Controlled breathing and pain

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Controlled breathing and pain: Respiratory rate and inspiratory loading modulate cardiovascular autonomic responses, but not pain

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Abstract

Slow, deep breathing (SDB) is a common pain self-management technique. Stimulation of the arterial baroreceptors and vagal modulation are suggested, among others, as potential mechanisms underlying the hypoalgesic effects of SDB. We tested whether adding an inspiratory load to SDB, which results in a stronger baroreceptor stimulation and vagal modulation, enhances its hypoalgesic effects. Healthy volunteers performed SDB (controlled at 0.1 Hz) with and without an inspiratory threshold load. Controlled breathing (CB) at a normal frequency (0.23 Hz) was used as an active control. Each condition lasted 90 s, included an electrical pain stimulation on the hand, and was repeated four times in a randomized order. Pain intensity, self-reported emotional responses (arousal, valence, dominance), and cardiovascular parameters (including vagally-mediated heart rate variability) were measured per trial. A cover story was used to limit the potential effect of outcome expectancy. Pain intensity was slightly lower during SDB with load compared with normal-frequency CB, but the effect was negligible (Cohens $d < 0.2$), and there was no other difference in pain intensity between the conditions. Heart rate variability was higher during SDB with/without load compared with normal-frequency CB. Using load during SDB was associated with higher heart rate variability, but less favorable emotional responses. These findings do not support the role of baroreceptor stimulation or vagal modulation in the hypoalgesic effects of SDB. Other mechanisms, such as attentional modulation, warrant further investigation.

KEYWORDS

autonomic, baroreflex, breathing exercises, pain, respiratory sinus arrhythmia, vagus nerve

1 | INTRODUCTION

Slow, deep breathing (SDB) is a common pain self-management technique. It is also a component of several non-pharmacological interventions such as hypnosis, yoga, and relaxation, which have shown by some studies to be effective for pain management (Garland et al., 2019; Tick et al., 2018). A number of clinical and experimental studies have supported the beneficial effects of SDB on pain (Jafari et al., 2017). However, the results of these previous studies have been equivocal. The underlying mechanisms are not clear and seem to be multifactorial (Jafari et al., 2017). These include cognitive (e.g., distraction, positive outcome expectancy), emotional (e.g., reduced arousal), and autonomic modulation (e.g., increased parasympathetic activity) (Jafari et al., 2017).

Slow, deep breathing has a profound impact on the cardiovascular and pulmonary systems including a phasic stimulation of the arterial baroreceptors and pulmonary stretch receptors, which increase vagal afferent signaling (Gholamrezaei et al., 2019). It is proposed that the increased pulmonary afferent signaling during deep breathing may inhibit the transmission of pain signals at the spinal or supraspinal levels (Jafari et al., 2017). Baroreceptors are a type of mechanoreceptors mainly located in the aortic arch and carotid sinus. An increase in blood pressure increases baroreceptors afferent signaling which is transmitted via the glossopharyngeal and vagus nerves to the nucleus of the solitary tract and ultimately leads to sympathoinhibition and increased cardiac vagal output (i.e., the arterial baroreceptor reflex, or baroreflex) (Benarroch, 2008). Baroreceptor and vagal nerve stimulation have shown to have hypoalgesic effects (Chakravarthy et al., 2015; Dworkin et al., 1994). The nucleus of the solitary tract and the caudal ventrolateral medulla are suggested as the main mediating pathways associating the cardiorespiratory afferents with pain processing and pain regulatory areas in the brain (Boscan et al., 2002; Tavares & Lima, 2002).

We have recently shown that adding an inspiratory threshold load to SDB is associated with increased vagal modulation. This effect seems to be mainly due to larger blood pressure swings over the respiratory cycles resulting in stronger stimulation of the arterial baroreceptors (Gholamrezaei et al., 2019). Hence, adding an inspiratory load to SDB has the potential to enhance its hypoalgesic effect, and can help to better investigate cardiovascular responses as the potential underlying mechanisms. Here we experimentally investigated the effect of SDB (with/without load) on pain. We hypothesized that SDB reduces pain, when compared with an active control condition (i.e., controlled breathing at normal frequency, CB), and that adding a load to SDB will increase its hypoalgesic effect. We also explored some putative mediating mechanisms including baroreceptor stimulation, vagal modulation, and emotional responses. By using an active control condition and a cover story to hide the true purpose of the study, we tried to limit the potential effect of expectancy on the outcomes, and to better investigate the potential underlying mechanisms beyond distraction and expectancy.

2 | METHOD

2.1 | Participants

This within-subject experimental study was conducted in the human psychophysiology laboratory of the Health Psychology Research Group, KU Leuven (Leuven, Belgium). Healthy volunteers, including males and females aged 18 to 45 years, were invited. Those who reported any of the following conditions were excluded: cardiovascular, respiratory, or neurological diseases, psychiatric disorders, acute/chronic pain, regular medication use (other than contraceptives), pregnancy, current smoking or any other nicotine consumption, and practicing breathing exercises on a regular basis. Also, those with a body mass index of <18.5 or >30 kg/m^2, score of ≥ 8 in either subscales of the Hospital Anxiety and Depression Scale (Bjelland et al., 2002; Spinhoven et al., 1997), or moderate-to-severe levels of nasal symptoms in the Nasal Obstruction Symptom Evaluation scale (Stewart et al., 2004; van Zijl et al., 2017) were excluded. Participants were asked to refrain from strenuous exercise and from caffeine and alcohol intake for at least 12 hr prior to testing. The study was approved by the Ethics Committee Research UZ/KU Leuven (#S62062), and informed consent was obtained from the participants. Testing was performed in a single 2-hr session, between 9 a.m. and 5 p.m., in a sound-attenuated and temperature-controlled (22–25°C) room.

2.2 | Instruments and measurements

2.2.1 | Respiratory measures and devices

Respiratory (chest) movement was recorded using an aneroid chest bellows attached to a pressure transducer and coupler (V94-19, V94-05, V72-25B, Coulbourn Instruments, Allentown, PA, US). A flow-independent threshold load (Threshold PEP, Respironics Inc., NJ, US) was used and set to a load of 10 cmH_2O based on our previous studies (Gholamrezaei et al., 2019, 2021). A nose clip was used to help the participant to breathe only through the mouth when using the load. A disposable filter with integrated oval shaped mouthpiece (MicroGard II Pulmonary Function Filter, Carefusion, Hoechberg, Germany) was connected to the threshold load.

2.2.2 | Electrocardiography and blood pressure

Disposable electrodes (H66LG, KendallTM, Covidien llc, MA, US) were placed in lead II configuration and the signal was amplified and band-pass filtered (gain 1 K, 0.1 to 150 Hz, V75-04, Coulbourn Instruments, Allentown, PA, US). Continuous noninvasive arterial pressure was recorded using the volume clamp method (Portapres Model-2, TNO TPD Biomedical Instrumentation, Amsterdam, the Netherlands). A cuff with appropriate size was attached to the middle phalanx of the middle or ring finger of the left hand. Arm blood pressure was measured using an automatic digital sphygmomanometer (Kodea KD-202F, Shanghai Kodea Economic & Trade Development Co., Ltd., Shanghai, China).

2.2.3 | Electrodermal activity

Disposable electrodes (EL507, Biopac Systems, Inc., Goleta, CA, US) were placed on the hypothenar palm of the left hand. Skin conductance was measured using the isolated skin conductance coupler (V71-23, Coulbourn Instruments, Allentown, PA, US).

The respiration, electrocardiography, arterial blood pressure, and skin conductance signals were recorded and digitized at 1 kHz sampling rate (16-bit PCI-6221 card, National Instruments, TX, US) using AFFECT software version 5.0 (KU Leuven, Belgium) (Spruyt et al., 2010).

2.2.4 | Pain stimulus calibration

Pain was induced using electrocutaneous stimulation (DS5, Digitimer Ltd, Hertfordshire, UK) at the wrist. Reusable electrodes (Digitimer Ltd, Hertfordshire, UK) were filled with the KY-gel (Johnson & Johnson, New Brunswick, NJ, US) and were placed on the left ulnar styloid process. To induce a dull pain, a sine wave pulse train was given at a frequency of 5 Hz (Beissner et al., 2010). Total stimulation duration was 6 s consisting of 2 s ramp up, 2 s plateau, and 2 s ramp down to zero.

