Published in final edited form as:
J Behav Ther Exp Psychiatry. 2015 June ; 47: 68–76. doi:10.1016/j.jbtep.2014.10.013.

Examining the Latent Class Structure of CO_2 Hypersensitivity using Time Course Trajectories of Panic Response Systems

Roxann Roberson-Nay[1], **Jessica R. Beadel**[2], **Eugenia I. Gorlin**[2], **Shawn J. Latendresse**[3], and **Bethany A. Teachman**[2]

[1]Virginia Commonwealth University, Department of Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics, Richmond, VA

[2]University of Virginia, Department of Psychology, Charlottesville, VA

[3]Baylor University, Department of Psychology and Neuroscience, Waco, Tx

Abstract

Background and Objectives—Carbon dioxide (CO_2) hypersensitivity is hypothesized to be a robust endophenotypic marker of panic spectrum vulnerability. The goal of the current study was to explore the latent class trajectories of three primary response systems theoretically associated with CO_2 hypersensitivity: subjective anxiety, panic symptoms, and respiratory rate (*f*R).

Methods—Participants (*n*=376; 56% female) underwent a maintained 7.5% CO_2 breathing task that included three phases: baseline, CO_2 air breathing, and recovery. Growth mixture modeling was used to compare response classes (1..n) to identify the best-fit model for each marker. Panic correlates also were examined to determine class differences in panic vulnerability.

Results—For subjective anxiety ratings, a three-class model was selected, with individuals in one class reporting an acute increase in anxiety during 7.5% CO_2 breathing and a return to pre-CO2 levels during recovery. A second, smaller latent class was distinguished by elevated anxiety across all three phases. The third class reported low anxiety reported during room air, a mild increase in anxiety during 7.5% CO_2 breathing, and a return to baseline during recovery. Latent class trajectories for *f*R yielded one class whereas panic symptom response yielded two classes.

Limitations—This study examined CO_2 hypersensitivity in one of the largest samples to date, but did not ascertain a general population sample thereby limiting generalizability. Moreover, a true resting baseline measure of *f*R was not measured.

Conclusions—Two classes potentially representing different risk pathways were observed. Implications of results will be discussed in the context of panic risk research.

Correspondence: Roxann Roberson-Nay, Ph.D., Department of Psychiatry, Virginia Commonwealth University, Virginia Institute for Psychiatric and Behavioral Genetics, P.O. Box 980489, Richmond, VA 23298, Fax (804) 828-0245, rrobersonnay@vcu.edu..

The authors have no financial conflicts to disclose.

Keywords

CO_2 hypersensitivity; panic risk; latent classes; anxiety; respiratory

Carbon dioxide (CO_2) reactivity reflects an individual difference trait in which persons exhibit differing emotional and/or physiological responses to breathing air containing increased concentrations of CO_2. Breathing CO_2 enriched air reliably produces some level of enhanced emotional and physiologic responding in general population samples and provokes panic attacks at high rates among persons with panic disorder (PD; Perna, Barbini, Cocchi, Bertani, & Gasperini, 1995; Rassovsky & Kushner, 2003) as well as individuals with non-clinical panic (i.e., occasional, unexpected panic attacks) (Griez, de Loof, Pols, Zandbergen & Lousberg, 1990), suggesting that CO_2 hypersensitivity relates to panic spectrum liability broadly. Understanding unique patterns of responding to CO_2 across multiple response systems may help to identify different risk profiles for panic. To this end, the current study examines the latent class structure of subjective anxiety, somatic and cognitive symptoms, and physiological response systems associated with CO_2 reactivity, a robust endophenotypic measure of panic risk.

There currently is no clear consensus regarding which outcome measure(s), including panic symptoms, respiratory measures, panic attack rate, or subjective anxiety, should be the basis for defining sensitivity to inhalation of CO_2 enriched air. Adding to this uncertainty, the literature generally observes modest to moderate associations between panic symptoms and subjective anxiety, with weak to modest levels of association between subjective and physiological measures. After careful review of the literature, subjective anxiety was selected as the primary CO_2 hypersensitivity outcome measure. This selection was based on a recent review of the CO_2 inhalation literature, which suggested that while no exemplary definition of CO_2 hypersensitivity has emerged, marked subjective anxiety post-CO_2 inhalation has the most support as a panic-relevant, putative trait marker (Coryell et al., 2001; Vickers, et al., 2011), including evidence for elevated self-reported anxiety post-CO2 inhalation as a trait marker of PD (Coryell et al., 2001). Moreover, self-reported anxiety post-35% CO_2 inhalation exhibited moderate heritability (Battaglia et al., 2007), predicts genetic risk status for PD (Schmidt et al., 2007) and robustly differentiates persons with and without PD during CO_2 challenge (Battaglia & Perna, 1995).

Beyond subjective anxiety, studies have relied on symptom report as both a continuous measure and to define panic attack or panic-like response status. A number of panic symptom measures have been used in studies of response to CO_2, with no gold-standard measure emerging. Panic symptom measures generally include DSM panic attack criteria, but some measures include additional symptom items (e.g., desire to escape). There is disagreement as to whether the total number of panic symptoms reported post-hypersonic challenge is a valid measure of CO_2 hypersensitivity (Vickers, et al., 2011). Arguments against use of panic symptoms as a primary definition of CO_2 hypersensitivity include the fact that inhalation of CO_2-enriched air produces some level of physiological arousal in most everyone (e.g., Argyropoulos et al., 2002) and use of a total score could obscure distinct group differences in symptoms (Vickers et al., 2011). For these reasons, measurement of panic symptoms has not emerged as a strong operational definition of CO_2 hypersensitivity. We assessed the latent class structure of reported symptoms to determine its association with to-be-determined anxiety classes.

Another plausible feature for defining CO_2 hypersensitivity is aberrant respiratory responding to CO_2 inhalation. Whether the susceptibility to experiencing panic symptoms following CO_2 inhalation reflects a perturbation within neural circuits responsible for respiratory functioning is not known, but it is a possibility (Klein, 1993). An abnormality in respiratory physiology may not be detectable, however, because there is some evidence to suggest that vulnerable persons engage in subtle respiratory maneuvering to avoid absorption of CO_2 into the blood (Coryell, et al, 2001; Roberson-Nay et al., 2010). Moreover, the respiratory measures that might underlie subjective hypersensitivity to breathing CO_2 enriched air have produced mixed findings, most notably tidal volume (i.e., measurement of the air expired in a breath). Because CO_2 enriched air is a respiratory stimulant, most people will exhibit changes in respiratory measures, indicating that a simple increase in respiratory frequency does not necessarily represent CO_2 hypersensitivity. Thus, similar to panic symptoms, we assess the latent class structure of respiratory frequency to determine its association with possible subjective anxiety classes.

