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Inhalation of 7.5% Carbon Dioxide Increases Threat Processing in Humans

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Inhalation of 7.5% CO_2 increases anxiety and autonomic arousal in humans, and elicits fear behavior in animals. However, it is not known whether CO_2 challenge in humans induces dysfunction in neurocognitive processes that characterize generalized anxiety, notably selective attention to environmental threat. Healthy volunteers completed an emotional antisaccade task in which they looked toward or away from (inhibited) negative and neutral stimuli during inhalation of 7.5% CO_2 and air. CO_2 inhalation increased anxiety, autonomic arousal, and erroneous eye movements toward threat on antisaccade trials. Autonomic response to CO_2 correlated with hypervigilance to threat (speed to initiate prosaccades) and reduced threat inhibition (increased orienting toward and slower orienting away from threat on antisaccade trials) independent of change in mood. Findings extend evidence that CO_2 triggers fear behavior in animals via direct innervation of a distributed fear network that mobilizes the detection of and allocation of processing resources toward environmental threat in humans.

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INTRODUCTION

The development of experimental models of anxiety that readily translate between animals and humans is required to better integrate and clarify the biological, behavioral, and cognitive mechanisms that underlie anxiety disorders and mediate response to treatment.

Inhalation of 7.5% CO_2 for 20 min increases self-report anxiety (eg, worry, tension) and autonomic arousal (eg, heart rate, blood pressure), and provides a novel experimental model of generalized anxiety disorder (GAD) in healthy humans (Bailey *et al*, 2005) that quantitatively and qualitatively differs from the established single vital capacity inhalation of 35% CO_2 model of panic (van den Hout and Griez, 1984; see Colasanti *et al*, 2008 for detailed characterization of panic symptoms following 35% challenge in healthy volunteers). Evidence that acute benzodiazepine administration and chronic administration of selective serotonin reuptake inhibitors both attenuate subjective response to 7.5% challenge in healthy volunteers is consistent with their efficacy in patients with GAD, and further validates the 7.5% CO_2 model in humans (Bailey *et al*, 2007).

In rodents, inhalation of 10% CO_2 triggers significant freezing behavior, reduced activity in an open-field test, and greater contextual fear conditioning, consistent with the anxiety phenotype (Ziemann *et al*, 2009). Recent evidence confirms the amygdala as an important chemosensor that directly detects increases in CO_2 (via the acid-sensing ion-channel ASIC1a, which is sensitive to localized reductions in pH) to elicit fear behavior in mice (Ziemann *et al*, 2009).

Human neurocognitive models of anxiety propose a common amygdala-prefrontal circuitry that underlies fear behaviour and dysfunctional cognitive processes that promote the detection and selection of threat, and increase distractibility to task-irrelevant information in anxiety (Bishop, 2007; Davis and Whalen, 2001; Davidson, 2002). Amygdala hyperactivity to threat has been observed in healthy individuals with high levels of state anxiety (Bishop *et al*, 2004a), high levels of generalized trait anxiety (Stein *et al*, 2007), and in patients with GAD (Nitschke *et al*, 2009), and is strongly correlated with attention to threat in GAD (Monk *et al*, 2008).

Dysfunction in prefrontal cortex is observed when anxious individuals process threat distracters (Bishop *et al*, 2004b) and lateral prefrontal cortex is implicated in mediating the modification of attentional bias to threat stimuli in healthy volunteers (Browning *et al*, 2010). Furthermore, high levels of generalized trait anxiety are

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associated with reduced structural integrity in amygdala-prefrontal pathways (assessed using diffusion tensor imaging; Kim and Whalen, 2009) and reduced (negative) functional connectivity between prefrontal cortex and amygdala during threat processing (Monk *et al*, 2008; for discussion of dysfunction in additional structures, eg, insula, see Paulus and Stein, 2006; Stein *et al*, 2007).

Together these findings are consistent with cognitive models of anxiety which emphasize increased activation of threat-related representations and a failure to use controlled processing to regulate attention and emotion in the etiology of anxiety (Eysenck *et al*, 2007), and with extensive behavioral evidence of increased attention to environmental threat in anxiety (review by Bar-Haim *et al*, 2007).