The threshold for moderate pain intensity was determined using the ascending method of limits. Stimulation was started at the current of 0 mA and increased with steps of 0.1 mA until the participant rated pain intensity at a score of ≥60 (out of 100) on the numerical rating scale (see below). The inter-stimulus interval was 15 s during stimulus calibration. Calibration was performed two times and the averaged current was used as the final threshold. Stimulation was terminated at a maximum allowed current of 50 mA for safety.

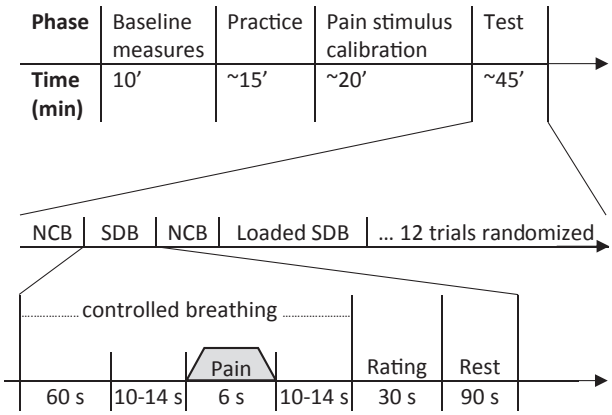

FIGURE 1 Study procedure. Each participant performed 12 trials, 90 s each. Each trial included one breathing condition and one painful stimulation. Order of conditions was randomized across the trials. There was 90 s rest between the trials. loaded SDB, slow, deep breathing with inspiratory load; NCB, normal controlled breathing; SDB, slow deep breathing

2.2.5 | Self-reported measurements

Pain intensity was assessed using a numerical rating scale with numbers visible at intervals of 5 and with anchors at 0 (no pain), 50 (moderate pain), and 100 (intolerable pain). The reliability of this scale in an experimental context has been shown by Ekblom and Hansson, who estimated that the minimum reliable change for this scale is 5 points (1988). Emotional responses were assessed in three dimensions of arousal, valence, and dominance using the 9-point Self-Assessment Manikin Scale. Scores ranged from 1 (extreme calm/pleasant/control) to 9 (extreme excited/unpleasant/lack of control) (Bradley & Lang, 1994). The Pain Catastrophizing Scale was used to measure the perceived threat value of pain in general, with a total score ranging from 0 to 52 (Severeijns et al., 2002; Sullivan et al., 1995). The short version of the Fear of Pain Questionnaire was used to measure fear of pain with the total score ranging from 9 to 45 (McNeil et al., 2018; Roelofs et al., 2005).

2.3 | Procedure

2.3.1 | Cover story

The procedure of the experiment (Figure 1) was explained to the participants. We hid the study aim from the participants to prevent the possible influence of expectation toward any of the breathing patterns on pain and other outcomes. The study was titled "pain perception and heart–lung interactions" in both the flyers and participant information/consent forms. Heart–lung coupling was briefly explained to the participants. They were told that the study's aim is to evaluate

the effect of experiencing pain on the heart–lung coupling. The efficacy of this method for blinding was checked at the end of the study (Figure 1).

2.3.2 | Baseline measures

After explaining the study's aim and procedure, electrodes/instruments were attached. Participants rested for 5 min while seated in a comfortable chair with the upper body and arms being supported. Arm blood pressure was measured two times at the end of the baseline period.

2.3.3 | Training for controlled breathing

Participants were trained to perform controlled (paced) breathing at different breathing frequencies. Using the AFFECT software version 5.0 (KU Leuven, Belgium), a visual cue (vertical bar) was continuously presented on a computer screen. Participants practiced breathing at a frequency of 0.23 Hz (14 breaths per minute), at 0.1 Hz (6 breaths per minute) without load, and then at 0.1 Hz with a load of 10 cmH_2O. The inspiration to expiration ratio was 1:2 for all conditions (Van Diest et al., 2014). Controlled breathing at a normal breathing frequency was used as an active control condition (Jafari et al., 2020). Normal breathing frequency was set at 0.23 Hz considering the average breathing rate of ~14 breaths per minute at rest among our potential participants (Van Diest et al., 2014).

For controlled breathing at the normal frequency, participants were asked to breathe in and out through the nose. For SDB without load, participants were asked to breathe in through the nose and breathe out through the mouth while pursing the lips (i.e., the pursed-lips breathing technique). For SDB with load, participants were instructed to breathe in deeply enough to open the load valve and keep it open during inspiration (Gholamrezaei et al., 2021). Tidal volume was not controlled; however, participants were asked to not perform deep breathing when breathing rate is controlled at 0.23 Hz and to perform deep but "comfortable" breathing during SDB (with/without load). The experimenter (AG) performed each breathing pattern in front of the participant for further clarification. Then, participants practiced each condition two times, each time for 1 min, under the supervision of the experimenter.

Using the modified BORG scale (Borg, 1982), dyspnea (phrased as "difficulty in breathing") was measured during the training phase to evaluate whether participants can comfortably perform SDB with a load of 10 cmH_2O. If a participant rated his/her dyspnea higher than "moderate," the load was reduced to 5 cmH_2O (this happened for two participants).

2.3.4 | Main test

The main test included three breathing conditions for all participants: controlled breathing at 0.23 Hz (or normal-frequency controlled breathing), SDB (at 0.1 Hz) without load, and SDB (at 0.1 Hz) with inspiratory threshold load of 10 cm H_2O. Each trial consisted of one type of breathing for 90 s and one painful stimulation. Pain stimulation was given at a random moment within a 30-s window at the end of the trial while the participant was performing controlled breathing. Participants rated the intensity of pain and emotional responses after each trial (Figure 1).

There was a 90-s rest period between the trials. The order of the breathing conditions was randomized across the trials for each participant. Four blocks, each composed of three trials, were generated separately for each participant using the Research Randomizer (www.randomizer.org). Having same breathing condition in two consecutive trials was avoided.

2.4 | Data reduction and analysis

All physiological signals were processed using custom-written algorithms in the MATLAB software (R2018b, Mathworks, Inc., Natick, MA, US) with the details explained in the Supporting Information. Briefly, the breathing rate and respiratory amplitude were extracted from the respiratory signal for each trial. R-wave peaks were determined in the electrocardiography signal and the RR-intervals were calculated. Considering the duration of the trial (90 s), analysis of heart rate variability (HRV) was performed for the time-domain indexes (Task Force of the European Society of Cardiology and the North American Society of Pacing & Electrophysiology, 1996). Average heart rate (representing tonic autonomic activity) and root mean square of the successive differences (RMSSD) in RR-interval (representing vagally-mediated HRV) were calculated for each trial. Studies have shown that RMSSD can be reliably measured from ultra-short records of 10 s to 1 min (Nussinovitch et al., 2011). Respiratory sinus arrhythmia (RSA, representing respiratory modulation of cardiac vagal activity) was calculated for each respiratory cycle using the peak-to-valley method and then was averaged for each trial (Gholamrezaei et al., 2019). Beat-to-beat systolic and diastolic blood pressure values were extracted from the arterial blood pressure signal, corrected based on simultaneous arm blood pressure measures at baseline, and averages of blood pressure values were calculated. The amplitude of systolic blood pressure variation during each respiratory cycle was measured using the peak-to-valley method and was averaged for each trial (Gholamrezaei et al., 2019). Cardiovagal baroreflex sensitivity was calculated using the sequence method and the following criteria were considered to define effective sequences:

≥1 mmHg change in successive systolic blood pressures; ≥5 msec change in successive RR-intervals; ≥3 heartbeats in each sequence; ≥0.8 coefficient of correlation between changes in systolic blood pressures and RR-intervals; and a lag (delay) of one beat between blood pressure and RR-interval time series (SBP_n influences RR-interval$_{n+1}$ [i.e., $tR_{n+1} - tR_n$]; see Figure S1 in the Supporting Information) (Parati et al., 1988). The amplitude of pain-evoked skin conductance response (SCR) was measured according to the available guidelines (Boucsein et al., 2012). Average skin conductance level at one second before each stimulation was taken as a baseline and a peak detection method (with a threshold of 0.03 micro siemens) was applied to find the stimulus-evoked SCR amplitude. Minimum and maximum distances between the stimulus event and SCR peak were set as 3 and 7 s, respectively.