In sum, response to breathing CO_2 enriched air may be characterized by multiple response trajectories. To our knowledge, this study is the first to test subjective anxiety, symptomatic, and physiological responses to breathing 7.5% CO_2, with the goal of identifying classes of response to CO_2 breathing, and then determining their relevance by examining their relationship to established correlates of panic. It was hypothesized that classes reflecting more intense reactivity to the CO_2 breathing challenge will be associated with panic related constructs (e.g., anxiety sensitivity). Given limited prior data-driven studies of CO_2 hypersensitivity, no *a priori* hypotheses were formulated regarding the number or structure of to-be-identified classes based on subjective anxiety, panic symptom endorsements, or respiratory response beyond a general expectation that at least one class would reflect elevated response during the CO_2 enriched air phase, relative to the pre-CO_2 room-air and recovery phases, indicating CO_2 sensitivity. Our methodological design allows us significant power to dissect responses to CO_2 given the repeated measurement of outcomes across three distinct phases, as compared to many prior designs, which focused almost exclusively on post-CO_2 challenge outcomes.

Methods

Participants

Participants included 382 young adults (see Table 1) from two large universities who participated in exchange for course credit or payment, with the majority (85%) receiving course credit. Two consented participants were excluded from the study based on their endorsement of one or more exclusion criteria (see below) on the screening form. Four enrolled participants opted out of participating in the CO_2 breathing task after signing the informed consent, yielding a final sample of 376 participants (site1 n=239; site 2 n=137) for analysis. Participants were recruited either based on their scores on the Anxiety Sensitivity Index (ASI; Reiss, Peterson, Gursky, & McNally, 1986), which they completed as part of a department-wide preselection survey, or via recruitment fliers. For participants completing the study for course credit, a stratified sampling approach was used, with recruitment e-mails sent to approximately equivalent numbers of students scoring within each quartile of the distribution of ASI scores (Peterson & Reiss, 1992). This was done to ensure adequate representation of low to high ASI scores in the sample. No prior ASI-based selection criteria were used for participants who participated for financial compensation. These individuals completed the ASI at the time of participation. ASI scores did not differ between participants participating for credit versus financial compensation ($t(360)=1.09$, $p=.277$, Cohen's $d=.17$). Sites also did not differ on ASI ($t(360) = -0.99$, $p=.321$, Cohen's $d=.11$) or other primary variables (e.g., Diagnostic Symptom Questionnaire [DSQ; Sanderson et al., 1989] score across time points; all *p*s>.265) or other measured outcomes (e.g., sex distribution ($\chi^2(1)=0.51$, $p=.477$).

Participants were excluded if they reported having currently treated asthma, a serious, unstable medical condition, or if they had taken an antidepressant or other psychotropic medication within the past four weeks. Participants taking benzodiazepines were eligible to participate if they had not taken a benzodiazepine medication for at least 48 hours prior to the day of the study. These medication criteria were included to reduce dampening effects (i.e., reduced physiological reactivity; c.f. Biber & Alkin, 1999). All exclusionary criteria were provided in the initial recruitment materials and reassessed on the day of participation.

Measures

Panic Response System Measures

Subjective Anxiety—The Subjective Units of Distress Scale (SUDS; Wolpe, 1969) is a verbally administered rating scale used to index self-reported anxiety, on a scale ranging from 0 (no anxiety) to 100 (extreme anxiety). SUDS were measured every two minutes during the 18-minute CO_2 challenge procedure, starting at minute 2 of the Room Air Breathing Period and ending at minute 5 of the Recovery Period, for a total of *nine* SUDS ratings during the challenge task (assuming participants did not terminate the task early). Participants also provided a baseline SUDS rating at the start of the experiment, approximately 15 to 20 minutes before the facemask was attached (No-Facemask Baseline Period). Given the high correlations between the three Room Air Breathing SUDS measurements (*r*s = .80 - .91, all *p*,s < .001), a mean Room Air Breathing SUDS score was created for analyses.

Panic Symptoms—The Diagnostic Symptom Questionnaire (DSQ) is a 26-item self-report measure that assesses the Diagnostic and Statistical Manual of Mental Disorders' (DSMIV; American Psychological Association, 1994) symptoms of a panic attack. The first 16 items assess the presence and severity of somatic or physical symptoms (e.g., "trembling or shaking," "pounding or racing heart") using a 0-8 Likert scale, and the next 10 items assess the presence and intensity of panic-related cognitions (e.g., "I feel like I might be dying," "I need help") using a 0-4 Likert scale. The DSQ was administered at multiple time points to assess subjective changes in panic symptoms during the CO_2 challenge. DSQ symptoms mapping onto the 13 DSM-V panic attack symptoms were used to create DSQ panic symptom sum scores. The DSQ was completed at four time points: 1) during the No-

Facemask Baseline Period, 2) at the start of the Room Air Breathing Period (following attachment of the facemask, but prior to CO_2 administration), 3) five minutes after the start of the CO2 Inhalation Period, and 4) immediately after the Recovery Period (while still wearing the facemask).

Respiratory Frequency—A respiratory transducer was placed around the abdomen/chest of the participant to measure respiratory frequency. Respiratory frequency (*f*R) was continuously measured by recording each breath during the task. Similar to SUDS ratings, a high correlation was observed between the two minute epochs of *f*R measured during the Room Air Breathing Period (rs = .86-.92, p > .000). Thus, a baseline *f*R mean was created and entered as the first data point in GMM. Two-minute averages were computed for *f*R assessed during the CO_2 and Recovery phases. This approach was taken to map as closely as possible to the timing of the SUDS ratings. A Biopac data acquisition unit with Acqknowledge 4.1 software (Biopac System, Inc, Goleta, CA) was used to measure and calculate *f*R.

Established Correlates of Panic

Several well-validated constructs associated with panic were assessed via self-report measures before the CO_2 challenge task.

Anxiety Sensitivity—The Anxiety Sensitivity Index (ASI; Reiss et al., 1986) is a 16-item Likert scale that assesses an individual's tendency to fear sensations or symptoms associated with anxiety. The scale has high internal consistency (.82-.91) and good test-retest reliability (.71-.75; Peterson & Reiss, 1992).

Fear Questionnaire—The Agoraphobia subscale of the Fear Questionnaire (FQ) includes five items designed to assess agoraphobic avoidance (e.g., avoidance of going alone far from home). The FQ has demonstrated good reliability over one week (.82-.96) as well as longer time intervals ranging from 3-16 weeks (.84-.90; Michelson & Mavissakalian, 1983)

Current Stress Levels—The Depression Anxiety Stress Scales – Stress subscale (DASS-Stress; Lovibond & Lovibond, 1993) contains seven items that participants rate using a four point Likert scale to indicate the extent to which they have experienced symptoms tied to stress over the past week. The Stress subscale assesses a number of different aspects of stress including difficulty relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatient. The DASS has demonstrated good test-retest reliability (.71-.81; Brown et al., 1997) over 2-weeks.