In the antisaccade task, top–down attention control is required to suppress (inhibit) reflexive saccades (eye movements) toward abrupt peripheral visual stimuli and instead generate a volitional saccade in the opposite direction ('antisaccade'). Enhanced activity in prefrontal cortex (dorsolateral and ventrolateral) during (correct) antisaccade trials supports the involvement of these regions in regulating attention allocation by inhibiting reflexive attentional capture by distracters (Ettinger *et al*, 2008). Previous research with the antisaccade task has revealed deficits in attention control in individuals with high generalized trait anxiety (Garner *et al*, 2009; Ansari *et al*, 2008).

Although inhalation of low concentrations of CO_2 increase anxiety and autonomic arousal in humans and triggers fear behavior coordinated by the amygdala in small rodents, its effects on human neurocognitive mechanisms considered to underlie anxiety (eg, selective attention to threat) are not known. The present study therefore examined whether 7.5% CO_2 challenge (relative to normal air) increases attention toward and impairs inhibition of threat stimuli within a modified emotional variant of the antisaccade task.

PARTICIPANTS AND METHODS

Participants

A total of 26 healthy volunteers (12 women; mean age 21.3 years, SD = 3.0 years) were recruited from the local community. Participants attended a pretest screening interview during which they underwent a short structured diagnostic interview based on DSM-IV criteria (Mini International Neuropsychiatric Interview; Sheehan *et al*, 1998). Exclusion criteria included recent use of medication (past 8 weeks bar local treatment, occasional aspirin or paracetamol, oral, injectable, or skin patch contraception), pregnancy, history of asthma/respiratory illness, high blood pressure ($>140/90$ mm Hg), cardiovascular disease, migraines, current or lifetime history of psychiatric illness (including lifetime history/family history of panic attacks), smoker, under- or overweight (body mass index <18 or $\geqslant 28\,kg/m^2$), current or past drug or alcohol dependence, and recent use of illicit drugs (urine screen) or alcohol (breath test). Participant levels of generalized trait anxiety (Spielberger *et al*, 1983: $m = 34.8$, SD = 7.2) and fear of anxiety sensations (anxiety sensitivity, Taylor and Cox, 1998; $m = 14.3$, SD = 6.2) are indicative of those reported in healthy control samples.

Procedure

Participants attended a single test session in which they completed an emotional variant of the antisaccade task during 20 min inhalation of 7.5% CO_2 and air. Gas was administrated blind to participants through an oronasal face mask with order of gas presentation counterbalanced across participants. Heart rate, diastolic and systolic blood pressure (Omron-M6, Medisave, UK), subjective ratings of current anxiety (state version of the STAI; Spielberger *et al*, 1983), and positive and negative affect (PANAS; Watson *et al*, 1988) were measured at pretest baseline and immediately following inhalation of air and 7.5% CO_2.

Antisaccade Task

Eight negative and eight neutral color images were selected from the International Affective Picture Set (Center for the Study of Emotion and Motivation, Gainesville, FL, 1999) on the basis of normative valence ratings (scale -4 to $+4$) and arousal ratings (0–8; negative images: mean valence $= -3.1$ and mean arousal = 5.8; neutral images: mean valence = 1.2 and mean arousal = 2.9). Images subtended 8×5.5 visual-deg (at 57 cm). On each trial, an instruction word (either 'TOWARDS' or 'AWAY') was presented at central fixation for 2000 ms. At 200 ms following word offset, the picture stimulus was presented for 600 ms (6° to the left or right of central fixation). On prosaccade ('TOWARDS') trials, participants were required to look toward the picture and on antisaccade ('AWAY') trials to look away from it (ie, to shift their gaze to the opposite side of the screen). Images were presented 6 times (balanced across conditions) throughout 96 fully randomized experimental trials. Participants completed eight practice trials in which they completed pro- and antisaccades to a peripheral yellow rectangle. To increase task demand on each trial, participants classified the direction of a small arrow (↑ or ↓) presented at 50 ms following picture offset (arrow-picture location congruent on 50% of trials per trial type). The mean intertrial interval was 1000 ms (range 750–1250 ms). Stimuli were presented using Inquisit 2 Computer software, Millisecond Software, Seattle, WA. Horizontal eye movements were measured by electro-oculography and sampled at 1000 Hz (MP150-amplifier and AcqKnowledge-3.8.1 software, Biopac-Systems, Goleta, CA).

During each inhalation period, participants also completed a short (5 min) face classification task. This task addressed different research questions to those of the present study and results are to be reported elsewhere by different authors. Task order was counterbalanced across participants and did not interact with or moderate the observed effects of CO_2 on eye movements to threat, reported below (ie, gas × trial type × image valence × task order, $F < 1$, $p = 0.98$).