2.5 | Statistical analyses

2.5.1 | Sample size

In a previous study on the effect of SDB on pain perception in our group, the mean difference between SDB and controlled breathing at a normal-frequency was 5 on a 100-point scale of pain intensity (Jafari et al., 2020) which is also the minimum reliable change for this scale in an experimental context (Ekblom & Hansson, 1988). We expected that using load during SDB would further decrease pain intensity by 5 points. The required sample size was calculated as 42 for the omnibus test on the main effect of breathing condition. We used the online tool GLIMMPSE (Kreidler et al., 2013) for linear mixed models. Alpha was considered as 0.05 and the desired power as 0.80. Considering a 10% drop-out rate, we aimed for 46 participants.

2.5.2 | Study outcomes

The study primary outcome was pain intensity. The secondary outcome was pain-evoked SCR to test whether SDB influences autonomic responses to pain, considering the interaction between pain and cardiovascular regulatory systems. Potential mediators included cardiovascular variables (e.g., RSA and respiratory-related blood pressure variation) and emotional responses (arousal, valence, dominance).

2.5.3 | Statistical methods

Data were averaged over (four) trials of each breathing condition. Marginal mixed models with breathing condition as a within-subject factor were performed. Log-transformation was used to approximate the residuals to normality. All pairwise contrasts were performed, and the Holm–Bonferroni (stepdown) method was applied for correcting p values for multiple comparisons (Holm, 1979). Cohen's d is reported as a measure of effect size (Lakens, 2013). Results of the contrasts are reported as differences of the least-squares means (DLSM) and standard error (SE). Demographic (gender) and psychometric (pain catastrophizing and fear of pain) and cardiovascular (HRV, systolic/diastolic blood pressure, and baroreflex sensitivity) characteristics measured at baseline were included in the models to explore possible factors associated with pain intensity and possible moderators of response to the intervention. For this aim, we used the standardized variables (to have a mean of 0 and a standard deviation of 1). Also, we performed multi-level mediation analyses, using data of all individual trials (not averaged), to test whether the difference between each pair of breathing conditions in pain is mediated via alterations in cardiovascular and/or emotional responses. This analysis contains three separate mixed models of (1) testing the effect of independent variable on dependent variable, (2) testing the effect of independent variable on mediator, and (3) testing the independent effects of the independent variable and mediator on dependent variable (see Figure 3, model A). One set of analyses was done for each potential mediator (for SAS code see ["Introduction to SAS. UCLA: Statistical Consulting Group," 2016]). Statistical significance was set at $p < .05$. All statistical analyses were performed using the SAS® Studio 3.8 (SAS Institute Inc., Cary, NC, US).

3 | RESULTS

3.1 | Participants

From a total of 72 screened volunteers, 49 were eligible and participated in the study. Five participants were excluded during the study; two because of a technical failure (and data loss), two because of adverse events (frequent coughing during SDB with load), and one due to extreme stress during pain stimulus calibration. Finally, 44 participants completed the study, including 27 females and 17 males with a mean age of 22 years (standard deviation 4, range 18 to 37). Physiological data for some participants were not appropriate for analysis due to extreme noise and their data were excluded (two SCR, one heart rate, and two blood pressure and baroreflex). Baseline and demographic parameters are summarized in Table 1.

3.2 | Manipulation check

Analysis of the respiratory measures indicates that participants could perform controlled breathing at the specified frequency and deep breathing during SDB with/without load

TABLE 1 Demographic data and baseline characteristics of the participants

	Female, $N = 27$	Male, $N = 17$
Age (year)	21.40 (3.54)	23.82 (5.36)
Weight (kg)	59.51 (9.41)	77.70 (9.15)
Height (m)	166.44 (6.87)	179.88 (5.58)
BMI (kg/m^2)	21.42 (2.86)	24.07 (3.21)
Baseline measures		
BR (per minute)	15.87 (3.18)	14.52 (2.83)
RR-interval (msec)	787.33 (108.02)	865.18 (138.39)
RMSSD (msec)	56.48 (17.61)	59.06 (17.82)
RSA (msec)	75.67 (37.20)	80.31 (44.47)
SBP (mmHg)[a]	107.0 (9.89)	116.82 (9.02)
DBP (mmHg)[a]	71.59 (6.92)	77.55 (7.86)
BRS (msec/mmHg)	11.09 (5.82)	12.84 (7.09)

Note: Data are presented as mean ± standard deviation or number (%).

Abbreviations: BMI, body mass index; BR, breathing rate; BRS, baroreflex sensitivity; DBP, diastolic blood pressure; RMSSD, root mean square of successive differences; RSA, respiratory sinus arrhythmia; SBP, systolic blood pressure.

[a]Measured at the arm level.

TABLE 2 Comparison of pain intensity and pain-evoked SCR between the breathing conditions

	NCB	SDB	Loaded SDB
Pain intensity	53.95 (2.38)	52.59 (2.36)	51.56 (2.16)[†]
SCR, μS	1.81 (0.31)	2.16 (0.34)	1.78 (0.30)

Note: Data are presented as least square means (standard error).

Abbreviations: loaded SDB, slow, deep breathing with inspiratory load; NCB, normal-frequency controlled breathing; SCR, skin conductance response; SDB, slow deep breathing.

[†]$p < .05$ between NCB and loaded SDB.

(see Table S1 in the Supporting Information). No habituation of pain ratings was observed during the test period (see Figure S2 in the Supporting Information).

3.3 | Effects of breathing condition on pain intensity and pain-evoked SCR

The effect of breathing condition on pain intensity was significant ($F(2,43) = 5.22$, $p = .009$). Pain intensity was slightly lower during SDB with load compared with normal-frequency controlled breathing (DLSM [SE] = 2.09 [0.66], $t(1,43) = 3.12$, $p = .009$, $d = 0.13$). There was no other difference between the breathing conditions for pain intensity (Table 2). The effect of breathing condition on pain-evoked SCR was not significant ($F(2,41) = 2.40$, $p = .103$, Table 2, also see Figure S3 in the Supporting Information).

3.4 | Effect of breathing condition on blood pressure

The effects of breathing condition on mean systolic ($F(2,41) = 3.56$, $p = .037$) and diastolic blood pressure ($F(2,41) = 8.47$, $p < .001$) were significant (Table 3). Compared with normal-frequency controlled breathing, mean systolic blood pressure was lower during SDB without load (DLSM [SE] = 2.12 [0.80] mmHg, $t(1,41) = 2.64$, $p = .034$, $d = 0.09$). Also, compared with normal-frequency controlled breathing, mean diastolic blood pressure was lower during SDB without load (DLSM [SE] = 2.50 [0.61] mmHg, $t(1,41) = 4.11$, $p < .001$, $d = 0.23$) and with load (DLSM [SE] = 1.91 [0.69] mmHg, $t(1,41) = 2.75$, $p = .017$, $d = 0.17$).

The effect of breathing condition on respiratory-related blood pressure variation was significant ($F(2,41) = 141.30$,

TABLE 3 Comparison of physiological measures between the breathing conditions

	NCB	SDB	Loaded SDB
Mean SBP (mmHg)[a]	118.91 (2.68)	116.79 (2.72)[*]	117.27 (2.56)
Mean DBP (mmHg)[a]	75.46 (1.63)	72.95 (1.63)[*]	73.54 (1.78)[*]
BPV (mmHg)[a]	9.82 (0.40)	23.63 (1.06)[*]	28.64 (1.30)[*,†]
HR (per minute)	72.01 (1.44)	70.26 (1.24)[*]	70.73 (1.21)[*]
RMSSD (msec)	49.22 (3.77)	82.57 (5.83)[*]	91.62 (6.14)[*,†]
RSA (msec)	97.13 (6.58)	320.64 (17.44)[*]	345.07 (17.14)[*,†]
BRS (msec/mmHg)	10.42 (0.91)	18.80 (1.60)[*]	18.44 (1.19)[*]

Note: Data are presented as least square means (standard error).

Abbreviations: BPV, blood pressure variation; BRS, baroreflex sensitivity; DBP, diastolic blood pressure; HR, heart rate; loaded SDB, slow, deep breathing with inspiratory load; NCB, normal-frequency controlled breathing; RMSSD, root mean square of the successive differences; RSA, respiratory sinus arrhythmia; SBP, systolic blood pressure; SDB, slow, deep breathing.

[a]Measured at the finger and then corrected based on measures at the arm level.

[*]$p < .05$ between NCB and SDB;

[†]$p < .05$ between NCB and Loaded SDB.