Behavioral Escape—Experimenters followed a prescribed script in which they informed participants on several occasions that they could stop the CO_2 challenge procedure at any time if they felt too uncomfortable.[1] Discontinuing the task early was used as a measure of escape. This outcome measure was not normally distributed, with 79.6% of participants

[1]If a subject appeared very distressed by the CO_2 challenge task (e.g., significantly labored breathing), experimenters were allowed to deviate from the study script and inform participants that they were allowed to stop.

completing the full 18-minute challenge procedure. For this reason, behavioral escape was coded as a dichotomous variable (i.e., stopped early/did not stop early).

CO_2 Challenge Task

All participants were informed that they would begin the task by breathing ambient room air through a facemask and that, at some point during the procedure, they would breath 7.5% CO_2-enriched air. Thus, participants were not informed of the timing of CO_2 versus ambient air exposure, minimizing the potential impact of expectancy effects. Participants also were told that the task would take a total of 18 minutes to complete, but that they could terminate the task at any time without penalty if they felt too uncomfortable. They were reminded of this on several, prescribed occasions throughout the challenge task.

During the challenge task, participants sat in a comfortable chair and were asked to remain as still as possible to reduce movement artifacts in physiological data recordings. Participants breathed through a silicone facemask (Hans Rudolph, Inc.) that covered their nose and mouth and was connected via gas impermeable tubing to a large multi-liter bag (Hans Rudolph, Inc.) that served as a reservoir for the 7.5% CO_2 enriched air. The experimenter manually turned a three-way stopcock valve (Hans Rudolph, Inc.) to switch from room-air to the CO_2 mixture. A research assistant remained in the room during the entire CO_2 procedure. Once fitted with an appropriately sized facemask, participants breathed ambient air for five minutes (Room Air Breathing Period), followed by eight minutes of 7.5% CO_2 enriched air (CO_2-Inhalation Period), followed by a five-minute recovery phase during which participants again breathed ambient air (Recovery Period). At the end of the recovery period, the facemask was removed.

Statistical Methods

Growth Mixture Model

Growth mixture modeling (GMM) identified latent class trajectories of SUDS, DSQ, and *f*R responses to the CO_2 challenge because this method allows for the identification of multiple, unique trajectories. The latent trajectory classes are formed on the basis of the growth factor means (i.e., means of intercept, slope) so that each class defines a different trajectory over time (Muthén, 2001; Muthén & Muthén, 2000). The selected modeling method was based on a chronometric approach (McArdle & Anderson, 1990; Meredith & Tisak, 1990; Ram & Grimm, 2007; Wood, 2011), where the repeated measures of observed variables are represented by chronometric common factors, which allow for individual differences in response over time (Duncan et al., 1997). These common factors are estimated as two primary growth factors (i.e., intercept and slope). The factor loadings of the repeated measures were set at 1 for each measurement and the slope was bound only at the end points, allowing each individual's curve to take shape without any *a priori* restrictions. No constraints were placed on class variances.

Currently, the preferred methods for determining the number of classes in a growth mixture model consists of finding the model with the smallest Bayesian information criteria (BIC) value and a significant Lo-Mendell-Rubin (LMR; 2001) and Vuong-Lo-Mendell-Rubin (VLMR) likelihood ratio test (LMR-LRT) statistic. A simulation study also indicated that while the BIC performed the best among the information criteria based indices, the bootstrap likelihood ratio test (BLRT) proved to be the best indicator of classes (Nylund, Asparouhov, & Muthén, 2007). Entropy also was used as an additional model selection measure. Entropy is a measure of classification certainty, with higher numbers reflecting higher certainty of classification. Entropy is not a measure of model fit, but is nonetheless useful for determining the number of best-fitting latent classes. Final class selection was based on a number of factors including fit indices, entropy, parsimony, theoretical justification, and interpretability (Bauer & Curran, 2003; Muthén, 2003; Rindskopf, 2003). To select the number of latent classes, a two-class model was first examined to determine whether two-classes provided better fit compared with a one-class model. If so, a three-class model was considered next and continued until the combination of model selection factors previously described no longer suggested examination of a larger number of classes.

When examining the relation of covariates with latent class trajectories, Muthén (2003, 2004) recommends that regression of trajectory class membership and growth factors on covariates should be included in GMM to correctly specify the model, find the proper number of classes, and correctly estimate class membership. Unfortunately, this is not always possible because some covariates cause shifting on persons amongst trajectories when class is regressed onto the covariate (Clark & Muthén, 2010). This issue was encountered when examining some of the panic correlates. When inclusion of covariates while forming the latent classes is not a viable option, covariates can be examined outside the model if entropy is at least .80 (Clark & Muthén, 2010). Using assignment to the most likely class membership is the best performing alternative method for examining covariates when entropy is high (Clark & Muthén, 2010). We therefore examined panic correlates using logistic regression where latent class served as the dependent variable, as recommended by Clark and Muthén (2010). All analyses were conducted in Mplus 6.0 (Muthen & Muthen 2007) using the maximum likelihood robust (MLR) estimator.

Results

Zero-order correlations are presented in Table 2. Subjective anxiety (SUDS) and panic symptom (DSQ) endorsements were significantly and positively correlated, with associations of moderate size. These measures did not correlate with physiological outcomes with the exception of *f*R during CO_2 breathing, which was modestly associated with SUDS (r=.18) and DSQ (r=.22). Next, Table 3 presents GM model outcomes for subjective anxiety, panic symptoms, and physiological response.

Subjective Anxiety Response

Fit indices in Table 3 suggest that the three-class model best fit the data, with a significant VLMR=.01 and LMR=.012 coupled with an entropy of .84 and a ΔBIC=51.91. Examination of a four-class model suggested no additional improvement in fit. Examination of the three-class model indicated that Class 1 (Low Anxiety) captured the largest portion of the sample (74.1%) and represented persons reporting low anxiety during the Room Air Breathing Period and a mild increase in anxiety during the CO_2 Inhalation Period, with a return to the No-Facemask Baseline following termination of CO_2 (see Figure 1). Class 2 (Acute

Anxiety) reported similar levels of room air anxiety as Class 1, but this class exhibited a robust increase in anxiety with 7.5% CO_2 air onset. Interestingly, class 2 also exhibited a rapid deceleration in SUDS after CO_2 termination, ultimately reporting about the same anxiety at the end of the recovery phase as to their No-Facemask Baseline and Room Air Breathing periods. Class 2 captured approximately 20.4% of the sample. Although the third latent class (High Persistent Anxiety; 5.5%) was distinguished by elevated anxiety across all three phases, this class' anxiety increased slightly during the CO_2 Inhalation Period from their facemask baseline.

