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RESPIRATORY, AUTONOMIC, AND EXPERIENTIAL RESPONSES TO REPEATED INHALATIONS OF 20% CO₂ ENRICHED AIR IN PANIC DISORDER, SOCIAL PHOBIA, AND HEALTHY CONTROLS

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Abstract

Inhalation of carbon-dioxide (CO₂) enriched air triggers anxiety in panic disorder (PD) patients, which is often interpreted as a sign of biological vulnerability. However, most studies have not measured respiration in these tasks. We compared patients with PD (N=20) and social phobia (SP, N=19) to healthy controls (N=18) during eight inhalations of 20% CO₂, preceded and followed by two inhalations of room air, while continuously measuring subjective anxiety and dyspnea as well as autonomic and respiratory variables. PD patients showed increased reactivity and delayed recovery during CO₂ inhalations for most measures. Unlike both other groups, the PD group's tidal volume responses did not habituate across CO₂ inhalations. However, PD patients did not differ from SP patients on most other measures, supporting a continuum model of CO₂ sensitivity across anxiety disorders. Both patient groups showed continued reactivity during the last air inhalations, which is unlikely to be due to a biological sensitivity.

Keywords

anxiety disorder; carbon dioxide; habituation; sensitization; psychophysiology; biological challenge

Introduction

Panic disorder (PD) is characterized by episodes of psychophysiological symptoms such as racing heart, breathlessness, and intense anxiety accompanied by feelings of dying or losing control. These panic attacks typically give rise to chronic fear about the meaning of the attacks and their possible recurrence (Diagnostic and Statistical Manual of Mental

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Disorders, DSM-IV, American Psychiatric Association, 1994). The last decades have seen considerable research aiming to unravel the processes underlying this debilitating disorder. Its impressive bodily symptoms and distorted cognitions have motivated researchers to search for both the biological and cognitive factors behind it.

Panic attacks, characterized by a massive autonomic surge, strong fight-or-flight behavioral action tendencies, and extreme fear (Barlow, Brown, & Craske, 1994), lie at the heart of PD and are thought to be crucial for its development and for the development of agoraphobia (Bouton, Mineka, & Barlow, 2001). Since capturing naturally occurring panic attacks has proven extremely difficult, researchers often have turned to laboratory provocations to trigger these attacks under controlled conditions. Among a variety of methods used to provoke panic symptoms, the inhalation of carbon dioxide (CO₂) enriched air is one of the most valid, since it induces a pattern of symptoms (including breathlessness, hyperventilation, and accelerated heart rate), that closely resembles naturally occurring attacks (Rassovsky & Kushner, 2003).

A number of studies using this provocation have reported that PD patients respond to CO₂ inhalation with more anxiety and panic symptoms than patients with other anxiety disorders or healthy individuals (see Rassovsky & Kushner, 2003, for review), which is one basis of the suffocation alarm theory (Klein, 1993). According to this theory, a brainstem-based suffocation alarm center is overly sensitive in PD, so that PD patients respond to rises in arterial CO₂ with exaggerated compensatory ventilation and with feelings of panic.

This theory has stimulated much research. Some pharmacologic and genetic studies argue for a genetic basis of such a suffocation alarm center (Bellodi et al., 1998; Bertani, Perna, Arancio, Caldirola, & Bellodi, 1997; Coryell, Dindo, Fyer, & Pine, 2006), while other studies question the specificity and causality of a suffocation alarm center as the sole mechanism underlying PD. First, PD patients respond anxiously not only to CO₂ inhalation but also to a wide range of chemical agents (see Rassovsky & Kushner, 2003, for review). Second, the finding that patients with social phobia, (SP, Caldirola, Perna, Arancio, Bertani, & Bellodi, 1997; Gorman et al., 1990), premenstrual dysphoric disorder (Kent et al., 2001), and bipolar disorder (Mackinnon, Craighead, & Lorenz, 2008) also respond to CO₂ with panic, suggests that CO₂ hypersensitivity is not unique to PD and not its sole cause. Third, cognitive factors are known to influence the anxiety response to CO₂ inhalation to a considerable degree (see Rapee, 1995, for review).

Most earlier studies used single or double breath inhalations of highly concentrated (35%) CO₂ enriched air (termed CO₂ in the following) and focused on whether participants reported panic attacks (e.g., Gorman et al., 1990; Griez, Deloof, Pols, Zandbergen, & Lousberg, 1990; van den Hout, van der Molen, Griez, Lousberg, & Nansen, 1987). In these paradigms, more frequent reports of panic or of greater anxiety increases in a given group were considered evidence of elevated CO₂ sensitivity. However, it soon became clear that focusing on the frequency of panic and degree of experienced anxiety was too narrow because it left out a host of psychological factors and respiratory processes before, during and after the exposures to CO₂. Thus, to capture more fully these factors and their time course, paradigms featuring repeated CO₂ inhalations came into use. Beck and coworkers, for example, examined PD patients and high PD risk participants during repeated exposures to 20% and 35% CO₂. They found that subgroups of high risk participants and PD patients showed an increase in anxiety and autonomic responses across repeated exposures, which they attributed to sensitization. Another subgroup showed the opposite pattern, habituation across exposures (Beck & Shipherd, 1997; Beck, Shipherd, & Read, 1999; Beck, Shipherd, & Zebb, 1997; Beck & Wolf, 2001). However, none of these reports included healthy or low

PD risk control groups, thus leaving the specificity of findings unclear. Furthermore, respiration, either as experienced or as physiologically measured, was often left unassessed.

The present study had three major aims. The first was to replicate earlier findings of *CO₂ sensitivity* in PD and to determine its specificity to PD by including a clinically anxious control group (patients with SP) in addition to a healthy control group (HC). Our task administered eight inhalations of 20% *CO₂*, a concentration that was lower than in many previous studies, and thus was less likely to produce the disruptive psychological and physiological ceiling effects of higher concentrations (cf, Gorman et al., 1990). Our second aim was to conduct a finer grained analysis of the *time course* of responding during *CO₂* exposure. By measuring subjective, autonomic, and respiratory responding at a higher temporal resolution, we are able to observe both processes *across* *CO₂* inhalations such as habituation and sensitization and *within* each inhalation such as reactivity to and recovery from individual inhalations. Sensitization (or lack of habituation) has been implicated in the development of increasing intensity of panic attacks in PD (Bouton et al., 2001) and therefore is a candidate etiological mechanism. Furthermore, on the experiential level, anxiety and dyspnea (breathlessness) were distinguished, the latter having proved specifically sensitive in our previous reports (Roth, Wilhelm, & Trabert, 1998; Wilhelm, Gerlach, & Roth, 2001a; Wilhelm, Trabert, & Roth, 2001b). Our third aim was to account for more cognitive factors that might influence anxious responding in the context of respiratory challenge. Thus, *CO₂* inhalations were preceded and followed by two inhalations of room air. Any group differences during these phases are less likely to be associated with biochemically mediated *CO₂* sensitivity than with a cognitive mechanism.

Our expectation was that *CO₂ sensitivity* would be higher in PD patients compared to controls in all response domains, however, SP patients might be intermediate (Caldirola et al., 1997; Gorman et al., 1990). Furthermore, this elevated *CO₂* sensitivity would manifest itself in increased reactivity and/or reduced recovery in anxious responding to individual *CO₂* inhalations and slower habituation or even sensitization across *CO₂* inhalations in PD patients compared to both control groups (cf, Beck & Shipperd, 1997; Beck et al., 1997). Both anxious groups might show greater anticipatory anxiety to air inhalations before the *CO₂* inhalations and less reduction of anxiety to air inhalations after the *CO₂* inhalations (Wilhelm et al., 2001a).