$p < .001$). Compared with normal-frequency controlled breathing, amplitude of blood pressure variation was higher during SDB without load (DLSM [SE] = 13.81 [0.88] mmHg, $t(1,41) = 15.63$, $p < .001$, $d = 2.64$) and SDB with load (DLSM [SE] = 18.82 [1.25] mmHg, $t(1,41) = 14.98$, $p < .001$, $d = 3.00$). Using load during SDB further increased respiratory-related blood pressure variation (DLSM [SE] = 5.00 [0.94] mmHg, $t(1,41) = 5.31$, $p < .001$. $d = 0.64$).

3.5 | Effects of breathing condition on heart rate and heart rate variability

Effect of breathing condition on heart rate was significant ($F(2,42) = 9.25$, $p < .001$, Table 2). Compared with normal-frequency controlled breathing, heart rate was lower during SDB without load (DLSM [SE] = 1.74 [0.40] bpm, $t(1,42) = 4.28$, $p < .001$, $d = 0.19$) and with load (DLSM [SE] = 1.27 [0.43] bpm, $t(1,42) = 2.96$, $p = .010$, $d = 0.14$). The effects of breathing condition on RMSSD ($F(2,42) = 50.26$, $p < .001$) and RSA ($F(2,42) = 161.48$, $p < .001$) were significant. Compared with normal-frequency controlled breathing, RMSSD was higher during SDB without load (DLSM [SE] = 33.34 [3.81] msec, $t(1,42) = 8.75$, $p < .001$, $d = 1.04$) and with load (DLSM [SE] = 42.39 [4.43] msec, $t(1,42) = 9.56$, $p < .001$, $d = 1.28$). Using load during SDB further increased RMSSD (DLSM [SE] = 9.05 [3.32] msec, $t(1,42) = 2.72$, $p = .009$, $d = 0.23$). Compared with normal-frequency controlled breathing, RSA was higher during SDB without load (DLSM [SE] = 223.50 [13.85] msec, $t(1,42) = 16.13$, $p < .001$, $d = 2.61$) and with load (DLSM [SE] = 247.93 [14.37] msec, $t(1,42) = 17.25$, $p < .001$, $d = 2.94$). Using load during SDB further increased RSA (DLSM [SE] = 24.42 [10.22] msec, $t(1,42) = 2.39$, $p = .021$, $d = 0.21$).

3.6 | Effect of breathing condition on baroreflex sensitivity

Effect of breathing condition on baroreflex sensitivity was significant ($F(2,42) = 42.33$, $p < .001$, Table 3). Compared with normal-frequency controlled breathing, baroreflex sensitivity was higher during SDB without load (DLSM [SE] = 8.38 [1.35] msec/mmHg, $t(1,41) = 6.17$, $p < .001$, $d = 0.99$) and SDB with load (DLSM [SE] = 8.02 [0.87] msec/mmHg, $t(1,41) = 9.20$, $p < .001$, $d = 1.16$), Table 3.

3.7 | Effects of breathing condition on emotional arousal, valence, and dominance

The effects of breathing condition on arousal ($F(2,43) = 17.26$, $p = .008$), valence ($F(2,43) = 15.97$, $p < .001$), and dominance ($F(2,43) = 14.85$, $p < .001$) were significant. Compared with SDB with load, normal-frequency controlled breathing (DLSM [SE] = 0.55 [0.11], $t(1,43) = 4.77$, $p < .001$, $d = 0.33$) and SDB without load (DLSM [SE] = 0.68 [0.13], $t(1,43) = 5.12$, $p < .001$, $d = 0.42$) were rated as more calming, Figure 2a. Similarly, normal-frequency controlled breathing (DLSM [SE] = 0.71 [0.15], $t(1,43) = 4.51$, $p < .001$, $d = 0.45$) and SDB without load (DLSM [SE] = 0.86 [0.15], $t(1,43) = 5.44$, $p < .001$, $d = 0.57$) were rated as more pleasant, Figure 2b. Also, compared with SDB with load, participants reported higher control during normal-frequency controlled breathing (DLSM [SE] =0.73 [0.18], $t(1,43) = 3.91$, $p < .001$, $d = 0.45$) and SDB without load (DLSM [SE] = 0.84 [0.15], $t(1,43) = 5.43$, $p < .001$, $d = 0.54$), Figure 2c.

3.8 | Exploring possible mechanisms of action

Multilevel mediation analysis was performed to explore whether the difference between SDB with load and normal-frequency controlled breathing in pain intensity was mediated by cardiovascular and/or emotional responses. Although the effect of SDB with load on pain was no longer significant with RSA as the possible mediator, RSA was not associated with pain intensity (Figure 3c). Arousal was positively associated with pain. However, SDB with load was associated with higher arousal (vs. normal-frequency controlled breathing), and the effect of SDB with load on pain remained significant after including arousal in the model (Figure 3d). None of the other evaluated factors mediated the influence of SDB with load on pain (see Figure S4 in the Supporting Information).

3.9 | Exploring demographic and psychophysiological factors associated with pain intensity

We explored whether demographic (gender), psychometric (pain catastrophizing, fear of pain), or baseline characteristics (HRV, systolic/diastolic blood pressure, baroreflex sensitivity) were associated with pain intensity or could moderate the effect of breathing condition on pain intensity. None of the evaluated factors were associated with pain intensity ratings or interacted with the breathing condition.

3.10 | Efficacy of the cover story

Out of 44 participants, 42 believed that the study's aim was to test the effect of experiencing pain on the heart–lung coupling. Two participants mentioned that they got suspicious

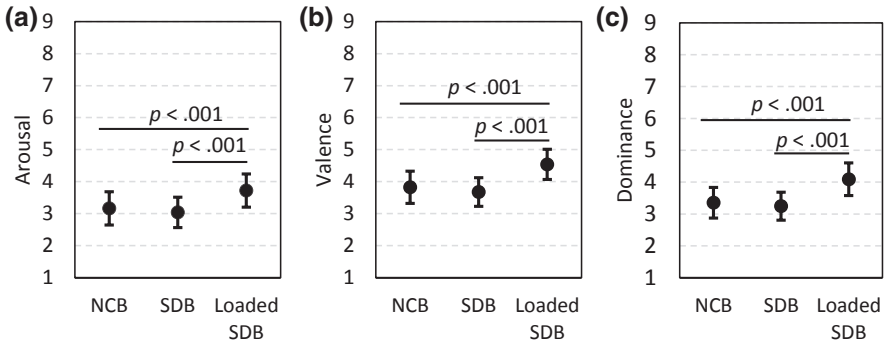

FIGURE 2 Comparison of arousal (a), valence (b), and dominance (c) between the breathing conditions. Data are presented as least square means with error bars (2 × standard error). Lower scores indicate more calm, pleasant, and perceived control states. NCB, normal controlled breathing; SDB slow deep breathing; loaded SDB, slow, deep breathing with inspiratory load

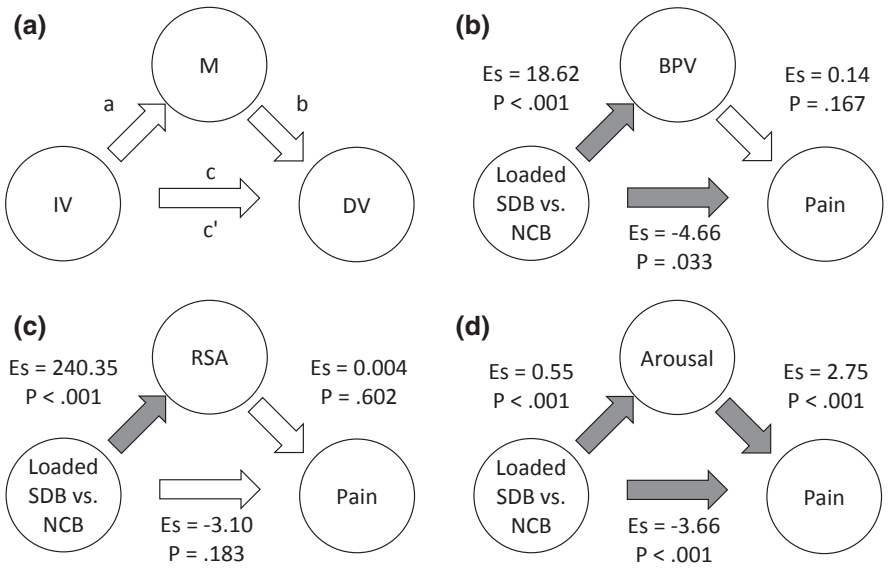

FIGURE 3 Mediation analysis. IV: independent variable, DV: dependent variable, M: mediator, a: IV → M relationship, b: M → DV relationship, c': unmediated IV → DV relationship (residual), c: IV → DV total relationship (model A). Examining the effect of loaded SDB (vs. NCB) on pain, having BPV (model B), RSA (model C), and arousal (model D) as possible mediators. BPV amplitude of systolic blood pressure variation; Es, Estimate; loaded SDB, slow, deep breathing with inspiratory load; NCB, normal controlled breathing; RSA respiratory sinus arrhythmia; SDB, slow deep breathing

about the aim of the study, but they didn't guess the actual aim and, therefore, were not excluded from the study.