Data Analysis

Saccade direction and latency were scored manually and blind to trial type and inhalation condition using AcqKnowledge software. Saccades with a latency less than 100 ms (ie, reflecting anticipatory eye movements = 2.1% of data) or that subtended less than 6 horizontal degrees (ie, did not terminate in the location/mirror location

Table 1 Anxiety, Mood, Blood Pressure, and Heart Rate at Baseline and Following 7.5% CO_2 and Normal Air Inhalation

	Baseline	Air	7.5% CO_2	ANOVA F(2,50)
State anxiety	32.7[a] (7.9)	36.9[a] (9.4)	46.2[b] (12.9)	16.79, $p<0.001$, $\eta_p^2=0.402$
Negative (PANAS)	11.9[a] (2.2)	12.6[a] (5.0)	17.0[b] (5.6)	11.19, $p<0.001$, $\eta_p^2=0.309$
Positive (PANAS)	29.0[a] (6.8)	24.8[b] (7.8)	21.3[c] (7.9)	27.67, $p<0.001$, $\eta_p^2=0.525$
Systolic BP	111.1[a] (15.8)	110.4[a] (10.6)	117.8[b] (15.5)	6.40, $p=0.003$, $\eta_p^2=0.204$
Diastolic BP	71.6 (10.3)	72.1 (7.6)	74.4 (8.6)	2.64, $p=0.080$, $\eta_p^2=0.096$
Heart rate	68.6 (14.1)	67.0[a] (12.8)	74.0[b] (18.2)	4.48, $p=0.016$, $\eta_p^2=0.152$

Within each measure (row), mean values with different superscripts (a, b, c) significantly differ from each other, p's<0.05.

of the picture = 0.8% of data) were removed from analyses. The amount of missing data did not vary across conditions. Saccade accuracy and latencies for correct saccades were entered into separate repeated-measures analysis of variance (ANOVA) with inhalation (7.5% CO_2 *vs* air), trial type (pro *vs* antisaccade), and image valence (negative *vs* neutral) as independent variables.

RESULTS

Inhalation of 7.5% CO_2 produced significant increases in anxiety, negative affect, blood pressure, and heart rate, and a significant decrease in positive affect compared with inhalation of air and pretest baseline, see Table 1.

Saccade Accuracy

A significant gas × trial type × image valence interaction ($F(1, 25)=6.761$, $p=0.015$, and $\eta_p^2=0.213$) was characterized by a significant gas × valence interaction for antisaccade ($F(1, 25)=6.882$, $p=0.015$, and $\eta_p^2=0.216$), but not prosaccade trials (F values <1). CO_2 inhalation significantly increased antisaccade errors, ie, erroneous eye movements toward negative stimuli compared with (i) neutral stimuli presented during CO_2 inhalation; $t(25)=2.17$, $p=0.04$, and $d=0.213$; and (ii) both negative and neutral stimuli presented during air inhalation; $t(25)$ values >2.35, p-values <0.027, and d values >0.20, see Figure 1 (panel a).

Saccade Latency

ANOVA revealed a significant gas × trial type interaction ($F(1, 25)=4.482$, $p=0.044$, and $\eta_p^2=0.152$). CO_2 inhalation significantly delayed the time taken to correctly initiate antisaccades (CO_2: $m=305$ ms and SD = 72; air: $m=289$ ms, SD = 50; $t(25)=2.04$; and $p=0.04$), but did not affect prosaccade latencies (CO_2: $m=201$ ms and SD = 54; air: $m=203$ ms and SD = 44; t <1). All other results were non-significant. Analysis of latencies for incorrect saccades did not reveal significant results.

Associations between Self-Report and Autonomic Response to CO_2 Challenge and Antisaccade Performance

Change scores were calculated to reflect the degree of CO_2-induced increases in (i) subjective (self-report state anxiety) and autonomic response, (ii) proportion of erroneous eye movements toward negative *vs* neutral stimuli on antisaccade trials, (iii) time taken to correctly orient away from (ie, inhibit) negative *vs* neutral stimuli on antisaccade trials, and (iv) speed to correctly orient toward negative *vs* neutral stimuli on prosaccade trials.

CO_2-induced increase in blood pressure was significantly associated with (i) erroneous eye movements toward negative relative to neutral stimuli on antisaccade trials ($r=0.47$, $p=0.01$), (ii) time taken to correctly orient away from negative relative to neutral stimuli on antisaccade trials (see Figure 1b), and (iii) speed to correctly initiate eye movements toward negative relative to neutral images on prosaccade trials (Figure 1 **c**). Change in blood pressure was not associated with subjective response to CO_2 challenge. Associations between autonomic response and both attention toward and impaired inhibition of threat remained significant after controlling for change in subjective anxiety (p-values $\leqslant 0.01$).