Methods

Participants

Twenty patients with PD (footnote 1) and 19 patients with SP (DSM-IV, APA, 1994) were recruited by advertisements in local newspapers offering evaluation and treatment (described in Meuret, Wilhelm, Ritz, & Roth, 2008; Meuret, Wilhelm, & Roth, 2004). In addition, 18 controls were selected to age- and sex-match the PD and SP patients. General exclusion criteria for all participants were a lifetime history of psychosis, bipolar disorder, mental disability, substance abuse or dependence, or a medical history of conditions that might affect the physiological systems under examination (e.g., angina, myocardial infarction, thyroid dysfunction, and asthma). Participants were then interviewed using the Structured Clinical Interview for DSM-IV, SCID (First, Spitzer, Gibbon, & Williams, 1995) by trained clinical psychologists. The following comorbid disorders were found in the PD/SP groups: Generalized Anxiety Disorder (2 in the PD group / 2 in the SP group), Major Depression (1/2), and Specific Phobia (3/0). Controls were selected to be without lifetime

¹Of 21 PD patients, originally included into the study, one PD patient declined to complete the inhalations due to a panic attack and was therefore excluded from all analyses.

psychiatric diagnoses. The PD/SP groups reported taking the following medications: benzodiazepines (3/0), selective serotonin reuptake inhibitors (2/2) (footnote 2). As seen in Table 1, groups were successfully selected not to differ in the proportion of women, age, education, smoking status, or body mass index, the latter two of which are known to influence respiratory function (Pelosi et al., 1998). In the week before testing, participants filled out several questionnaires that had been mailed to them (footnote 3). These included the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) and the State-Trait Anxiety Inventory (STAI) Trait Form (Spielberger, Gorsuch, & Luchene, 1970). We further included the Anxiety Sensitivity Index (ASI, Reiss, Peterson, Gursky, & McNally, 1986), which was shown to have good validity and reliability (Peterson & Heilbronner, 1987; Reiss et al., 1986) (footnote 4). Table 1 shows that the PD and SP groups had similarly elevated BDI-depression and STAI-trait levels compared to controls. The PD group scored highest on the ASI, followed by the SP and the HC group.

Procedures

The study was approved by the local ethics committee for medical research and participants gave written consent before participating. Testing took place in a sound-attenuated chamber partitioned into testing and experimenter rooms. The experimenter could communicate with participants by intercom and observe them through a one-way mirror. The following seven assessments were done in fixed order: quiet sitting (15 min), attention task (9 min), comfortable paced breathing (4 min), giving a speech (3 min), quiet sitting (10 min), the inhalation task (36 min) that is the topic of this report, fast breathing (3 min), and quiet sitting (18 min).

The inhalation task comprised 12 inhalations: inhalation 1, 2, 11, and 12 were of room air, and 3 through 10 were of 20% CO₂ + 80% O₂. This concentration of CO₂ is able to trigger substantial anxiety symptoms, but not with an intensity that would preclude repeated application (Forsyth & Eifert, 1998). Participants were informed in advance about the order of gases and reminded by the rating log sheet, which indicated which gas would occur on each inhalation. The taste and symptoms produced by inhaling 20% CO₂ are unmistakably different from room air, so blinding would have been difficult. Each inhalation followed the same sequence: at the beginning of a 15 s acoustic countdown, the experimenter entered the room, placed a clip on the participant's nose, and handed him/her a tube which came out of a hole in the partition. Five seconds before the end of the countdown, participants were instructed to exhale completely, and at the end of the countdown to take as deep a breath as possible from the tube and to hold it for 3 seconds before exhaling. The experimenter then put the tube back in place and left the testing room. After 2 minutes and 45 s, a 15-s countdown began the next inhalation.

Self-report measures

In line with our previous research, anxiety and shortness of breath (termed “dyspnea” in the following) were chosen as main subjective dependent variables (Wilhelm et al., 2001a; Wilhelm et al., 2001b). Participants rated these two items (first anxiety, second dyspnea) during the procedure on 11 point scales from 0 (“not at all”) to 10 (“extremely”) by circling

²Because benzodiazepines are known to influence psychophysiological responding, the analyses were rerun omitting the 3 PD patients taking them. The general pattern of results remained unaltered, although the RSA group difference fell below the level of statistical significance.

³Although it is preferable to administer questionnaires during laboratory testing, questionnaires were sent by mail for practical reasons.

⁴The original version of the ASI has been criticized for its factor structure, and has been revised (e.g., Deacon, Abramowitz, Woods, & Tolin, 2003; Taylor & Cox, 1998; Taylor et al., 2007). However, to maintain comparability with older studies we used the original version.

the appropriate numbers on a rating sheet every 15 s (signaled on a computer display). The first rating was instructed 15 s after the inhalation. It took participants about 2 s to conduct these ratings.

In addition to these “on-line” symptom measures, a retrospective, more detailed *symptom questionnaire* was administered after the completion of the 12 inhalations. Participants completed this questionnaire twice: once for any experiences during the CO₂ inhalations and again for any experiences during the air inhalations. It encompassed the following questions. Three anxiety items (“anxious”, “worried” and “relaxed” [coded negatively]) rated on a 0 (not at all) to 10 (extremely) scale) were averaged in a composite anxiety score (Cronbach’s Alpha=.84). In addition, 6 of the 13 DSM-IV panic symptoms were rated on a 0 (not at all) to 4 (extremely) scale. The following symptoms were rated: (1) dyspnea/smothering/feeling of suffocation; (2) heart pounding or racing; (3) chest pain or discomfort; (4) dizziness, unsteadiness, lightheadedness, or faintness; (5) numbness or tingling sensations; and (6) trembling or shakiness. These symptoms, most of which are known to be sensitive to the effects of CO₂, were averaged for a composite *panic symptom score* (Cronbach’s Alpha=.89). Participants also rated each symptom on how anxious it made them (how much did this scare you?” 0 (not at all) – 4 (extremely)), yielding a second composite score, *panic symptom sensitivity* (Cronbach’s Alpha=.89). Three further items assessed *awareness of breathing* and *conscious control of breathing* on a 0 (not at all) to 4 (completely) scale. In addition, participants were asked to rate the *familiarity* of the CO₂-induced symptoms compared to those experienced during their normal lives when anxious (0 - “I never felt anything like that before” to 4 - “I have sensations like that quite often”). Participants were then asked to indicate whether they had a *panic attack* at any point during or between the inhalations (defined as a “discrete period of intense fear or discomfort in which a variety of physical symptoms appear abruptly and reach a peak within 10 minutes”). If they indicated yes, they were given a separate panic attack questionnaire listing all DSM-IV symptoms of attacks. Endorsement of 4 or more symptoms qualified as a panic attack.

Physiological measures and apparatuses

The following dependent measures were chosen in line with our previous studies and with the aim of capturing both sympathetic and parasympathetic autonomic activation, and respiratory functioning (for a more detailed description, see Wilhelm et al., 2001a; Wilhelm et al., 2001b).

1. From the electrocardiogram lead-II, the interbeat interval (IBI) was calculated in milliseconds between successive R-waves, from which instantaneous heart rate (HR) was calculated.
2. Respiratory sinus arrhythmia (RSA) as a measure of cardiac vagal control was calculated using complex demodulation of IBI time series for the frequency band of 0.15–0.4 Hz. Complex demodulation is a method less vulnerable to violations of the stationarity assumption than power spectral analysis and is suitable for quantification of rapid alterations in HR variability that are characteristic of the current data (Hayano et al., 1994; Wilhelm, Grossman, & Roth, 2005).
3. Two channels of respiration were measured with inductive plethysmography using Respibands (Respitrace Corporation, Ardsley, NY) placed around the chest and abdomen. Calibration against spirometry was accomplished by the least-squares method. Several respiratory parameters were calculated breath-by-breath using customized programs: instantaneous respiratory rate, tidal volume, minute ventilation, and mean inspiratory flow rate.