Panic Symptom Response

Model outcomes suggested that the two-class solution provided better fit over a one-class solution based on LRTs (VLMR=.027; LMR=.022); high entropy (.90) also was observed. Examination of a three-class model suggested improved fit over the two-class model (VLMR=.005; LMR=.004) and high entropy (.93). Non-significant LRTs were observed for the four-class model (VLMR=.236; LMR=.249). Although the three-class model provided better fit compared to the two-class model, one of the three classes was quite small, capturing less than 3% (n<10 persons) of the sample. Class one under the two-class model consumed this small class. For this reason, the two-class model was selected and is presented in Figure 2. Similar to subjective anxiety, Class 1 (Moderate Panic Symptoms) captured the largest portion of the sample (90.3%) and represented persons reporting a low level of panic symptoms during the No-Facemask Baseline and Room Air Breathing Periods along with a moderate increase (i.e., doubling) in panic symptoms during the CO_2 Inhalation Period, with a near return to the No-Facemask Baseline following discontinuation of CO_2. Class 2 (Acute Panic Symptoms) reported panic symptoms levels similar to Class 1 during No-Facemask Baseline and Room Breathing Periods, with a sharp increase in panic symptoms during CO2 breathing, and a modest deceleration of panic symptoms with 7.5% CO_2 air termination, such that panic symptom report during recovery remained quite elevated.

Respiratory Response

The two-class solution was first examined and suggested that two-classes did not fit better than one class (VLMR=.360; LRM=.380). The one class solution was selected as best fitting and is presented in Figure 3. This class exhibited *f*R of about 15 breaths per minute (BPM) during the Facemask Baseline with a peak of approximately 21 BPM, which, unlike SUDS ratings occurred at the end of the 8 minutes of CO_2 breathing. *f*R returned to baseline levels during Recovery.

Overlap in Subjective and Panic Symptom Latent Class Trajectories

No cross-tabulations were possible with *f*R. Cross-tabulations for the three subjective anxiety classes and the two panic symptom classes are presented at the bottom of Table 3. Only 1.6% of the sample fell in the Acute Anxiety and Acute Panic Symptom cell and 1.3% fell in the High Baseline Anxiety and Acute Panic Symptom cell. The Low Anxiety and Moderate Panic Symptom Class crossing captured the largest percent (70%) of individuals.

Association between Latent Class Trajectories and Established Correlates of Panic

Next, the association between subjective anxiety and panic symptom latent class trajectories and established correlates of panic were examined using multinomial or binary logistic regression. Panic variables served as predictors of latent class membership (Clark & Muthen, 2010). Table 4 contains results of these analyses for the SUDS class trajectories. For the ASI, DASS, and FQ, the Low Anxiety (*M*=17.0, *M*=4.93, *M*=4.79 respectively) class differed significantly from the Acute (*M*=20.0, *M*=6.07, *M*=6.97, respectively) and High Persistent Anxiety classes (*M*=25.4, *M*=8.36, *M*=46.46, respectively) trajectories, with individuals in the High and Acute Anxiety classes' self-reporting higher levels of anxiety sensitivity and current stress compared with the Low Anxiety Class. The High Persistent Anxiety class (53.3%) also escaped from CO_2 inhalation at a significantly higher rate compared to the Low (19.8%) and Acute Anxiety (11.7%) classes.

For the two panic symptom latent class trajectories, all regression findings were non-significant with the exception of CO_2 escape where the High Symptom class (34.6%) escaped the CO_2 challenge almost two and half times more often than the Moderate Symptom class (18%). See lower portion of Table 3.

Discussion

The purpose of the current study was to examine the latent structure of responses to breathing CO_2 enriched air to characterize the variability in responding to an endophenotypic marker of panic spectrum vulnerability, and to understand if this variability predicts established correlates of panic. Model outcomes for subjective anxiety suggested a three-class solution as best fitting. The Acute Anxiety class was characterized by low baseline anxiety, with a slight increase in anxiety noted during the Room Air Breathing Period (i.e., upon attachment of the facemask). A sharp rise in anxiety was then observed with CO_2 onset, along with a rapid deceleration of anxiety upon CO_2 termination. Moreover, estimated means suggested a slight habituation pattern, with peak anxiety occurring after approximately three minutes of CO_2 exposure. This class generally scored between the Low and High Persistent Anxiety classes on established correlates of panic. A second, smaller class (High Persistent Anxiety) was identified, which included a group of people reporting moderately high anxiety during the No-Facemask Baseline and Room Air Breathing periods, followed by a modest rise in anxiety during CO_2 inhalation, and a slight decline in anxiety during the Recovery period. Like the Acute Anxiety class, this class exhibited a very slight decrease of anxiety during the course of CO_2 breathing. The High Persistent Anxiety class endorsed greater levels of agoraphobia related avoidance, anxiety sensitivity, and greater stress levels compared to the Low Anxiety class. Moreover, a very robust difference between the classes emerged for the escape measure, with a considerable number (i.e., approximately half) of persons in the High Persistent Anxiety class requesting to terminate the CO_2 challenge early compared to the Acute and Low Anxiety classes. These results suggest that two classes of people were hypersensitive to CO_2, but in differing ways.

The High Persistent Anxiety class may represent a more generally anxiety-vulnerable group, rather than showing particular sensitivity to the CO_2 challenge. Thus, this class may be capturing a group of individuals characterized by fairly consistently activated vulnerabilities to anxiety and stress (such that the anticipation of an upcoming physiological stressor elicits anxiety levels nearly equivalent to the stressor itself). By contrast, persons in the Acute Anxiety class may carry a somewhat heightened risk for panic in the form of somatic/ interoceptive vulnerability that is activated strongly in response to a panic stressor (i.e., the onset of CO_2 enriched air), but does not result in ongoing heightened anxiety. It is likely that the Acute Anxiety class also may include some level of heterogeneity in anxious responding. For example, there may be individuals who start to exhibit a habituation pattern during CO_2 breathing while others do not, and we speculate that this latter pattern will be associated with particularly increased panic risk.

Given the habituation pattern observed in the Acute and High Persistent Anxiety classes' profiles, post-hoc examinations of habituation patterns were performed at the individual level. Habituation was defined as a decrease in subjective anxiety of one standard deviation (SD) of the Acute Anxiety group mean (SD=15). Persons in the Acute Anxiety class were considered to achieve habituation if their SUDS score decreased by 15 points from one rating to another during CO_2 breathing or if their anxiety decreased a total of 15 points after the first CO_2 inhalation SUDS rating. Using this approach, an adjusted Acute Anxiety class prevalence of 14.3% was determined. This same approach was applied to the High Persistent Anxiety class (SD=22), resulting in an adjusted rate of 3.7%, with a combined adjusted rate of 18% of participants considered at-risk.

The largest portion of the sample was captured in the Low Anxiety class. This class exhibited a mild increase in subjective anxiety from the No-Facemask Baseline and Room Air Breathing periods to CO_2 inhalation, and a return to baseline levels of anxiety upon CO_2 discontinuation. Interestingly, the size of this class indicates that the majority of persons undergoing the 7.5% CO_2 challenge do not find inhalation of CO_2 enriched air strongly anxiety provoking. Moreover, this class was associated with the lowest levels of self-reported stress and anxiety sensitivity, suggesting minimal panic vulnerability. These findings highlight that response to CO_2 is quite heterogeneous in that three unique response trajectories were identified, indicating that researchers should consider this variability when using the CO_2 hypersensitivity challenge to study panic risk.