4 | DISCUSSION

4.1 | Study hypotheses

We investigated whether adding an inspiratory load to SDB, which results in stronger stimulation of the arterial baroreceptors and vagal modulation (Gholamrezaei et al., 2019), enhances its hypoalgesic effects. Our first hypothesis was that SDB (with or without load) is associated with lower pain intensity compared with normal-frequency controlled breathing. Although both SDB conditions were associated with a profound increase in respiratory-related blood pressure variation and vagally-mediated HRV, only SDB with load was associated with lower pain intensity. However, the effect was small and negligible in this experimental context (Cohen's $d = 0.13$), even though it was statistically significant. The observed difference (2 points out of 100) was smaller than what can be considered a reliable change in pain report (5 points out of 100) (Ekblom & Hansson, 1988). Our second hypothesis was that pain intensity is lower during SDB with load compared with SDB without load. This was not supported by the results, even though cardiovascular responses were augmented by using a load during SDB. Based on the mediation analyses, none of the (evaluated) cardiovascular

or emotional parameters could explain (mediate) the effect of SDB with load on pain. Overall, the findings of this study suggest that the previously reported hypoalgesic effect of *controlled* breathing may not be dependent on the breathing frequency (slow or normal). Also, our findings do not support the proposed role of baroreceptor stimulation or modulation of vagal activity in the hypoalgesia associated with SDB.

4.2 | Possible mechanisms for controlled breathing hypoalgesia

4.2.1 | Stimulation of baroreceptors and vagal modulation

Stimulation of the arterial baroreceptors, mechanically (Dworkin et al., 1994; Reyes Del Paso et al., 2014) or by experimentally increasing blood pressure (D'Antono et al., 2000; Sévoz-Couche et al., 2002), has been shown to have hypoalgesic effects. Also, invasive and non-invasive vagal nerve stimulation has been shown to reduce pain (Busch et al., 2013; Chakravarthy et al., 2015). Breathing methods have profound influences on the cardiovascular system. Breath-holding after a deep inhalation or at early exhalation strongly stimulates arterial baroreceptors (Reyes del Paso et al., 2015), similar to the phase I of the Valsalva maneuver (Looga, 2005). Study by Reyes del Paso et al. found lower pain intensity during breath-holding after a deep inhalation compared with during slow inhalation (2015). By contrast, a study in our group found no difference in pain during breath-holding at different stages (following exhalation, at mid-inhalation and at full-capacity inhalation) (Jafari et al., 2016). There was no mediation or correlational analysis reported in these previous studies testing the association between pain and cardiovascular modulation. Considering the inconsistencies in findings and other factors that might explain the results (difference in attentional demand and expectancy with breath-holding) the role of baroreceptor stimulation in pain modulation by breath-holding cannot be confirmed. Regarding SDB, an increase in blood pressure and heart rate variability indexes at the frequency of breathing have consistently been shown by previous studies (Kromenacker et al., 2018; Pitzalis, 1998; Sin et al., 2010). Our previous studies suggest involvement of the baroreflex pathway and stimulation of the arterial baroreceptors in cardiovascular responses to SDB (Gholamrezaei et al., 2019, 2021). Increased parasympathetic activity is proposed as one of the mechanisms underlying the effect of SDB on pain (Jafari et al., 2017). Lower heart rate and blood pressure during the SDB conditions in our study are suggestive of an increase in parasympathetic and/or a decrease in sympathetic tonic activity (Adler et al., 2019). However, we found no association between the cardiovascular responses to SDB and pain intensity.

Previous studies on the effect of SDB on pain have reported an increase in HRV during the intervention; however, either SDB was not effective (vs. an active control condition) in reducing pain (Arsenault et al., 2013; Courtois et al., 2019; Zunhammer et al., 2013) or there was no statistical association between reduction in pain and an increase in HRV (Jafari et al., 2020; Martin et al., 2012). Whereas these studies have explored potential autonomic mechanisms statistically, Botha and colleagues applied a pharmacological manipulation to address the same question. These authors demonstrated that SDB inhibits the development of secondary visceral hyperalgesia, and that this effect is attenuated by injection of atropine (an anticholinergic agent which can inhibit vagal effects) (Botha et al., 2015). Although this finding strongly supports the involvement of autonomic modulation in the effect of SDB on pain, several factors needs to be considered. In their study, hyperalgesia was only partially prevented during SDB when atropine was injected, suggesting the involvement of other potential mechanisms in addition to autonomic modulation (Botha et al., 2015). Moreover, since no control (breathing) condition was used, it is not clear whether the antagonizing effect of atropine is specific to the slow breathing frequency and its associated autonomic responses. As atropine can act at the level of both the peripheral and central nervous systems (Green & Kitchen, 1986), it remains to be investigated at what level(s) atropine can counteract the hypoalgesic effects of SDB, and whether such an effect is specific to controlled and deep breathing at a slow frequency. Finally, Botha et al. included a selective sample of participants in whom central sensitization could be achieved by distal esophagus acid infusion which may limit the generalizability of their results. Overall, despite the strong evidence of increased baroreceptor stimulation and autonomic modulation by SDB, our findings do not support them as underlying mechanisms for the effect of SDB on pain.

4.2.2 | Expectancy and placebo effect

Despite the powerful effect of expectancy on pain (Atlas & Wager, 2012), it has not been systematically addressed, or properly controlled, in previous experiments on the influence of controlled breathing on pain. Like many other non-pharmacological interventions, controlled breathing can hardly, if at all, be blinded from the participants (Whitehead, 2004). To control the effect of expectation, an active control condition similar and credible as the main intervention should be used. Some of the previous experiments have used controlled breathing at a normal breathing frequency as an active control condition (Jafari et al., 2017). However, whether it is creating a similar outcome expectancy as with SDB has not been evaluated. Moreover, the experimenter's expectation of the intervention effect, via non/-verbal

behaviors, may influence participants' expectations and modulate pain reports (Chen et al., 2019). Accordingly, we hid the study aim from the participants using a cover story. Since blindness was successful for almost all participants, the observed effect of SDB with load on pain, though small, is less likely to be due to expectation.

4.2.3 | Attentional modulation

Under certain conditions, re-allocating attention (distraction) away from pain can reduce perceived pain intensity in a dose–response relationship (Torta et al., 2017). A possible mechanism for controlled breathing hypoalgesia is attentional modulation. Controlled breathing requires sustained attention to an external (visual, auditory) or internal (counting) cues and altering and monitoring breathing behavior. This can re-allocate attention from pain to breathing which in turn may influence pain perception.

Controlled breathing at both a normal frequency (~14 breaths/min) and a slow frequency (6 breaths/min) has been found to reduce pain compared with *uncontrolled* breathing (Jafari et al., 2020). This effect may be attributed to attentional modulation of pain. However, the results of previous experiments on the effect of *controlled* breathing at a slow frequency (vs. at a normal frequency) on pain measures have not been consistent, and some studies found no specific effect for breathing frequency (Jafari et al., 2017). The effect of attention modulation on pain depends on distracter characteristics, and to what extent it interferes with attention to pain (Torta et al., 2017). Accordingly, the reported differences between controlled breathing at slow and normal frequencies in pain might be due to different attentional demands by these breathing patterns. However, as we mentioned above, the role of expectancy has not been ruled out in these previous studies. We do not know whether the small and negligible difference in pain when adding load to SDB in this study was due to increasing the attentional demand required for loaded breathing. To our knowledge, there is no report on the degree of distraction during controlled breathing at different frequencies or with different techniques. Measuring evoked-related potentials (Legrain et al., 2013) can help to better evaluate attentional modulation during controlled breathing exercises in future studies.