4. End-tidal partial pressure of expired CO₂ (pCO₂) was measured continuously using acalibrated infrared capnograph (N-1000, Nellcor, Hayward, CA) and a dual nostril prong. End-tidal values, which are close to arterial values, were scored only for breaths with a distinct plateau.
5. Electrodermal activity was recorded from the middle phalanx of the index and middle finger of the left hand using 11 mm inner diameter Ag/AgCl electrodes filled with isotonic electrode paste. Skin conductance level (SCL) and number of non-specific skin conductance fluctuations (NS-SCR, number of deflections from a zero-slope baseline exceeding 0.02 μ Siemens) were calculated.

Data reduction and analysis

In parallel with subjective ratings, mean scores for physiological channels were extracted for 15-s segments. One inhalation cycle was broken down into three consecutive *epochs*, each containing 4 physiological segments and 4 subjective ratings. The 1-minute *baseline* epoch preceded the inhalation and contained the 15 s countdown. Baseline was followed by the *peak* epoch, containing the gas inhalation (footnote 5) which in turn was followed by the *recovery* epoch. Across all 12 inhalations preliminary trend analyses showed that all group effects could be captured by a simplified analysis averaging across pertinent inhalations: air inhalations 1 and 2 were averaged into AIR-A, air inhalations 3 and 4 (following CO₂ inhalations): AIR-B, CO₂ inhalations 1–4: CO₂-A, and CO₂ inhalations 5–8: CO₂-B). The resulting means scores were then screened for outliers and non-normality. Outliers outside of ± 3 SDs were excluded. NS-SCRs were logarithm transformed [$\lg 10 (1 + \text{NS-SCR})$]. Due to technical errors physiological data were lost for some participants (footnote 6). Table 2 displays means and standard deviations for all measures during baseline, peak, and recovery epochs, averaged across the first two air inhalations for descriptive purposes.

To protect against cumulative alpha error due to multiple testing, multivariate analyses of variance (MANOVAs) preceded ANOVAs for individual measures. Separate repeated measures MANOVAs tested for overall Group effects on ratings of anxiety and dyspnea, physiological measures, and items of the symptom questionnaire. When significant on the Group factor (Wilks' criterion), MANOVAs were followed by ANOVAs for the individual measures. Tukey's Honest Significant Differences tests were used to specify Group effects, and *t*-tests to follow up on ANOVA effects.

Air inhalations were analyzed separately from CO₂ inhalations in a $2 \times 3 \times 3$, Time (AIR-A, AIR-B) $\times$ Epoch (baseline, peak, recovery) $\times$ Group (PD, SP, HC) analyses of variance (ANOVA) with repeated measures on Time and Epoch. CO₂ inhalations were analyzed in a $2 \times 3 \times 3$, Time (CO₂-A, CO₂-B) $\times$ Epoch (baseline, peak, recovery) $\times$ Group (PD, SP, HC) ANOVAs. In these analyses, increased reactivity (baseline to peak increases) and/or reduced recovery (baseline to recovery increases) would be evident in Group $\times$ Epoch interactions, and habituation/sensitization would give rise to Group $\times$ Time interactions.

Generally, for reasons of brevity, only effects involving the Group factor are reported. Epoch main effects were strong on all measures indicating that the inhalations reliably induced subjective and physiological changes. Time effects during CO₂ inhalations, indicative of habituation/sensitization, are reported only when significant or when interacting with Group. The alpha level was set to .05. When the sphericity assumption was

⁵Ratings and physiological data during the 15 s segment containing the tube handling and gas inhalation were excluded from analysis, resulting in 3 segments (45 s) of physiological data and 3 ratings in the peak epoch.

⁶Physiological data were lost for 3 participants (1 PD and 2 SP patients). Further missing values/outliers on individual measures: flow rate, 0/1; minute ventilation, 0/1; SCL, 0/1; NS-SCRs, 1/2; pCO₂, 5/1; RSA, 1/1

violated, the Greenhouse-Geisser correction for repeated measures was applied. Nominal df values and effect sizes are reported (partial η^2 -squared, in % [η^2] for ANOVAs, indicating percentage of explained variance, and Cohen's d for post-hoc t -tests). A general alpha level of .05 determined statistical significance, which was Bonferroni adjusted for non-independent follow-up analyses.

Results

Ratings of anxiety and dyspnea

The MANOVA of anxiety and dyspnea ratings over all inhalations showed a significant Group effect, $F(4,104)=5.27$, $p=.001$, $\eta^2=17.1\%$ (Wilks Lambda), which was followed up with ANOVAs for individual measures, separately for air and CO₂ inhalations.

Anxiety ratings during air inhalations—The Time $\times$ Epoch $\times$ Group ANOVA showed significant effects for Group, $F(2, 53)=8.84$, $p<.001$, $\eta^2=25.0\%$, the Time $\times$ Group interaction, $F(2, 53)=3.97$, $p=.025$, $\eta^2=13.0\%$, and the Time $\times$ Group $\times$ Epoch interaction, $F(4, 106)=2.71$, $p=.030$, $\eta^2=9.3\%$. The graph in Figure 1a suggests that the 3-way interaction was due to stronger Epoch effects in the SP group during AIR-B compared to AIR-A. Post-hoc tests confirmed an increase in anxiety from baseline to peak in the SP group during AIR-B, $t(18)=2.83$, $p=.030$, $d=0.24$, that was absent during AIR-A, $t(18)=0.58$, $p=.56$, $d=0.03$. Epoch effects were weak or absent in the other two groups, $ps>.081$. Figure 1a further suggests that the Time $\times$ Group interaction was due to a *general* decline in anxiety from AIR-A to AIR-B in the SP and HC group, but a slight increase in the PD group, possibly related to slowed recovery in this group. To test this impression, one anxiety score for AIR-A and one anxiety score for AIR-B was created by averaging across epochs. This anxiety mean score slightly *increased* in the PD group from AIR-A (mean[M]=1.94, standard deviation [SD]=1.63) to AIR-B (M=2.56, SD=2.41), $t(19)=1.75$, $p=.096$, $d=0.30$, while it slightly *decreased* in the SP group (M_{AIR-A}=1.76, SD_{AIR-A}=1.68, M_{AIR-B}=1.45, SD_{AIR-B}=1.68), $t(18)=1.66$, $p=.115$, $d=0.18$, and the HC group (M_{AIR-A}=0.25, SD_{AIR-A}=0.42, M_{AIR-B}=0.13, SD_{AIR-B}=0.29), $t(17)=1.44$, $p=.169$, $d=0.33$.

Anxiety ratings during CO₂ inhalations—The Time $\times$ Epoch $\times$ Group ANOVA showed significant effects for Group, $F(2, 53)=12.0$, $p<.001$, $\eta^2=31.1\%$ and the Group $\times$ Epoch interaction, $F(4,106)=5.79$, $p=.003$, $\eta^2=17.9\%$. Inspection of Figure 1a suggests that both anxiety groups showed larger anxiety increases from baseline to peak and incomplete recoveries thereafter. This effect was specified with two follow-up ANOVAs assessing baseline to peak responses and baseline to recovery differences separately. A 2 (Epoch: baseline, peak) $\times$ 3 (Group) ANOVA yielded a significant Epoch $\times$ Group interaction, $F(2, 54)=5.89$, $p=.010$, $\eta^2=17.9\%$, supporting larger increase from baseline to peak in the anxiety groups. Similarly, the 2 $\times$ 3 ANOVA including the baseline and recovery epochs showed a significant Epoch $\times$ Group interaction, $F(2, 54)=6.98$, $p=.004$, $\eta^2=20.5\%$, demonstrating incomplete recovery in the anxiety groups. A significant Time effect indicated habituation across trials, $F(1, 54)=5.08$, $p=.019$, $\eta^2=10.0\%$ that did not differ by group, $F<1.00$.