Although subjective anxiety was regarded as the primary measure of CO_2 hypersensitivity, panic symptoms also were examined as this outcome has been used in a number of previous studies of CO_2 hypersensitivity. A two-class model was selected as the best fit, with one class (Acute Panic Symptoms) exhibiting a robust increase (a near tripling) in panic symptoms with CO_2 onset and a slight deceleration in symptoms when CO_2 was stopped. Thus, this class continued to experience panic symptoms well after CO_2 discontinuation. This observation is similar to that observed in children with two known panic risk factors - a diagnosis of separation anxiety disorder and a parent with PD - where scores on panic symptoms following inhalation of 5% CO_2 remained high after CO_2 was turned off (Roberson-Nay, et al, 2010). The Moderate Panic Symptom class manifested an increase in panic symptoms from the No-Facemask Baseline and Room Air Breathing periods to the CO_2 breathing phase along with a return to baseline during recovery, suggesting no lasting residual of CO_2-induced panic symptoms once CO_2 was discontinued. Despite the drastic differences in panic symptom trajectories over time, no differences emerged between these two classes on sex, anxiety sensitivity, or current stress levels. The Acute Panic Symptom class did escape the CO_2 task at significantly higher rates, however. Together, these mostly null results for the relationships between the symptom-based classes and the established correlates of panic raise important questions about the utility of panic symptom measures to identify panic risk conferred by CO_2 hypersensitivity.

Next, subjective anxiety and panic symptom classes were cross-tabulated to determine whether a panic risk class would emerge wherein persons express both elevated subjective anxiety and panic symptom response. Results revealed that a very small percent (2.9%) was captured by the crossing of the Acute Anxiety / High Persistent Anxiety subjective anxiety classes and Acute panic symptom class. That is, persons reporting acute panic symptom increases did not necessarily fall in the High or Acute subjective anxiety classes and vice versa, suggesting discordance of these two response systems. In all, the cross-tabulation did not reveal a consistent at-risk group(s) of persons. This may be partly explained by the minimal evidence for relationships between the panic symptom classes and correlates of panic.

Finally, a link between panic and respiration has been suggested by the extant literature (Klein, 1993), and we were interested to learn if a similar number and structure of classes would emerge using a physiological marker, rather than a self-reported subjective response. Unlike results for subjective anxiety and panic symptoms, only one respiratory class was identified. This finding suggests little evidence for respiratory frequency as a clear marker of CO_2 hypersensitivity. Instead, subjective anxiety response showed a more reliable pattern of CO_2 hypersensitivity and associated panic outcomes, although there appears to be both an acute and a more chronically high anxiety response that may be associated with different risk pathways. Finally, desynchrony among the three selected CO_2 hypersensitivity markers was observed, which is not wholly unexpected as this pattern is frequently observed across differing response systems associated with anxiety and fear expression (cf. Lang, 1988).

Limitations and Conclusion

There are several limitations to note. Although a resting measure (i.e., No-facemask baseline) of subjective anxiety and panic symptoms were collected, there was no equivalent measure for respiratory frequency. Instead, respiratory rate was assessed only starting with the Room Air Breathing Period, during which some participants may have already felt threatened by the unknown timing of the CO_2 enriched air. We also did not measure end-tidal CO_2 levels, which would have provided additional insight into the “dosage” of CO_2 received by persons in the three anxiety classes. Moreover, although this study examined CO_2 hypersensitivity in one of the largest samples to date, replication of class trajectories is needed. The study sample also was not a general population sample, which somewhat limits our ability to generalize study findings. Finally, at first glance, the percent of persons considered at-risk may appear high. Our adjusted and unadjusted estimates are rather consistent with epidemiological data indicating that approximately 20% of the population has experienced at least one panic attack in their lifetime (Kessler et al., 2006). Moreover, panic plays an etiologic role in multiple disorders other than PD (e.g., social phobia; Kinley, Walker, Enns, & Sareen, 2011). For these reasons, the combined prevalence of the Acute and High Persistent Anxiety classes appears to be an appropriate approximation of risk.

A potential next step is to first replicate the class structures observed here as well as determine whether the identified latent class trajectories relate to distal outcomes (e.g., development of panic attacks or PD). Moreover, it would be valuable to examine whether these classes also represent persons at risk for other psychopathology, including conditions whose development may be potentiated by panic and its pathophysiology (e.g., PTSD among trauma-exposed persons). Using a repeated measures design that tracks multiple panic responses has considerable potential to advance identification of the classes of people who are most at risk, ultimately generating new opportunities for focused prevention and intervention efforts.

Acknowledgements

Portions of these data also were presented at the 32nd annual meeting of the Anxiety Disorders Association of America, Arlington, Virginia; the 46th annual meeting of the Association for Cognitive-Behavioral Therapy, National Harbor, MD; and the 53rd annual meeting of the Society for Psychophysiological Research, Florence, Italy.

Financial Support: Preparation of this manuscript was supported by grant K01-MH-080953 from the National Institute of Mental Health (RRN) and grant R01AG033033 from the National Institute of Aging (BT).

References

Battaglia M, Ogliari A, Harris J, Spatola CA, Pesenti-Gritti P, Reichborn-Kjennerud T, Tambs K. A genetic study of the acute anxious response to carbon dioxide stimulation in man. Journal of Psychiatric Research. 2007; 41(11):906–917. [PubMed: 17254605]

Battaglia M, Perna G. The 35% CO2 challenge in panic disorder: Optimization by receiver operating characteristic (ROC) analysis. Journal of Psychiatric Research. 1995; 29(2):111–119. [PubMed: 7666379]

Biber B, Alkin T. Panic disorder subtypes: Differential responses to CO2 challenge. The American Journal of Psychiatry. 1999; 156(5):739–744. [PubMed: 10327907]

Brown TA, Chorpita BF, Korotitsch W, Barlow DH. Psychometric properties of the Depression Anxiety Stress Scales (DASS) in clinical samples. Behaviour Research and Therapy. 1997; 35:79–89. [PubMed: 9009048]

Clark, S.; Muth_en, B. Relating latent class analysis results to variables not included in the analysis. 2009. https://www.statmodel.com/download/relatinglca.pdf

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]

Coryell W, Pine D, Fyer A, Klein D. Anxiety responses to CO2 inhalation in subjects at high-risk for panic disorder. Journal of Affective Disorders. 2006; 92(1):63–70. [PubMed: 16527360]

Duncan, TE.; Duncan, SC.; Strycker, LA.; Li, F.; Alpert, A. An introduction to latent variable growth curve modeling: Concepts, issues, and applications. Lawrence Erlbaum Associates; Mahwah NJ: 1999.

Gottesman II, Gould TD. The endophenotype concept in psychiatry: Etymology and strategic intentions. The American Journal of Psychiatry. 2003; 160(4):636–645. [PubMed: 12668349]

Gorman JM, Kent J, Martinez J, Browne S, Coplan J, Papp LA. Physiological changes during carbon dioxide inhalation in patients with panic disorder, major depression, and premenstrual dysphoric disorder: evidence for a central fear mechanism. Archives of General Psychiatry. 2001; 58:125–131. [PubMed: 11177114]

Griez E, de Loof 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]

Klein DF. False suffocation alarms, spontaneous panics, and related conditions: An integrative hypothesis. Archives of General Psychiatry. 1993; 50:306–318. [PubMed: 8466392]

Lovibond, SH.; Lovibond, PF. Manual for the Depression Anxiety Stress Scales (DASS). Psychology Foundation Monograph; NSW, Australia: 1993.