4.2.4 | Emotional modulation

Breathing behavior can influence emotion (Del Negro et al., 2018) which in turn can modulate pain perception (Kornelsen et al., 2019). Lowering breathing rate may reduce the excitatory input to the locus coeruleus and reduce arousal (Yackle et al., 2017). Slow, deep breathing can reduce arousal in a threatening situation such as pain anticipation, though this effect may be, at least in part, due to distraction (McCaul et al., 1979). It may also influence emotion by increasing parasympathetic activity and decreasing sympathetic activity. This may be through projections of the cardiorespiratory vagal afferents, via nucleus of the solitary tract, to the primary interoceptive cortex (insula), and asymmetric central processing and regulation of emotions and autonomic activity (sympathovagal balance) (Strigo & Craig, 2016). However, we found no effect of SDB without load (vs. control) on emotional responses. In the current study, and for feasibility reasons, we evaluated emotional responses retrospectively after, but not during, each trial. Therefore, emotional responses were perhaps influenced by both the breathing condition and the perceived pain intensity. Findings of the current study suggest that simply reducing the breathing rate may not be able to alter emotional state when anticipating/experiencing a painful stimulation. To induce an emotional state with SDB that can reduce pain, it is perhaps necessary to use specific instructions that induce positive emotion, such as relaxation (Busch et al., 2012).

In our study, adding load to SDB had a negative impact on the emotional state. This might decrease its potential hypoalgesic effect. Some studies have shown that heightened arousal may attenuate baroreceptor modulation of nociception (Martins et al., 2009; McIntyre et al., 2006). We tried to minimize such possible counteracting effect by using a low-to-moderate load (Ubolsakka-Jones et al., 2017). Practice for a longer period may be able to modulate the emotional responses to loaded breathing. It is warranted to test whether adding specific instructions for emotion induction by SDB enhances its hypoalgesic effect (Busch et al., 2012).

4.3 | Study limitations

Our study had strengths as well as limitations. We used a cover story to blind participants about the study aim and used an active control condition to limit the potential effect of expectancy on the outcomes. Accordingly, we could not measure the outcome expectancy before the test. We did not include an uncontrolled breathing condition in this study since previous studies, including ours (Jafari et al., 2020), have shown the effect of controlled (vs. uncontrolled) breathing on pain. We did not measure attentional demand in different breathing conditions. Therefore, whether the small effect of SDB with load on pain was due to differences in attentional demand could not be evaluated. We measured emotion retrospectively after each trial. Therefore, responses might be influenced by both the breathing condition and perceived pain intensity. Another potential limitation was the negative impact of adding a load to SDB on the emotional state which might attenuate its hypoalgesic effects (Martins et al., 2009;

McIntyre et al., 2006). Also, the duration of each trial was limited to 90 s for the feasibility of loaded breathing and the study was done in a single session. Therefore, or results cannot be generalized to practices that involve longer duration of SDB (e.g., 15 min) or when it is practiced for long-term (Lehrer et al., 2003).

5 | CONCLUSIONS

We found no hypoalgesic effect for SDB when it is compared with an active control breathing condition. Adding an inspiratory load to SDB increased baroreceptor stimulation and modulation of vagal activity and slightly reduced pain intensity. However, the latter effect on pain was negligible and cannot be attributed to the cardiovascular/autonomic responses. Whether and to what extent the hypoalgesic effect of SDB reported by previous studies is due to distraction and/or expectation needs further careful investigation. Considering its clinical utility (Ublosakka-Jones et al., 2018), it is warranted to test whether and through which psychophysiological mechanisms SDB can influence pain when practiced for a longer period of time, and in individuals with various acute and chronic pain conditions.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Ali Gholamrezaei: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Software; Writing-original draft; Writing-review & editing. **Ilse Van Diest:** Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Writing-review & editing. **Qasim Aziz:** Conceptualization; Methodology; Supervision; Writing-review & editing. **Johan W. S. Vlaeyen:** Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Writing-review & editing. **Lukas Van Oudenhove:** Conceptualization; Funding acquisition; Methodology; Resources; Supervision; Writing-review & editing.

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REFERENCES

Adler, T. E., Coovadia, Y., Cirone, D., Khemakhem, M. L., & Usselman, C. W. (2019). Device-guided slow breathing reduces blood pressure and sympathetic activity in young normotensive individuals of both sexes. *Journal of Applied Physiology*, *8*(2), 2019. https://doi.org/10.1152/japplphysiol.00442.2019

Arsenault, M., Ladouceur, A., Lehmann, A., Rainville, P., & Piché, M. (2013). Pain modulation induced by respiration: Phase and frequency effects. *Neuroscience*, *252*, 501–511. https://doi.org/10.1016/j.neuroscience.2013.07.048

Atlas, L. Y., & Wager, T. D. (2012). How expectations shape pain. *Neuroscience Letters*, *520*(2), 140–148. https://doi.org/10.1016/j.neulet.2012.03.039

Beissner, F., Brandau, A., Henke, C., Felden, L., Baumgärtner, U., Treede, R.-D., Oertel, B. G., & Lötsch, J. (2010). Quick discrimination of A(delta) and C fiber mediated pain based on three verbal descriptors. *PLoS ONE*, *5*(9), e12944. https://doi.org/10.1371/journal.pone.0012944

Benarroch, E. E. (2008). The arterial baroreflex: Functional organization and involvement in neurologic disease. *Neurology*, *71*(21), 1733–1738. https://doi.org/10.1212/01.wnl.0000335246.93495.92

Bjelland, I., Dahl, A. A., Haug, T. T., & Neckelmann, D. (2002). The validity of the hospital anxiety and depression scale. *Journal of Psychosomatic Research*, *52*(2), 69–77. https://doi.org/10.1016/S0022-3999(01)00296-3

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise*, *14*(5), 377–381. https://doi.org/10.1249/00005768-198205000-00012

Boscan, P., Pickering, A. E., & Paton, J. F. R. (2002). The nucleus of the solitary tract: An integrating station for nociceptive and cardiorespiratory afferents. *Experimental Physiology*, *87*(2), 259–266. https://doi.org/10.1113/eph8702353

Botha, C., Farmer, A. D., Nilsson, M., Brock, C., Gavrila, A. D., Drewes, A. M., Knowles, C. H., & Aziz, Q. (2015). Preliminary report: Modulation of parasympathetic nervous system tone influences oesophageal pain hypersensitivity. *Gut*, *64*(4), 611–617. https://doi.org/10.1136/gutjnl-2013-306698

Boucsein, W., Fowles, D. C., Grimnes, S., Ben-Shakhar, G., Roth, W. T., Dawson, M. E., & Filion, D. L. (2012). Publication recommendations for electrodermal measurements. *Psychophysiology*, *49*(8), 1017–1034. https://doi.org/10.1111/j.1469-8986.2012.01384.x

Bradley, M. M., & Lang, P. J. (1994). Measuring emotion: The self-assessment manikin and the semantic differential. *Journal of Behavior Therapy and Experimental Psychiatry*, *25*(1), 49–59. https://doi.org/10.1016/0005-7916(94)90063-9

Busch, V., Magerl, W., Kern, U., Haas, J., Hajak, G., & Eichhammer, P. (2012). The effect of deep and slow breathing on pain perception, autonomic activity, and mood processing—An experimental study. *Pain Medicine*, *13*(2), 215–228. https://doi.org/10.1111/j.1526-4637.2011.01243.x

Busch, V., Zeman, F., Heckel, A., Menne, F., Ellrich, J., & Eichhammer, P. (2013). The effect of transcutaneous vagus nerve stimulation on pain perception—An experimental study. *Brain Stimulation*, *6*(2), 202–209. https://doi.org/10.1016/j.brs.2012.04.006

Chakravarthy, K., Chaudhry, H., Williams, K., & Christo, P. J. (2015). Review of the uses of vagal nerve stimulation in chronic pain management. *Current Pain and Headache Reports*, *19*(12), 54. https://doi.org/10.1007/s11916-015-0528-6

Chen, P. A., Cheong, J. H., Jolly, E., Elhence, H., Wager, T. D., & Chang, L. J. (2019). Socially transmitted placebo effects. *Nature Human Behaviour*, *3*, 1295–1305. https://doi.org/10.1038/s41562-019-0749-5