Dyspnea ratings during air inhalations—For dyspnea ratings, the Time $\times$ Epoch $\times$ Group ANOVA of air inhalations showed a significant main effect for Group, $F(2, 53)=7.06$, $p=.002$, $\eta^2=21.0\%$. A marginally significant 3-way interaction, $F(4,106)=2.27$, $p=.077$, $\eta^2=7.9\%$ was followed up for exploratory reasons. Figure 1b suggests Epoch effects primarily in the patient groups during AIR-A, which were more pronounced during AIR-B. Thus, post-hoc t -tests assessed the increase from baseline to peak separately for AIR-A and AIR-B in the three groups. This analysis found weak increases during AIR-A in all three groups, $ps>.051$, but strong increases during AIR-B in the PD and SP groups, $t(19)=3.17$,

$p=.005$, $d=0.64$ and $t(18)=3.61$, $p=.002$, $d=0.91$, respectively, but not in the HC group, $p=.44$.

Dyspnea ratings during CO₂ inhalations—For dyspnea ratings during CO₂ inhalations, the Time $\times$ Epoch $\times$ Group ANOVA yielded a highly significant Group effect, $F(2, 53)=8.56$, $p=.0012$, $\eta^2=24.4\%$, which interacted with Epoch, $F(4, 106)=5.35$, $p=.004$, $\eta^2=16.8\%$. Similarly to the anxiety ratings, means for dyspnea in Figure 1b suggest that both anxious groups showed a larger increase from baseline to peak and incomplete recovery. Follow-up ANOVAs confirmed this: a 2×3 , Epoch (baseline, peak) $\times$ Group ANOVA yielded a significant Epoch $\times$ Group interaction, $F(2, 53)=6.00$, $p=.008$, $\eta^2=18.5\%$, signifying larger increase in dyspnea in the patient groups than controls. The 2×3 ANOVA including the baseline and recovery epochs yielded a Epoch $\times$ Group that only approached significance, $F(2, 53)=3.24$, $p=.094$, $\eta^2=10.9\%$, suggesting a trend of incomplete return to baseline in the patient groups. Similar to the anxiety ratings, a significant Time effect indicated habituation across trials, $F(1, 54)=5.53$, $p=.022$, $\eta^2=9.4\%$ that did not differ by group, $F<1.00$.

Physiological responses

The MANOVA of the physiological measures during all inhalations showed a significant Group effect, $F(18,72)=1.85$, $p=.035$, $\eta^2=31.7\%$, which was followed by ANOVAs for the individual measures.

Physiological responses during air inhalations—Neither Group main effects nor its interactions with other factors reached significance during air inhalations on the physiological variables.

Physiological responses during CO₂ inhalations—Analysis of *HR* during CO₂ inhalations in a Time $\times$ Epoch $\times$ Group ANOVA revealed a marginally significant Group effect, $F(2, 50)=2.79$, $p=.071$, $\eta^2=10.0\%$, indicating a trend towards higher HR in PD patients compared to controls.

Whereas Group effects were absent on *SCL*, the ANOVA of *NS-SCRs* showed a significant Group $\times$ Epoch interaction (Figure 1c), $F(4,98)=3.10$, $p=.020$, $\eta^2=11.2\%$, pointing to a stronger recovery in the HC group than in the anxious groups. Post-hoc *t*-tests comparing baseline and recovery epochs separately in each group indicated that only the HC group had fewer NS-SCRs during recovery than during baseline, $t(18)=2.83$, $p=.033$, $d=0.22$, while values remained at baseline level in both patient groups ($ps>.36$).

For *RSA*, a significant Group effect was found during CO₂ inhalations, $F(2,49)=3.53$, $p=.037$, $\eta^2=9.2\%$. Post-hoc Tukey tests indicated that PD patients had persistently lower RSA values than healthy controls during baseline, peak, and recovery epochs ($p<.05$).

For *tidal volume*, a 3-way, Time $\times$ Epoch $\times$ Group interaction was found, $F(4,102)=4.14$, $p=.011$, $\eta^2=14.0\%$. Inspection of the means in Figure 1d suggests strong Epoch effects in all groups during CO₂-A. During CO₂-B only PD patients continued to show a marked response to the inhalations while the other two groups showed a blunted response. Post-hoc *t*-tests confirmed this impression: all groups showed strong increases in tidal volume during CO₂-A ($ps>.001$) but during CO₂-B, only PD patients continued to have strong increases, $t(18)=3.97$, $p>.001$, $d=0.69$, while the HC and SP groups responses were strongly reduced, $ps>.068$.

A very similar pattern emerged for *inspiratory flow rate* with continued responses to the gas only in the PD group during CO₂-B, but the respective 3-way interaction only approached

significance, $F(4,102)=2.17$, $p=.084$, $\eta^2=7.8\%$ (not shown in Figure 1). No significant effects involving the Group factor were found for *respiration rate*, *minute ventilation*, or *pCO₂*.

The absence of a group difference on pCO₂ and the increased peak tidal volume values suggest that patients did fully comply with the inhalation instructions and did not avoid inhaling the CO₂-O₂ mixture as sometimes reported (Coryell, Fyer, Pine, Martinez, & Arndt, 2001; Fannes et al., 2008).

Symptom Questionnaire

Table 3 presents information on the retrospective symptom questionnaire for inhalations of air and of CO₂. The MANOVA showed a significant effect for Group; $F(10, 98)=4.13$, $p<.001$, $\eta^2=29.7\%$. Follow-up ANOVAs and Tukey tests indicated that during *air inhalations*, PD patients scored higher than either other group on anxiety, panic symptoms, control of breathing, and familiarity of CO₂-induced sensations. SP patients gave higher symptom sensitivity ratings than controls. No differences were observed for awareness of breathing. During *CO₂ inhalations*, both patient groups had equally higher scores than controls on all items except awareness of breathing (no differences) and control of breathing (SP patients higher than controls).

During the inhalation task, 15.8% of PD patients reported a panic attack but none of the SP or control participants did ($\chi^2=6.01$, $p=.049$). One PD patient was not able to continue the experiment after the 5th inhalation of CO₂ because of panic and was excluded from the analyses.

Discussion

The present study aimed at replicating and extending previous research on CO₂ sensitivity in PD in a paradigm featuring a sequence of air and 20% CO₂ inhalations. The inclusion of a clinical control group (SP) as well as a healthy control group allowed a test of the specificity of findings to PD. Subjective and psychophysiological measures were assessed at high temporal resolution to capture changes reliably during this dynamic protocol. During CO₂ inhalations the micro level within-inhalation processes of *reactivity* (baseline to peak of inhalation) and *recovery* (baseline to recovery epoch) could be distinguished from the macro level across-inhalation changes of habituation or sensitization. Responses to air inhalations were analyzed separately from those to CO₂ inhalations.

Contrary to our hypothesis we found no evidence for group differences in sensitization/habituation across the eight CO₂ inhalation cycles (no Group $\times$ Time interactions). We had expected a slower habituation across CO₂ inhalations in PD patients. Habituation in anxiety and dyspnea ratings was present in all groups between the first and second block of CO₂ inhalations, albeit with small effect sizes. In contrast most physiological measures were temporally stable and did not habituate, attesting to the robustness of the physiological response to 20% CO₂.

