrated that in case of thermal pain (study 2), self-reported pain significantly decreased for the first four stimuli, regardless of breathing condition.

Conclusion

Overall, the present study provided no evidence for a hypoalgesic effect of SDB on electrocutaneous, mechanical, and thermal pain, despite significant increases in BRS and HRV. The current study provides several unique contributions. First of all, manipulating breathing frequency affects behavioral outcomes and respiratory parameters, which in turn affect cardiovascular parameters. In addition, manipulating breathing frequency was not considered as relaxing neither as comfortable for novices in these experiments. Although neither spontaneous breathing, nor SDB *without* breathing circuitry were included in the present study, we speculate that the additional dead space induced by the routinely used breathing circuitry may have contributed to this. Second, merely slowing down breathing frequency to .1 Hz is not sufficient to induce a reduction in pain, regardless of pain modality. Other potential mechanisms or components should be considered, such as behavioral and cognitive modulators including relaxation¹¹ and

treatment expectation.⁶² In the present study, beliefs or expectancies associated with SDB were not measured, neither manipulated experimentally, but they might be important components of SDB interventions. Further research should focus on (sub)components of SDB, and investigate how they might interact.

Authors' Contribution

J.V. is the guarantor; Study design: I.C., S.L., I.V.D., L.V.O., J.V.; Data collection: I.C.; Data processing, parameter extraction and preparing figures: I.C., A.G., H.J.; statistical analyses: I.C., A.G., H.J., L.V.O.; critically reviewing and correcting earlier drafts of the manuscript: all authors. All authors approved the final manuscript.

Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jpain.2019.10.002>.

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