Courtois, I., Gholamrezaei, A., Jafari, H., Lautenbacher, S., Van Diest, I., Van Oudenhove, L., & Vlaeyen, J. W. S. (2019). Respiratory hypoalgesia? The effect of slow deep breathing on electrocutaneous, thermal, and mechanical pain. *The Journal of Pain: Official Journal of the American Pain Society*, *21*, 616–632. https://doi.org/10.1016/j.jpain.2019.10.002

D'Antono, B., Ditto, B., Sita, A., & Miller, S. B. (2000). Cardiopulmonary baroreflex stimulation and blood pressure-related hypoalgesia. *Biological Psychology*, *53*(2–3), 217–231. https://doi.org/10.1016/S0301-0511(00)00044-2

Del Negro, C. A., Funk, G. D., & Feldman, J. L. (2018). Breathing matters. *Nature Reviews Neuroscience*, *19*(6), 351–367. https://doi.org/10.1038/s41583-018-0003-6

Dworkin, B. R., Elbert, T., Rau, H., Birbaumer, N., Pauli, P., Droste, C., & Brunia, C. H. (1994). Central effects of baroreceptor activation in humans: Attenuation of skeletal reflexes and pain perception. *Proceedings of the National Academy of Sciences of the United States of America*, *91*(14), 6329–6333. https://doi.org/10.1073/pnas.91.14.6329

Ekblom, A., & Hansson, P. (1988). Pain intensity measurements in patients with acute pain receiving afferent stimulation. *Journal of Neurology Neurosurgery and Psychiatry*, *51*(4), 481–486. https://doi.org/10.1136/jnnp.51.4.481

Garland, E. L., Brintz, C. E., Hanley, A. W., Roseen, E. J., Atchley, R. M., Gaylord, S. A., Faurot, K. R., Yaffe, J., Fiander, M., & Keefe, F. J. (2019). Mind-body therapies for opioid-treated pain: A systematic review and meta-analysis. *JAMA Internal Medicine*, *84112*, 1–15. https://doi.org/10.1001/jamainternmed.2019.4917

Gholamrezaei, A., Van Diest, I., Aziz, Q., Vlaeyen, J. W. S., & Van Oudenhove, L. (2019). Influence of inspiratory threshold load on cardiovascular responses to controlled breathing at 0.1 Hz. *Psychophysiology*, *56*(11), e13447. https://doi.org/10.1111/psyp.13447

Gholamrezaei, A., Van Diest, I., Aziz, Q., Vlaeyen, J. W. S., & Van Oudenhove, L. (2021). Psychophysiological responses to various slow, deep breathing techniques. *Psychophysiology*, *58*(2), e13712. https://doi.org/10.1111/psyp.13712

Green, P. G., & Kitchen, I. (1986). Antinociception opioids and the cholinergic system. *Progress in Neurobiology*, *26*(2), 119–146. https://doi.org/10.1016/0301-0082(86)90002-X

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Source: Scandinavian Journal of Statistics*, *6*(2), 65–70. https://doi.org/10.2307/4615733

Introduction to SAS. (2016). Introduction to SAS. UCLA: Statistical consulting. https://stats.idre.ucla.edu/sas/faq/how-can-i-perform-mediation-with-multilevel-data-method-2/

Jafari, H., Courtois, I., Van den Bergh, O., Vlaeyen, J. W. S., & Van Diest, I. (2017). Pain and respiration: A systematic review. *Pain*, *158*(6), 995–1006. https://doi.org/10.1097/j.pain.0000000000000865

Jafari, H., Gholamrezaei, A., Franssen, M., Van Oudenhove, L., Aziz, Q., Van den Bergh, O., Vlaeyen, J. W. S., & Van Diest, I. (2020). Can slow deep breathing reduce pain? An experimental study exploring mechanisms. *The Journal of Pain: Official Journal of the American Pain Society*, *21*, 1018–1030. https://doi.org/10.1016/j.jpain.2019.12.010

Jafari, H., Van de Broek, K., Plaghki, L., Vlaeyen, J. W. S., Van den Bergh, O., & Van Diest, I. (2016). Respiratory hypoalgesia? Breath-holding, but not respiratory phase modulates nociceptive flexion reflex and pain intensity. *International Journal of Psychophysiology*, *101*, 50–58. https://doi.org/10.1016/j.ijpsycho.2016.01.005

Kornelsen, J., McIver, T. A., & Stroman, P. W. (2019). Unique brain regions involved in positive versus negative emotional modulation of pain. *Scandinavian Journal of Pain*, *19*(3), 583–596. https://doi.org/10.1515/sjpain-2018-0341

Kreidler, S. M., Muller, K. E., Grunwald, G. K., Ringham, B. M., Coker-Dukowitz, Z. T., Sakhadeo, U. R., & Glueck, D. H. (2013). GLIMMPSE: Online power computation for linear models with and without a baseline covariate. *Journal of Statistical Software*, *54*(10), i10. https://doi.org/10.18637/jss.v054.i10

Kromenacker, B. W., Sanova, A. A., Marcus, F. I., Allen, J. J. B., & Lane, R. D. (2018). Vagal mediation of low-frequency heart rate variability during slow yogic breathing. *Psychosomatic Medicine*, *80*(6), 581–587. https://doi.org/10.1097/PSY.0000000000000603

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*(November), 1–12. https://doi.org/10.3389/fpsyg.2013.00863

Legrain, V., Crombez, G., Plaghki, L., & Mouraux, A. (2013). Shielding cognition from nociception with working memory. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *49*(7), 1922–1934. https://doi.org/10.1016/j.cortex.2012.08.014

Lehrer, P. M., Vaschillo, E., Vaschillo, B., Lu, S.-E., Eckberg, D. L., Edelberg, R., Shih, W. J., Lin, Y., Kuusela, T. A., Tahvanainen, K. U. O., & Hamer, R. M. (2003). Heart rate variability biofeedback increases baroreflex gain and peak expiratory flow. *Psychosomatic Medicine*, *65*(5), 796–805. https://doi.org/10.1097/01.psy.0000089200.81962.19

Looga, R. (2005). The Valsalva manoeuvre—Cardiovascular effects and performance technique: A critical review. *Respiratory Physiology and Neurobiology*, *147*(1), 39–49. https://doi.org/10.1016/j.resp.2005.01.003

Martin, S. L., Kerr, K. L., Bartley, E. J., Kuhn, B. L., Palit, S., Terry, E. L., DelVentura, J. L., & Rhudy, J. L. (2012). Respiration-induced hypoalgesia: Exploration of potential mechanisms. *Journal of Pain*, *13*(8), 755–763. https://doi.org/10.1016/j.jpain.2012.05.001

Martins, A. Q., Ring, C., McIntyre, D., Edwards, L., & Martin, U. (2009). Effects of unpredictable stimulation on pain and nociception across the cardiac cycle. *Pain*, *147*(1–3), 84–90. https://doi.org/10.1016/j.pain.2009.08.016

McCaul, K. D., Solomon, S., & Holmes, D. S. (1979). Effects of paced respiration and expectations on physiological and psychological responses to threat. *Journal of Personality and Social Psychology*, *37*(4), 564–571. https://doi.org/10.1037//0022-3514.37.4.564

McIntyre, D., Edwards, L., Ring, C., Parvin, B., & Carroll, D. (2006). Systolic inhibition of nociceptive responding is moderated by arousal. *Psychophysiology*, *43*(3), 314–319. https://doi.org/10.1111/j.1469-8986.2006.00407.x

McNeil, D. W., Kennedy, S. G., Randall, C. L., Addicks, S. H., Wright, C. D., Hursey, K. G., & Vaglienti, R. (2018). Fear of pain questionnaire-9: Brief assessment of pain-related fear and anxiety. *European Journal of Pain*, *22*(1), 39–48. https://doi.org/10.1002/ejp.1074

Nussinovitch, U., Elishkevitz, K. P., Katz, K., Nussinovitch, M., Segev, S., Volovitz, B., & Nussinovitch, N. (2011). Reliability of ultra-short ECG indices for heart rate variability. *Annals of Noninvasive Electrocardiology*, *16*(2), 117–122. https://doi.org/10.1111/j.1542-474X.2011.00417.x

Parati, G., Di Rienzo, M., Bertinieri, G., Pomidossi, G., Casadei, R., Groppelli, A., Pedotti, A., Zanchetti, A., & Mancia, G. (1988). Evaluation of the baroreceptor-heart rate reflex by 24-hour intra-arterial blood pressure monitoring in humans. *Hypertension*, *12*(2), 214–222. https://doi.org/10.1161/01.hyp.12.2.214