More robust and consistent group differences were found at the micro level, i.e. in changes within each inhalation (Group $\times$ Epoch or 3-way interactions). A pattern of increased reactivity (baseline-to-peak differences) to the CO₂ inhalation (for anxiety and dyspnea ratings) and incomplete recovery (baseline-to-recovery differences) of anxiety and electrodermal responding was found in both anxiety disorder groups. CO₂ sensitivity has been defined either as an elevated panic rate or a greater change in anxiety ratings to the challenge (see Rassovsky & Kushner, 2003, for a review and issues of definition). The pattern of results from this study is generally in line with previous findings of CO₂

sensitivity in PD, in that both reactivity and recovery processes are abnormal in these patients. In addition, we replicated previous findings that PD patients share many of these characteristics with SP (Griez et al., 1990; Perna, Bussi, Allevi, & Bellodi, 1999; van den Hout et al., 1987, see also discussion below). Some findings were larger or more reliable in PD than SP, being significant for PD vs. HC only. For example, parasympathetic activity, indexed by RSA was lower, and HR tended to be higher in PD compared to controls throughout the CO₂ inhalations. Thus, autonomic control in PD patients was characterized by a coupled reciprocal mode of increased sympathetic (elevated NS-SCRs) and decreased parasympathetic activity (Berntson, Cacioppo, & Quigley, 1993). The finding of lower RSA is in line with other research reporting lower cardiac parasympathetic activity in PD (Friedman & Thayer, 1998, but see Blechert et al. 2007). This must be interpreted with caution, however, since we did not correct for the influences of respiration depth and rate as is advisable, particularly during respiratory challenges (Grossman & Taylor, 2007; Ritz, 2009; Ritz & Dahme, 2006). On a related note, though, this finding underscores the utility of complex demodulation for quantifying heart rate variability during non-stationary conditions (Wilhelm et al., 2005).

A strength of the present study is the detailed assessment of respiratory measures. The finding of continued tidal volume responses throughout the eight CO₂ inhalations in PD represents one of the few PD-specific findings and lends some support for a respiratory hypersensitivity in these patients. Previous studies had reported altered respiratory behavior (increased reactivity, delayed recovery) in PD compared to SP and healthy control groups (e.g., Papp et al., 1993; Papp et al., 1997; Wilhelm et al., 2001a).

In sum, results across response domains during CO₂ inhalations suggest some specific CO₂ sensitivity in PD, particularly for reports of panic (only in the PD group) and for continued tidal volume responses. In addition, on the post-experimental questionnaire, PD patients rated the similarity between task-induced symptoms and everyday anxiety experiences as higher than did either other group. However, for all other measures, including ratings of anxiety and dyspnea, SP patients response profiles paralleled those of the PD patients, which is consistent with previous reports (Caldirola et al., 1997; Gorman et al., 1990; Kent et al., 2001; Wilhelm et al., 2001a; Wilhelm et al., 2001b, but see Papp et al., 1993). These findings support a continuum model of CO₂ sensitivity, with SP occupying an intermediate position between PD and healthy controls. Panic attacks also occur in SP, which might sensitize SP patients to respiratory sensations. There is some evidence linking CO₂ sensitivity to non-clinical panic attacks in healthy controls (Perna, Gabriele, Caldirola, & Bellodi, 1995). CO₂ sensitivity is found in healthy individuals with elevated anxiety sensitivity (AS; Perna, Romano, Caldirola, Cucchi, & Bellodi, 2003). PD patients with higher AS report more symptoms during 35% CO₂ inhalations than patients with medium or low AS (Perna et al., 2003). We recently found higher anxiety ratings and autonomic arousal in individuals with high AS than in their low AS counterparts during a CO₂ inhalation similar to the one described here (Blechert, Wilhelm, Meuret, & Roth, submitted). In the present study, AS was highest in the PD patients, intermediate in SP and low in the HC group, paralleling the results from the inhalations. Future studies could try to separate the effects of AS and diagnosis by comparing SP and PD groups equated on AS and by comparing these two groups with healthy controls with elevated AS levels.

Peak epoch anxiety ratings for the last two air inhalations were selectively increased compared to ratings during the first two air inhalations in the SP group, while the PD group reported tonically higher anxiety for all epochs of the last two air inhalations. Peak epoch dyspnea ratings were higher in both anxious groups although with small effects sizes. This increased reactivity during AIR-B is interesting because sensitization or slowed recovery would have produced tonically elevated ratings. The selectiveness of elevated peak epoch

dyspnea ratings in the patient groups suggests that some psychological or physiological process was at work that is independent of CO₂ sensitivity. Respiratory conditioning might be one such process: patients may have associated the tube handling, countdown, and the inhalation with the anxiety provoking sensations of the CO₂. Although patients knew that room air was being delivered, air inhalations may still have triggered conditioned anxiety and dyspnea responses. However, without an adequate control stimulus, this is uncertain. Differential conditioning paradigms typically use two different odors, one mixed with CO₂ and the other without, in order to distinguish genuine conditioning effects from sensitization and other non-associative learning processes (e.g., van den Bergh, Kempynck, van de Woestijne, Baeyens, & Eelen, 1995; van den Bergh et al., 1999; Van Diest et al., 2006).

Several other novel aspects of our methodology deserve comment. First, using 20% rather than 35% CO₂ made repeated presentation of the challenge acceptable to almost all participants. Higher concentrations are more aversive and may trigger panic attacks resulting in overt or covert avoidance (e.g., failure to fully inspire the gas). Second, the high temporal resolution of anxiety and dyspnea ratings could reliably detect fairly brief symptoms at the peak of inhalation. However, other features of our methodology may have reduced anxious responding. Because we were unable to blind the delivery of the CO₂ enriched air, we clearly informed our participants about the sequence of air and CO₂ inhalations to be expected. This lent a high level of predictability to symptoms, which will reduce anxiety and which is uncharacteristic of real-life panic. Future research could attempt to blind CO₂ delivery by using odorous CO₂-O₂ mixtures in a differential conditioning design (e.g., van den Bergh, Stegen, & van de Woestijne, 1997) or in a single cue design including a contrast group receiving unpaired conditioned (e.g. countdown) and unconditioned (CO₂ inhalations) presentation (see Acheson, Forsyth, Prenoveau, & Bouton, 2007, for a similar design). In one study, PD patients extinguished more slowly to electrocutaneous unconditioned stimuli than controls (Michael, Bleichert, Vriends, Margraf, & Wilhelm, 2007). The use of CO₂ as unconditioned stimulus in a differential conditioning design would be a stronger test of an interoceptive conditioning account of PD (cf, Bouton et al., 2001).

Our rather small sample size, which is, however, not untypical for experimental pathophysiological studies, limited our statistical power. In addition, some effect sizes may have been low because patients were recruited by advertisements in local newspapers and not from psychiatric clinics and thus tended to have only mild to moderate symptom severity, low comorbidity, and infrequent medication use. The study would have been improved with a measure of physical fitness, since fitness can differ between groups and influence autonomic and respiratory functioning.

In sum, our results are most consistent with a continuum model of CO₂ sensitivity across anxiety disorders. PD and SP patients both show enhanced reactivity and slowed recovery of anxiety and dyspnea ratings during CO₂ inhalations. Respiratory and autonomic dysregulation was most pronounced in PD. Continued reactivity to air inhalations after CO₂ inhalations in both patient groups suggests that processes such as fear conditioning/symptom learning may be involved independently from the physiological effects of CO₂. The existence of a continuum can be used to challenge the widely accepted categorical classification of anxiety disorders in the DSM and biological theories postulated to underlie specific disorders. However, unequivocal refutation of categorical distinctions would require much more evidence than our data can provide (Brown & Barlow, 2005, 2009; Haslan, 2003).