Meyer-Lindenberg A, Weinberger DR. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nature Reviews Neuroscience. 2006; 7:818–827.

Michelson L, Mavissaklian M. Temporal stability of self-report measures in agoraphobia research. Behaviour Research and Therapy. 1983; 21:695–698. [PubMed: 6661155]

Muthén, B. Latent variable mixture modeling.. In: Marcoulides, GA.; Schumacker, RE., editors. New Developments and Techniques in Structural Equation Modeling. Lawrence Erlbaum Associates; 2001. p. 1-33.

Muthén, B. Second-generation structural equation modeling with a combination of categorical and continuous latent variables: New opportunities for latent class/latent growth modeling.. In: Collins, LM.; Sayer, A., editors. New Methods for the Analysis of Change. APA; Washington, D.C.: 2001. p. 291-322.

Muthén B. Statistical and substantive checking in growth mixture modeling. Psychological Methods. 2003; 8:369–377. [PubMed: 14596497]

Muthén, B. Latent variable analysis: Growth mixture modeling and related techniques for longitudinal data.. In: Kaplan, D., editor. Handbook of quantitative methodology for the social sciences. Sage Publications; Newbury Park, CA: 2004. p. 345-368.

Muthén B, Muthén L. The development of heavy drinking and alcohol-related problems from ages 18 to 37 in a U.S. national sample. Journal of Studies on Alcohol. 2000; 61:290–300. [PubMed: 10757140]

Muthén B, Muthén L. Integrating person-centered and variable-centered analyses: Growth mixture modeling with latent trajectory classes. Alcoholism: Clinical and Experimental Research. 2000; 24:882–891.

Muthén, LK.; Muthén, BO. Mplus user's guide. Muthén & Muthén; Los Angeles: 2007.

Nagin DS. Analyzing developmental trajectories: A semi-parametric, group-based approach. Psychological Methods. 1999; 4:139–157.

Nylund KL, Asparouhov T, Muthén BO. Deciding on the number of classes in laten class analysis and growth mixture modeling: A Monte Carlo simulation study. Structural Equation Modeling: A Multidisciplinary Journal. 2007; 14(4):535–569.

Papp LA, Klein DF, Gorman JM. Carbon dioxide hypersensitivity, hyperventilation, and panic disorder. The American Journal of Psychiatry. 1993; 150(8):1149–1157. [PubMed: 8392296]

Perna G, Barbini B, Cocchi S, Bertani A, Gasperini M. 35% CO2 challenge in panic and mood disorders. Journal of Affective Disorders. 1995; 33(3):189–194. [PubMed: 7790671]

Perna G, Bertani A, Arancio C, Ronchi P, Bellodi L. Laboratory response of patients with panic and obsessive-compulsive disorders to 35% CO2 challenges. The American Journal of Psychiatry. 1995; 152(1):82–89.

Peterson, RA.; Reiss, S. Anxiety sensitivity index manual. 2nd ed.. International Diagnostic Systems; Worthington, OH: 1992.

Pine DS, Klein RG, Coplan JD, Papp LA, Hoven CW, Martinez J, et al. Differential Carbon Dioxide Sensitivity in Childhood Anxiety Disorders and Nonill Comparison Group. Archives of General Psychiatry. 2000; 57:960–967. [PubMed: 11015814]

Pine DS, Klein RG, Roberson-Nay R, Mannuzza S, Moulton JL III, Woldehawariat G, et al. Response to 5% Carbon Dioxide in Children and Adolescents: Relationship to Panic Disorder in Parents and Anxiety Disorders in Subjects. Archives of General Psychiatry. 2005; 62:73–80. [PubMed: 15630075]

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. Behavior Research and Therapy. 1986; 24:1–8.

Roberson-Nay R, Klein DF, Klein RG, Mannuzza S, Moulton JL 3rd, Guardino M, Daniel S. Carbon dioxide hypersensitivity in separation-anxious offspring of parents with panic disorder. Biological Psychiatry. 2010; 67(12):1171–1177. [PubMed: 20172505]

Roberson-Nay R, Moruzzi S, Ogliari A, Pezzica E, Tambs K, Kendler KS, Battaglia M. Evidence for distinct genetic effects associated with response to 35%CO_2. Depression and Anxiety. 2013; 30(3):259–266. [PubMed: 23349098]

Sanderson WC, Rapee RM, Barlow DH. The influence of perceived control on panic attacks induced via inhalation of 5.5% CO_2-enriched air. Archives of General Psychiatry. 1989; 46:157–162. [PubMed: 2492423]

Schmidt NB, Zvolensky MJ. Anxiety sensitivity and CO_2 challenge reactivity as unique and interactive prospective predictors of anxiety pathology. Depression and Anxiety. 2007; 24:527–536. [PubMed: 17136755]

Verburg C, Griez E, Meijer J. A 35% carbon dioxide challenge in simple phobias. Acta Psychiatrica Scandinavica. 1994; 90(6):420–423. [PubMed: 7892773]

Verburg K, Pols H, de Leeuw M, Griez E. Reliability of the 35% carbon dioxide panic provocation challenge. Psychiatry Research. 1998; 78(3):207–214. [PubMed: 9657425]

Vickers K, Jafarpour S, Mofidi A, Rafat B, Woznica A. The 35% carbon dioxide test in stress and panic research: Overview of effects and integration of findings. Clinical Psychology Review. 2012; 32(3):153–164. [PubMed: 22322014]

Wolpe, J. The practice of behavior therapy. Pergamon Press; New York: 1969.

Highlights

- Carbon dioxide (CO_2) hypersensitivity is a robust endophenotypic marker of panic spectrum vulnerability.
- The underlying structure of CO_2 hypersensitivity is unknown.
- Three latent class trajectories best explain subjective anxiety whereas two latent class trajectories best capture panic symptom response and only one class best reflects respiratory rate.
- Two subjective anxiety class trajectories potentially representing different panic risk pathways.