Pitzalis, M. (1998). Effect of respiratory rate on the relationships between RR interval and systolic blood pressure fluctuations: A frequency-dependent phenomenon. *Cardiovascular Research*, *38*(2), 332–339. https://doi.org/10.1016/S0008-6363(98)00029-7

Reyes del Paso, G. A., Ladrón, M., de Guevara, C., & Montoro, C. I. (2015). Breath-holding during exhalation as a simple manipulation to reduce pain perception. *Pain Medicine*, *16*(9), 1835–1841. https://doi.org/10.1111/pme.12764

Reyes del Paso, G. A., Montoro, C., Muñóz Ladrón de Guevara, C., Duschek, S., & Jennings, J. R. (2014). The effect of baroreceptor stimulation on pain perception depends on the elicitation of the reflex cardiovascular response: Evidence of the interplay between the two branches of the baroreceptor system. *Biological Psychology*, *101*(1), 82–90. https://doi.org/10.1016/j.biopsycho.2014.07.004

Roelofs, J., Peters, M. L., Deutz, J., Spijker, C., & Vlaeyen, J. W. S. (2005). The fear of pain questionnaire (FPQ): Further psychometric examination in a non-clinical sample. *Pain*, *116*(3), 339–346. https://doi.org/10.1016/j.pain.2005.05.003

Severeijns, R., Van Den Hout, M. A., Vlaeyen, J. W. S., & Picavet, H. S. J. (2002). Pain catastrophizing and general health status in a large Dutch community sample. *Pain*, *99*(1–2), 367–376. https://doi.org/10.1016/S0304-3959(02)00219-1

Sévoz-Couche, C., Hamon, M., & Laguzzi, R. (2002). Antinociceptive effect of cardiopulmonary chemoreceptor and baroreceptor reflex activation in the rat. *Pain*, *99*(1–2), 71–81. https://doi.org/10.1016/S0304-3959(02)00055-6

Sin, P. Y. W., Galletly, D. C., & Tzeng, Y. C. (2010). Influence of breathing frequency on the pattern of respiratory sinus arrhythmia and blood pressure: Old questions revisited. *American Journal of Physiology. Heart and Circulatory Physiology*, *298*(5), H1588–H1599. https://doi.org/10.1152/ajpheart.00036.2010

Spinhoven, P., Ormel, J., Sloekers, P. P., Kempen, G. I., Speckens, A. E., & Van Hemert, A. M. (1997). A validation study of the hospital anxiety and depression scale (HADS) in different groups of Dutch subjects. *Psychological Medicine*, *27*(2), 363–370. https://doi.org/10.1017/s0033291796004382

Spruyt, A., Clarysse, J., Vansteenwegen, D., Baeyens, F., & Hermans, D. (2010). Affect 4.0: A free software package for implementing psychological and psychophysiological experiments. *Experimental Psychology*, *57*(1), 36–45. https://doi.org/10.1027/1618-3169/a000005

Stewart, M. G., Witsell, D. L., Smith, T. L., Weaver, E. M., Yueh, B., & Hannley, M. T. (2004). Development and validation of the nasal obstruction symptom evaluation (NOSE) scale. *Otolaryngology - Head and Neck Surgery*, *130*(2), 157–163. https://doi.org/10.1016/j.otohns.2003.09.016

Strigo, I. A., & Craig, A. D. (Bud). (2016). Interoception, homeostatic emotions and sympathovagal balance. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *371*(1708), 1–9. https://doi.org/10.1098/rstb.2016.0010

Sullivan, M. J. L., Bishop, S. R., & Pivik, J. (1995). The pain catastrophizing scale: Development and validation. *Psychological Assessment*, *7*(4), 524–532. https://doi.org/10.1037/1040-3590.7.4.524

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation and clinical use. *Circulation*, *93*(5), 1043–1065. https://doi.org/10.1161/01.CIR.93.5.1043

Tavares, I., & Lima, D. (2002). The caudal ventrolateral medulla as an important inhibitory modulator of pain transmission in the spinal cord. *Journal of Pain*, *3*(5), 337–346. https://doi.org/10.1054/jpai.2002.127775

Tick, H., Nielsen, A., Pelletier, K. R., Bonakdar, R., Simmons, S., Glick, R., Ratner, E., Lemmon, R. L., Wayne, P., & Zador, V. (2018). Evidence-based nonpharmacologic strategies for comprehensive pain care: The consortium pain task force white paper. *Explore*, *14*(3), 177–211. https://doi.org/10.1016/j.explore.2018.02.001

Torta, D. M., Legrain, V., Mouraux, A., & Valentini, E. (2017). Attention to pain! A neurocognitive perspective on attentional modulation of pain in neuroimaging studies. *Cortex*, *89*, 120–134. https://doi.org/10.1016/j.cortex.2017.01.010

Ublosakka-Jones, C., Tongdee, P., Pachirat, O., & Jones, D. A. (2018). Slow loaded breathing training improves blood pressure, lung capacity and arm exercise endurance for older people with treated and stable isolated systolic hypertension. *Experimental Gerontology*, *108*(March), 48–53. https://doi.org/10.1016/j.exger.2018.03.023

Ubolsakka-Jones C., Sangthong B., Khrisanapant W., Jones D. A. (2017). The effect of slow-loaded breathing training on the blood pressure response to handgrip exercise in patients with isolated systolic hypertension. *Hypertension Research*, *40*, (10), 885–891. http://dx.doi.org/10.1038/hr.2017.54.

Van Diest, I., Verstappen, K., Aubert, A. E., Widjaja, D., Vansteenwegen, D., & Vlemincx, E. (2014). Inhalation/Exhalation ratio modulates the effect of slow breathing on heart rate variability and relaxation. *Applied Psychophysiology and Biofeedback*, *39*(3–4), 171–180. https://doi.org/10.1007/s10484-014-9253-x

van Zijl, F. V. W. J., Timman, R., & Datema, F. R. (2017). Adaptation and validation of the Dutch version of the nasal obstruction symptom evaluation (NOSE) scale. *European Archives of Oto-Rhino-Laryngology*, *274*(6), 2469–2476. https://doi.org/10.1007/s00405-017-4486-y

Whitehead, W. E. (2004). Control groups appropriate for behavioral interventions. *Gastroenterology*, *126*(1 Suppl. 1), S159–S163. https://doi.org/10.1053/j.gastro.2003.10.038

Yackle, K., Schwarz, L. A., Kam, K., Sorokin, J. M., Huguenard, J. R., Feldman, J. L., Luo, L., & Krasnow, M. A. (2017). Breathing control center neurons that promote arousal in mice. *Science*, *355*(6332), 1411–1415. https://doi.org/10.1126/science.aai7984

Zunhammer, M., Eichhammer, P., & Busch, V. (2013). Do cardiorespiratory variables predict the antinociceptive effects of deep and slow breathing? *Pain Medicine*, *14*(6), 843–854. https://doi.org/10.1111/pme.12085

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

FIGURE S1 Alignment of systolic blood pressure (SBP) and RR-interval time series for analyses. Arrows indicate the assumed effect of SBP on RR-interval via the arterial baroreflex

having a lag (delay) of +1 beat; SBP *n* was assumed to influence RR-interval *n*+1 (i.e., *t*R *n*+1 – *t*R *n*) (Davies et al., 2001; La Rovere, Pinna, & Raczak, 2008)

TABLE S1 Comparison of respiratory measures between the breathing conditions

FIGURE S2 Pain intensity across 12 trials. Results show no habituation during the study, but a small increase from trial 1 to 12

FIGURE S3 Skin conductance response (SCR, averaged signal) between breathing conditions. ITL inspiratory threshold load; NCB normal controlled breathing; SDB slow deep breathing

TABLE S2 Comparison of pain intensity and pain-evoked SCR between the breathing conditions

TABLE S3 Comparison of physiological measures between the breathing conditions

FIGURE S4 Analysis of possible mediators for the effect of loaded SDB vs. NCB on pain intensity. BRS, baroreflex sensitivity; DBP, diastolic blood pressure; HR, heart rate; ITL, inspiratory threshold load; NCB, normal controlled breathing; RMSSD, root mean square of successive difference; SBP, systolic blood pressure; SDB, slow deep breathing

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