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References

- Acheson DT, Forsyth JP, Prenoveau JM, Bouton ME. Interoceptive fear conditioning as a learning model of panic disorder: an experimental evaluation using 20% CO₂-enriched air in a non-clinical sample. *Behaviour Research and Therapy* 2007;45(10):2280–2294. [PubMed: 17548049]
- American Psychiatric Association, A. P. A.. *Diagnostic and Statistical Manual of Mental Disorders*. 4. Washington, DC: American Psychiatric Association; 1994.
- Barlow DH, Brown TA, Craske MG. Definitions of panic attacks and panic disorder in the DSM-IV: implications for research. *Journal of Abnormal Psychology* 1994;103(3):553–564. [PubMed: 7930055]
- Beck AT, Ward CH, Mendelson M, Mock JE, Erbaugh JK. An inventory for measuring depression. *Archives of General Psychiatry* 1961;4:561–571. [PubMed: 13688369]
- Beck JG, Shipherd JC. Repeated exposure to interoceptive cues: does habituation of fear occur in panic disorder patients? A preliminary report. *Behaviour Research and Therapy* 1997;35(6):551–557. [PubMed: 9159979]
- Beck JG, Shipherd JC, Read J. Response patterns to repeated CO₂ inhalation in individuals with high anxiety sensitivity. *Behaviour Research and Therapy* 1999;37(11):1073–1089. [PubMed: 10500321]
- Beck JG, Shipherd JC, Zebb BJ. How does interoceptive exposure for panic disorder work? An uncontrolled case study. *Journal of Anxiety Disorders* 1997;11(5):541–556. [PubMed: 9407272]
- Beck JG, Wolf MS. Response to repeated CO₂ in individuals with elevated anxiety sensitivity: replication with 20% CO₂. *Journal of Behavior Therapy and Experimental Psychiatry* 2001;32(1):1–16. [PubMed: 11729942]
- Bellodi L, Perna G, Caldirola D, Arancio C, Bertani A, Di Bella D. CO₂-induced panic attacks: a twin study. *American Journal of Psychiatry* 1998;155(9):1184–1188. [PubMed: 9734540]
- Berntson GG, Cacioppo JT, Quigley KS. Cardiac psychophysiology and autonomic space in humans: empirical perspectives and conceptual implications. *Psychological Bulletin* 1993;114(2):296–322. [PubMed: 8416034]
- Bertani A, Perna G, Arancio C, Caldirola D, Bellodi L. Pharmacologic effect of imipramine, paroxetine, and sertraline on 35% carbon dioxide hypersensitivity in panic patients: a double-blind, random, placebo-controlled study. *Journal of Clinical Psychopharmacology* 1997;17(2):97–101. [PubMed: 10950471]
- Blechert J, Michael T, Grossman P, Lajtman M, Wilhelm FH. Autonomic and respiratory characteristics of posttraumatic stress disorder and panic disorder. *Psychosomatic Medicine* 2007;69(9):935–943. [PubMed: 17991823]
- Blechert J, Wilhelm FH, Meuret AE, Roth WT. CO₂ sensitivity and respiratory conditionability in individuals with elevated anxiety sensitivity. *Journal of Anxiety Disorders*. (submitted).
- Bouton ME, Mineka S, Barlow DH. A modern learning theory perspective on the etiology of panic disorder. *Psychological Review* 2001;108:4–32. [PubMed: 11212632]
- Brown TA, Barlow DH. Dimensional versus categorical classification of mental disorders in the fifth edition of the *Diagnostic and Statistical Manual of Mental Disorders* and beyond: comment on the special section. *Journal of Abnormal Psychology* 2005;114(4):551–556. [PubMed: 16351377]
- Brown TA, Barlow DH. A proposal for a dimensional classification system based on the shared features of the DSM-IV anxiety and mood disorders: implications for assessment and treatment. *Psychol Assess* 2009;21(3):256–271. [PubMed: 19719339]
- Caldirola D, Perna G, Arancio C, Bertani A, Bellodi L. The 35% CO₂ challenge test in patients with social phobia. *Psychiatry Research* 1997;71(1):41–48. [PubMed: 9247980]
- Coryell W, Dindo L, Fyer A, Pine DS. Onset of spontaneous panic attacks: a prospective study of risk factors. *Psychosomatic Medicine* 2006;68(5):754–757. [PubMed: 17012529]

- Coryell W, Fyer A, Pine D, Martinez J, Arndt S. Aberrant respiratory sensitivity to CO₂ as a trait of familial panic disorder. *Biological Psychiatry* 2001;49(7):582–587. [PubMed: 11297715]
- Deacon BJ, Abramowitz JS, Woods CM, Tolin DF. The Anxiety Sensitivity Index - Revised: psychometric properties and factor structure in two nonclinical samples. *Behaviour Research and Therapy* 2003;41(12):1427–1449. [PubMed: 14583412]
- Fannes S, Van Diest I, Meulders A, De Peuter S, Vansteenwegen D, Van den Bergh O. To inhale or not to inhale: conditioned avoidance in breathing behavior in an odor--20% CO₂ paradigm. *Biological Psychology* 2008;78(1):87–92. [PubMed: 18316154]
- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM-IV Axis I Disorders. Washington, D. C.: American Psychiatric Press; 1995.
- Forsyth JP, Eifert GH. Response intensity in content-specific fear conditioning comparing 20% versus 13% CO₂-sub-2-enriched air as unconditioned stimuli. *Journal of Abnormal Psychology*. 1998
- Friedman BH, Thayer JF. Autonomic balance revisited: Panic anxiety and heart rate variability. *Journal of Psychosomatic Research*. 1998
- Gorman JM, Papp LA, Martinez J, Goetz RR, Hollander E, Liebowitz MR, et al. High-dose carbon dioxide challenge test in anxiety disorder patients. *Biological Psychiatry* 1990;28:743–757. [PubMed: 2124151]
- Griez E, Deloof C, Pols H, Zandbergen J, Lousberg H. Specific sensitivity of patients with panic attacks to carbon dioxide inhalation. *Psychiatry Research* 1990;31(2):193–199. [PubMed: 2109330]
- Grossman P, Taylor EW. Toward understanding respiratory sinus arrhythmia: relations to cardiac vagal tone, evolution and biobehavioral functions. *Biological Psychology* 2007;74(2):263–285. [PubMed: 17081672]
- Haslan N. Categorical versus dimensional models of mental disorder- the taxonomic evidence. *Australian and New Zealand Journal of Psychiatry* 2003;37(696–704):696. [PubMed: 14636384]
- Hayano J, Taylor JA, Mukai S, Okada A, Watanabe Y, Takata K, et al. Assessment of frequency shifts in R-R interval variability and respiration with complex demodulation. *J Appl Physiol* 1994;77(6):2879–2888. [PubMed: 7896636]
- Kent JM, Papp LA, Martinez JM, Browne ST, Coplan JD, Klein DF, et al. Specificity of panic response to CO₂ inhalation in panic disorder: a comparison with major depression and premenstrual dysphoric disorder. *American Journal of Psychiatry* 2001;158(1):58–67. [PubMed: 11136634]
- Klein DF. False suffocation alarms, spontaneous panics, and related conditions. An integrative hypothesis. *Archives of General Psychiatry* 1993;50(4):306–317. [PubMed: 8466392]
- Mackinnon DF, Craighead B, Lorenz L. Carbon dioxide induces erratic respiratory responses in bipolar disorder. *Journal of Affective Disorders*. 2008
- Meuret AE, Wilhelm FH, Ritz T, Roth WT. Feedback of end-tidal pCO₂ as a therapeutic approach for panic disorder. *Journal of Psychiatric Research* 2008;42(7):560–568. [PubMed: 17681544]
- Meuret AE, Wilhelm FH, Roth WT. Respiratory feedback for treating panic disorder. *Journal of Clinical Psychology* 2004;60(2):197–207. [PubMed: 14724927]
- Michael T, Blechert J, Vriends N, Margraf J, Wilhelm FH. Fear conditioning in panic disorder: Enhanced resistance to extinction. *Journal of Abnormal Psychology* 2007;116(3):612–617. [PubMed: 17696717]
- Papp LA, Klein DF, Martinez J, Schneier F, Cole R, Liebowitz MR, et al. Diagnostic and substance specificity of carbon-dioxide-induced panic. *American Journal of Psychiatry* 1993;150(2):250–257. [PubMed: 8123056]
- Papp LA, Martinez JM, Klein DF, Coplan JD, Norman RG, Cole R, et al. Respiratory psychophysiology of panic disorder: three respiratory challenges in 98 subjects. *American Journal of Psychiatry* 1997;154(11):1557–1565. [PubMed: 9356564]
- Pelosi P, Croci M, Ravagnan I, Tredici S, Pedoto A, Lissoni A, et al. The effects of body mass on lung volumes, respiratory mechanics, and gas exchange during general anesthesia. *Anesth Analg* 1998;87(3):654–660. [PubMed: 9728848]
- Perna G, Bussi R, Allevi L, Bellodi L. Sensitivity to 35% carbon dioxide in patients with generalized anxiety disorder. *Journal of Clinical Psychiatry* 1999;60(6):379–384. [PubMed: 10401916]