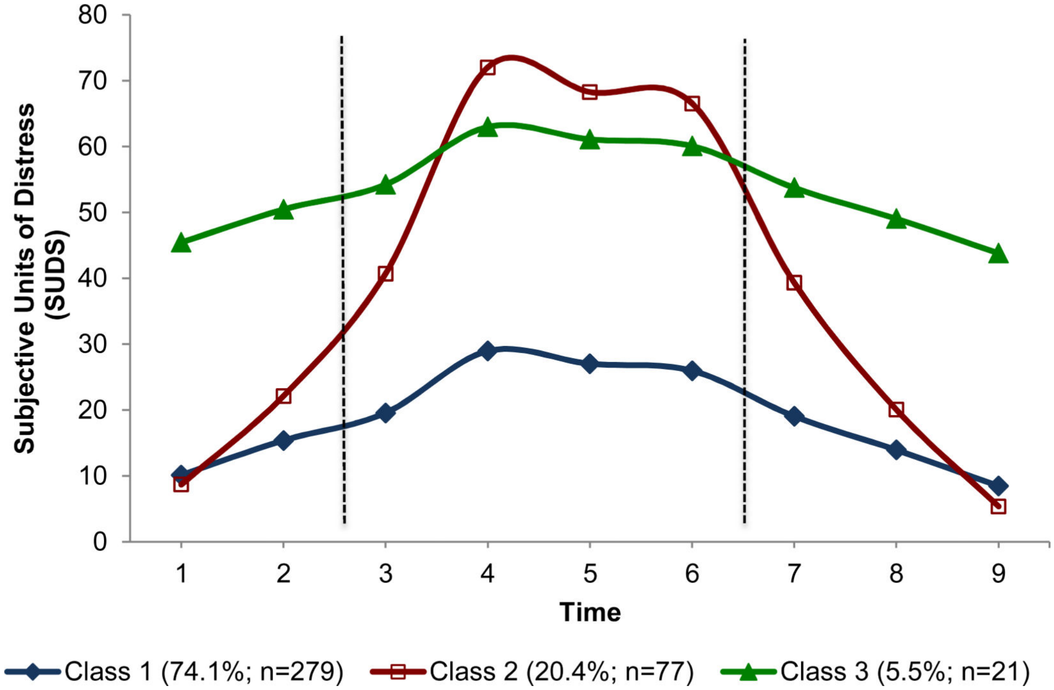

Figure 1.

Estimated Means of Latent Class Trajectories for Subjective Anxiety during Pre-CO_2 Room Air, 7.5% CO_2 Breathing, and Post-CO_2 Recovery.

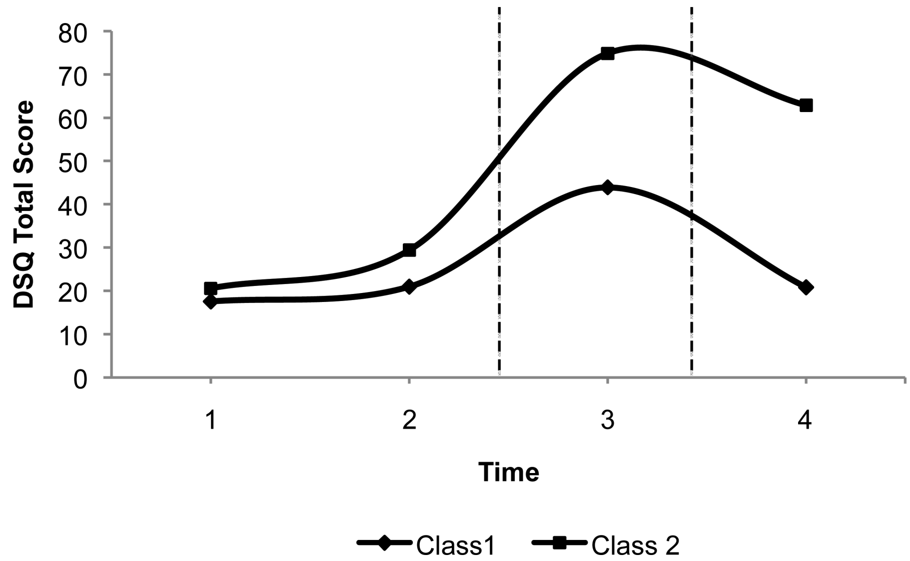

Figure 2.

Estimated Means of Latent Class Trajectories for Panic Symptoms during Baseline, Pre-CO_2 Room Air, 7.5% CO_2 Breathing, and Post-CO_2 Recovery.

NIH-PA Author Manuscript

NIH-PA Author Manuscript

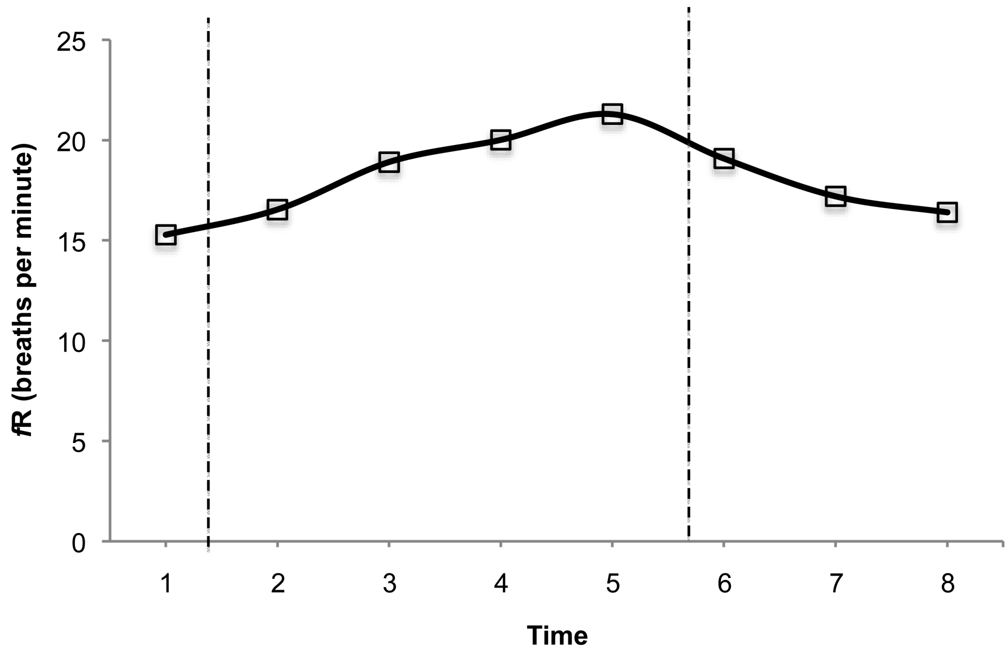

Figure 3.

Estimated Means of Latent Class Trajectory of Respiratory Rate (*f*R) during Pre-CO_2 Room Air Breathing, 7.5% CO_2 Breathing, and Post-CO_2 Recovery.

Table 1

Sample demographics and characteristics.

Sex-% female	56.1
Age, years-Mean (SD; Range)	19.8 (2.7; 18-49)
Race	
Caucasian	57.6
African American	18.4
Asian	18.7
Native American	0.6
Mixed Race	5.0
Did not report race	9.6
Ethnicity-% Hispanic	4.9
ASI* Quartiles, *n*	
<13	107
14-19	111
20-16	88
>27	56

* missing 14 cases.

Table 2

Zero-order Correlations between CO2 Hypersensitivity Markers.

	1	2	3	4	5	6	7	8	9	10
1. SUDS Mean Bsln	-----	.59**	.68**	.37**	.59**	.25**	.126*	0.02	−0.02	−0.03
2. SUD Mean CO_2		-----	.68**	.36**	.45**	.58**	.32**	0.08	.18**	0.07
3. SUDS Mean Rec			-----	.28**	.48**	.42**	.35**	0.06	0.10	0.04
4. DSQ No Facemask Bsln				-----	.47**	.33**	.26**	0.01	0.08	−0.05
5. DSQ Facemask Bsln					-----	.44**	.36**	0.04	0.03	0.04
6. DSQ CO_2						-----	.48**	0.04	.22**	0.10
7. DSQ Rec							-----	0.02	0.09	0.01
8. RR Mean Bsln								-----	.35**	.35**
9. RR Mean CO_2									-----	.53**
10. RR Mean Rec										-----

Note: Bsln=Baseline; CO2=CO2 breathing phase; Rec=Recovery Phase; SUDS=Subjective units of distress.

** $p<0.01$

* $p<0.05$.

Table 3

Fit Indices and Class Statistics of Growth Mixture Models for Subjective Anxiety, Respiratory Frequency, and Panic Symptoms (upper). The Percent of Participants Falling into Cross-tabulated Subjective Anxiety and Panic Symptoms Latent Class Trajectory Cells (lower).

# of Classes	# of params	BIC	Δ BIC	LL	VLMR	LMR	BLRT	Entropy
Subjective Anxiety-SUDS								
1	28	24188.71	-----	−12011.12	-----	-----		-----
2	31	24121.83	66.88	−11968.76	.019	.023	.0000	.93
3***	***34***	***24069.92***	***51.91***	***−11933.89***	***.010***	***.012***	***.0000***	***.84***
4	37	24055.78	14.14	−11917.90	.529	.531	.0000	.78
Respiratory Frequency-*f*R								
1***	***23***	***10423.05***	-----	***−5146.28***	-----	-----	-----	-----
2	26	10299.30	123.75	−5075.90	.36	.38	.0000	.84
3[¥]	-----	-----	-----	-----	-----	-----	-----	-----
Panic Symptoms-DSQ								
1	12	8862.31	-----	−4396.53	-----	-----	-----	-----
2***	***15***	***8736.28***	***126.03***	***−4324.85***	***.022***	***.027***	***.0000***	***.90***
3	18	8609.29	126.99	−4252.70	.004	.005	.0000	.93
4	21	8596.74	12.55	−4237.77	.236	.249	.0000	.92

			Panic Symptom (DSQ) Latent Class Trajectories	
		C1 Moderate Panic Sxs	**C2 Acute Panic Sxs**	
Subjective Anxiety (SUDS) Latent Class Trajectories (percent)		**C1-Low Anxiety**	70.0	5.5
		C2-Acute Anxiety	17.9	1.6
		C3-High Anxiety	3.6	1.3

Note: SUDS=Subjective Units of Distress Scale; Params=Parameters; BIC=Bayesian Information Criterion; LL=Log likelihood; VLMR=Vuong-Lo-Mendell-Rubin Likelihood Ratio Test; LMR= Lo-Mendell-Rubin Adjusted Likelihood Ratio Test; BLRT=Bootstrap; Likelihood Ratio Test; SUDS=Subjective Units of Distress Scale; DSQ=Diagnostic Symptom Questionnaire; Sxs=Symptoms; C1=Class 1; C2=Class 2; C3=Class 3

*Best fitting model

¥Model did not converge.

Table 4

Multinomial and Binary Logistic Regression, with Panic Correlates Predicting Subjective Anxiety (SUDS) Latent Classes and Panic Symptom (DSQ) Latent Classes, respectively.

Subjective Anxiety (SUDS) Class Trajectories						
Panic Risk Factor	**β**	**SE β**	**Wald**	***p***	**Exp(β)/OR**	**95%CI**
Demographic						
Sex						
Acute vs. Low§	−.411	.31	1.79	.181	0.66	0.36-1.21
High vs. Low§	−.580	.62	0.89	.346	0.56	0.17-1.87
Acute vs. High€	.169	.66	0.07	.798	1.18	0.32-4.30
Self-Report Measures						
ASI Total						
Acute vs. Low§	***.037***	***.02***	***5.30***	***.021***	***1.04***	***1.01-1.07***
High vs. Low§	***.087***	***.03***	***10.87***	***.001***	***1.09***	***1.04-1.15***
Acute vs. High€	−.049	.03	3.19	.074	0.95	0.90-1.01
DASS, Stress						
Acute vs. Low§	***.089***	***.04***	***4.46***	***.035***	***1.09***	***1.01-1.19***
High vs. Low§	***.241***	***.08***	***8.84***	***.003***	***1.27***	***1.09-1.49***
Acute vs. High€	−.152	.09	3.17	.075	0.86	0.73-1.02
FQ, Agoraphobic Avoidance						
Acute vs. Low§	***.075***	***.027***	***7.99***	***.005***	***1.08***	***1.02-1.14***
High vs. Low§	***.118***	***.042***	***7.81***	***.005***	***1.13***	***1.04-1.22***
Acute vs. High€	−.043	.043	1.01	.315	0.96	0.88-1.04
Behavioral						
CO_2 Escape (% stopped CO_2 early)						
Acute vs. Low§	.628	.43	2.09	.148	1.87	0.80-4.38

Subjective Anxiety (SUDS) Class Trajectories						
Panic Risk Factor	**β**	**SE β**	**Wald**	***p***	**Exp(β)/OR**	**95%CI**
High vs. Low[§]	***−1.53***	***.54***	***7.96***	***.005***	***0.22***	***0.08-0.63***
Acute vs. High[€]	***2.16***	***.66***	***10.84***	***.001***	***8.65***	***2.40-31.27***
Panic Symptom (DSQ) Class Trajectories						
Demographic						
Sex[*]						
Acute vs. Moderate	−.383	.45	.741	.389	1.47	0.61-3.51
Self-Report Measures						
ASI Total						
Acute vs. Moderate	.012	.02	.303	.582	0.99	0.95-1.03
DASS, Stress						
Acute vs. Moderate	.104	.06	3.104	.078	0.90	0.80-1.01
FQ, Agoraphobic Avoidance						
Acute vs. Moderate	.000	.007	.003	.957	1.00	0.99-1.01
Behavioral[*]						
CO_2 Escape (% stopped CO_2 early)						
Acute vs. Moderate	***−.883***	***.44***	***4.04***	***.044***	***2.42***	***1.02-5.72***

Note-For Subjective Anxiety: Low=Low Anxiety Class; Acute=Acute Anxiety Class; High=High Anxiety Class; OR=Odds Ratio; ASI=Anxiety Sensitivity Index; DASS=Depression, Anxiety, Stress Scale, FQ=Fear Questionnaire.

For Panic Symptoms: Moderate=Moderate Panic Symptoms Class; Acute=Acute Panic Symptom Class.

§ The Low Anxiety Class serves as the reference class.

€ The Acute Anxiety Class serves as the reference class.

* In binary regression models, female sex and positive escape behavior served as the reference group.