- Perna G, Gabriele A, Caldirola D, Bellodi L. Hypersensitivity to inhalation of carbon dioxide and panic attacks. *Psychiatry Research* 1995;57(3):267–273. [PubMed: 7501737]
- Perna G, Romano P, Caldirola D, Cucchi M, Bellodi L. Anxiety sensitivity and 35% CO₂ reactivity in patients with panic disorder. *Journal of Psychosomatic Research* 2003;54(6):573–577. [PubMed: 12781312]
- Peterson RA, Heilbronner RL. The Anxiety Sensitivity Index: Construct validity and factor analytic structure. *Journal of Anxiety Disorders* 1987;1(2):117–121.
- Rapee RM. Psychological factors influencing the affective response to biological challenge in panic disorder. *Journal of Anxiety Disorders* 1995;9(1):59–74.
- Rassovsky Y, Kushner MG. Carbon dioxide in the study of panic disorder: issues of definition, methodology, and outcome. *Journal of Anxiety Disorders* 2003;17(1):1–32. [PubMed: 12464286]
- Reiss S, Peterson RA, Gursky DM, McNally RJ. Anxiety sensitivity, anxiety frequency, and the prediction of fearfulness. *Behaviour Research and Therapy* 1986;24:1–8. [PubMed: 3947307]
- Ritz T. Studying noninvasive indices of vagal control: the need for respiratory control and the problem of target specificity. *Biological Psychology* 2009;80(2):158–168. [PubMed: 18775468]
- Ritz T, Dahme B. Implementation and interpretation of respiratory sinus arrhythmia measures in psychosomatic medicine: practice against better evidence? *Psychosomatic Medicine* 2006;68(4):617–627. [PubMed: 16868273]
- Roth WT, Wilhelm FH, Trabert W. Voluntary breath holding in panic and generalized anxiety disorders. *Psychosomatic Medicine* 1998;60(6):671–679. [PubMed: 9847025]
- Spielberger, CD.; Gorsuch, RL.; Luchene, RE. State-Trait Anxiety Inventory. Palo Alto: Consulting Psychology Press; 1970.
- Taylor S, Cox BJ. An expanded anxiety sensitivity index: evidence for a hierarchic structure in a clinical sample. *Journal of Anxiety Disorders* 1998;12(5):463–483. [PubMed: 9801964]
- Taylor S, Zvolensky MJ, Cox BJ, Deacon B, Heimberg RG, Ledley DR, et al. Robust dimensions of anxiety sensitivity: development and initial validation of the Anxiety Sensitivity Index-3. *Psychol Assess* 2007;19(2):176–188. [PubMed: 17563199]
- van den Bergh O, Kempynck PJ, van de Woestijne KP, Baeyens F, Eelen P. Respiratory learning and somatic complaints: a conditioning approach using CO₂-enriched air inhalation. *Behaviour Research and Therapy* 1995;33(5):517–527. [PubMed: 7598672]
- van den Bergh O, Stegen K, van de Woestijne KP. Learning to have psychosomatic complaints: conditioning of respiratory behavior and somatic complaints in psychosomatic patients. *Psychosomatic Medicine* 1997;59(1):13–23. [PubMed: 9021862]
- van den Bergh O, Stegen K, Van Diest I, Raes C, Stulens P, Eelen P, et al. Acquisition and extinction of somatic symptoms in response to odours: a Pavlovian paradigm relevant to multiple chemical sensitivity. *Occupational and Environmental Medicine* 1999;56(5):295–301. [PubMed: 10472302]
- van den Hout MA, van der Molen GM, Griez E, Lousberg H, Nansen A. Reduction of CO₂-induced anxiety in patients with panic attacks after repeated CO₂ exposure. *American Journal of Psychiatry* 1987;144(6):788–791. [PubMed: 3109265]
- Van Diest I, De Peuter S, Piedfort K, Bresseleers J, Devriese S, Van de Woestijne KP, et al. Acquired lightheadedness in response to odors after hyperventilation. *Psychosomatic Medicine* 2006;68(2):340–347. [PubMed: 16554402]
- Wilhelm FH, Gerlach AL, Roth WT. Slow recovery from voluntary hyperventilation in panic disorder. *Psychosomatic Medicine* 2001a;63(4):638–649. [PubMed: 11485118]
- Wilhelm FH, Grossman P, Roth WT. Assessment of heart rate variability during alterations in stress: complex demodulation vs. spectral analysis. *Biomedical Sciences Instrumentation* 2005;41:346–351. [PubMed: 15850130]
- Wilhelm FH, Trabert W, Roth WT. Physiological instability in panic disorder and generalized anxiety disorder. *Biological Psychiatry* 2001b;49(7):596–605. [PubMed: 11297717]

Figure 1.

Means and standard errors for self-reported and psychophysiological responses to air and CO₂ inhalations (each triplet consisting of averages for baseline, peak, and recovery epochs) in patients with panic disorder (PD) or social phobia (SP), and healthy controls (HC). Panels depict self-reported anxiety (a) and dyspnea (b), number N of nonspecific skin conductance responses (transformed by logarithm of N+1) (c), and tidal volume (d).

Note: AIR-A = first set of two inhalations of air; CO₂-A = first set of four inhalations of CO₂; CO₂-B = second set of four inhalations of CO₂; AIR-B = second set of two inhalations of air. See text for details.

Table 1Means $\pm$ SD of sample characteristics

	PD	SP	HC	Statistic	Post-hoc
Number in sample	20	19	18		
% women	55.0	52.6	61.1	$\chi^2(2)=0.28, p=.87$	
% smoker	10.0	10.5	11.1	$\chi^2(4)=2.59, p=.63$	
Age (years)	39.1 $\pm$ 11.3	39.5 $\pm$ 10.6	37.4 $\pm$ 10.2	$F(2,57)=0.20, p=.81$	
Years of education	16.5 $\pm$ 1.9	15.2 $\pm$ 3.0	16.5 $\pm$ 3.5	$F(2,49)^I=1.15, p=.33$	
Body Mass Index (kg/m ²)	25.1 $\pm$ 6.5	24.6 $\pm$ 4.5	25.4 $\pm$ 4.1	$F(2,47)=0.08, p=.92$	
Beck Depression Inventory	13.3 $\pm$ 8.1	14.3 $\pm$ 8.9	2.8 $\pm$ 2.9	$F(2,51)=13.4, p<.001$	PD=SP>HC
STAI Trait Form	46.4 $\pm$ 7.7	48.5 $\pm$ 14.8	35.5 $\pm$ 4.7	$F(2,49)=3.54, p=.037$	PD=SP>HC
Anxiety Sensitivity Index	30.1 $\pm$ 12.6	21.6 $\pm$ 10.5	7.5 $\pm$ 4.8	$F(2,44)=23.0, p<.001$	PD>SP>HC

Note. PD, panic disorder; SP, social phobia; HC, healthy controls; STAI = State-Trait Anxiety Inventory

^I Unfortunately, questionnaire data for some participants were lost.

Table 2

Means $\pm$ SD of experiential, autonomic, and respiratory measures during the initial set of two air inhalations (for baseline, peak, and recovery epochs)

	PD (n=20)			SP (n=19)			HC (n=18)		
	Baseline	Peak	Recovery	Baseline	Peak	Recovery	Baseline	Peak	Recovery
<i>Experiential</i>									
Anxiety	1.81 $\pm$ 1.58	2.11 $\pm$ 1.89	1.77 $\pm$ 1.61	1.88 $\pm$ 1.83	1.81 $\pm$ 1.73	1.59 $\pm$ 1.64	0.35 $\pm$ 1.53	0.27 $\pm$ 0.55	0.13 $\pm$ 0.38
Dyspnea	1.03 $\pm$ 1.19	1.33 $\pm$ 1.33	1.16 $\pm$ 1.09	0.64 $\pm$ 0.99	0.79 $\pm$ 1.07	0.58 $\pm$ 1.07	0.03 $\pm$ 0.08	0.24 $\pm$ 0.72	0.02 $\pm$ 0.08
<i>Autonomic</i>									
HR (bpm)	77.4 $\pm$ 8.15	78.6 $\pm$ 9.01	76.1 $\pm$ 8.96	73.5 $\pm$ 10.8	72.5 $\pm$ 9.83	72.7 $\pm$ 10.9	72.3 $\pm$ 10.5	72.9 $\pm$ 9.73	71.3 $\pm$ 10.6
RSA (ms)	16.4 $\pm$ 8.14	16.7 $\pm$ 9.36	16.2 $\pm$ 10.7	16.9 $\pm$ 9.14	16.9 $\pm$ 10.1	17.6 $\pm$ 11.4	23.6 $\pm$ 14.4	24.8 $\pm$ 16.4	22.4 $\pm$ 14.7
SCL (μ S)	10.6 $\pm$ 5.66	14.5 $\pm$ 8.07	12.6 $\pm$ 6.81	9.49 $\pm$ 7.04	12.6 $\pm$ 8.07	11.5 $\pm$ 7.32	10.3 $\pm$ 6.71	13.9 $\pm$ 8.07	12.4 $\pm$ 7.43
NS-SCR (1/min)	0.39 $\pm$ 0.29	0.45 $\pm$ 0.34	0.28 $\pm$ 0.19	0.27 $\pm$ 0.18	0.37 $\pm$ 0.29	0.24 $\pm$ 0.15	0.23 $\pm$ 0.21	0.29 $\pm$ 0.35	0.18 $\pm$ 0.11
<i>Respiratory</i>									
Respiratory rate (c/min)	14.0 $\pm$ 3.96	11.6 $\pm$ 2.78	12.9 $\pm$ 3.46	14.0 $\pm$ 2.61	12.5 $\pm$ 3.18	13.2 $\pm$ 2.70	12.1 $\pm$ 3.80	11.3 $\pm$ 2.67	12.7 $\pm$ 3.55
pCO ₂ (mm Hg)	37.7 $\pm$ 7.00	37.0 $\pm$ 7.29	37.9 $\pm$ 6.89	36.3 $\pm$ 7.71	34.7 $\pm$ 7.82	36.4 $\pm$ 7.96	39.2 $\pm$ 7.44	37.8 $\pm$ 7.29	39.4 $\pm$ 7.86
Tidal volume (ml)	557 $\pm$ 234	921 $\pm$ 353	669 $\pm$ 337	470 $\pm$ 271	841 $\pm$ 493	501 $\pm$ 358	441 $\pm$ 183	637 $\pm$ 198	473 $\pm$ 254
Minute ventilation (L/min)	7.92 $\pm$ 4.07	9.37 $\pm$ 4.39	8.34 $\pm$ 6.38	5.75 $\pm$ 2.53	8.04 $\pm$ 3.66	5.64 $\pm$ 3.40	5.25 $\pm$ 1.99	7.01 $\pm$ 2.79	5.47 $\pm$ 2.81
Inspiratory flow rate (ml/s)	352 $\pm$ 131	485 $\pm$ 205	383 $\pm$ 129	301 $\pm$ 111	447 $\pm$ 186	304 $\pm$ 165	287 $\pm$ 101	415 $\pm$ 126	324 $\pm$ 172

Note. PD, panic disorder; SP, social phobia; HC, healthy controls; HR, heart rate; RSA, respiratory sinus arrhythmia (computed with complex demodulation); SCL, skin conductance level; NS-SCR, number of non-specific skin conductance fluctuations; pCO₂, end-tidal partial pressure of expired CO₂;

Table 3

Means $\pm$ SD of symptom questionnaire data

	PD (n=20)	SP (n=19)	HC (n=18)	F, p	Post-hoc
<i>Inhalations of AIR</i>					
Anxiety	4.38 $\pm$ 2.29	2.49 $\pm$ 1.57	1.61 $\pm$ 1.07	12.5, <.001	PD>SP=HC
Panic symptoms	0.64 $\pm$ 0.73	0.18 $\pm$ 0.37	0.14 $\pm$ 0.22	6.20, .004	PD>SP=HC
Symptom sensitivity	0.30 $\pm$ 0.49	0.06 $\pm$ 0.26	0.01 $\pm$ 0.01	4.79, .012	PD=SP>HC
Awareness of breathing	2.05 $\pm$ 1.31	1.58 $\pm$ 1.17	1.56 $\pm$ 1.38	0.89, .416	-
Control of breathing	1.63 $\pm$ 1.30	0.74 $\pm$ 0.93	0.56 $\pm$ 1.04	5.10, .009	PD>SP=HC
<i>Inhalations of CO₂</i>					
Anxiety	6.31 $\pm$ 1.95	5.28 $\pm$ 1.70	2.57 $\pm$ 1.54	22.5, <.001	PD=SP>HC
Panic symptoms	1.46 $\pm$ 0.69	1.29 $\pm$ 0.87	0.66 $\pm$ 0.68	5.83, .005	PD=SP>HC
Symptom sensitivity	0.72 $\pm$ 0.59	0.71 $\pm$ 0.73	0.24 $\pm$ 0.60	3.32, .044	PD=SP>HC
Awareness of breathing	2.47 $\pm$ 0.90	2.79 $\pm$ 1.32	2.28 $\pm$ 1.45	0.81, .452	-
Symptom Control of breathing sensitivity	1.78 $\pm$ 1.13	2.42 $\pm$ 1.46	1.11 $\pm$ 1.23	4.80, .012	SP>HC
Familiarity of symptoms	2.63 $\pm$ 1.06	1.42 $\pm$ 1.12	0.72 $\pm$ 0.90	17.4, <.001	PD>SP=HC
Panic attack	15.8%	0%	0%	See text	PD>SP=HC

Note. PD, panic disorder; SP, social phobia; HC, healthy controls.