Increased heart rate following CO_2 inhalation (relative to baseline) was associated with increased state anxiety (relative to baseline, $r=0.44$ and $p<0.05$) but neither measure was correlated with performance measures on the antisaccade task. Individual differences in trait anxiety and anxiety sensitivity were not associated with subjective or physiological response to CO_2, nor antisaccade performance.

CO_2 Challenge in Males and Females

Mixed-design ANOVA with participant gender as a between-subject factor confirmed that subjective, autonomic, and behavioral responses to CO_2 challenge were unaffected by the gender of the participant (effects of gender on subjective measures: F values (2, 48) <1.95 and p-values >0.15; autonomic measures: F values (2, 48) <1.99 and p-values >0.15; and eye-movement measures: F values <1).

Reliability Analysis

Split half correlations confirmed high levels of reliability for saccade accuracy scores (per gas × trial type × valence condition: r values >0.68 and p-values <0.001) and saccade latency scores (per gas × trial type × valence condition: r values >0.61 and p-values $\leqslant 0.001$), and recommend our task for future within-subject (pharmacological) challenge studies that wish to index attentional bias to threat. Furthermore the effects of 7.5% CO_2 inhalation on

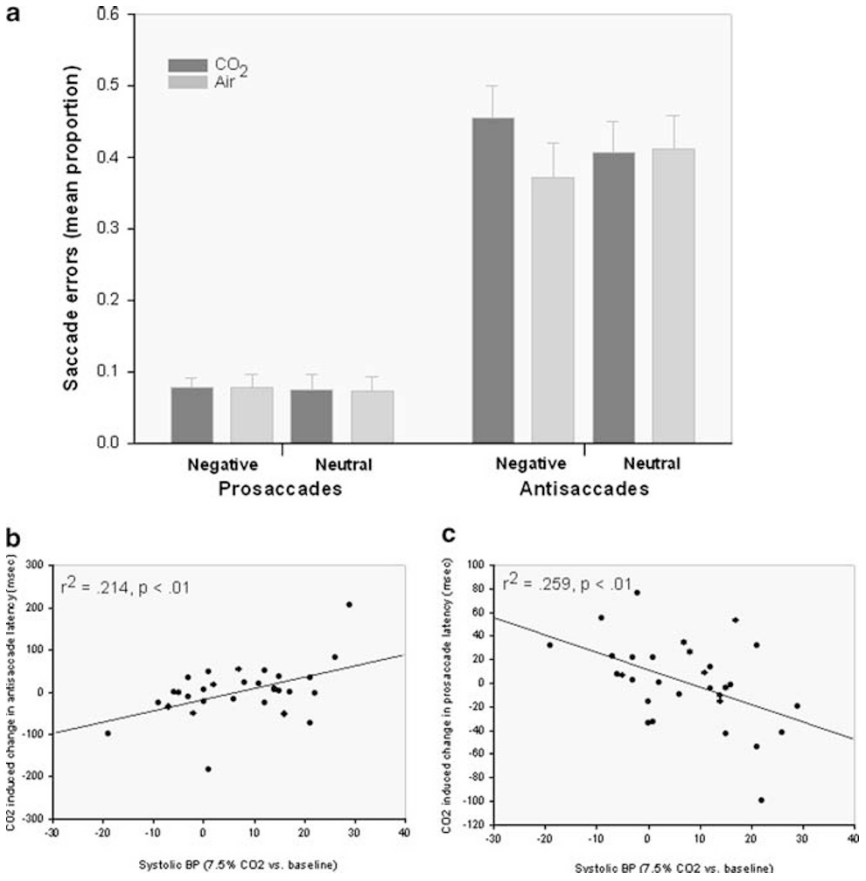

Figure 1 Prosaccade and antisaccade errors in response to negative and neutral stimuli presented during inhalation of 7.5% CO_2 and air (a). Relationships between systolic blood pressure response to CO_2 challenge and latency to initiate correct antisaccades away from (b) and correct prosaccades toward (c) negative (*vs* neutral stimuli) within CO_2 relative to air.

erroneous eye movements to threat did not differ between the first and second half of the antisaccade task (gas × trial type × image valence × task half: $F(1, 25) = 1.21$ and $p = 0.28$), further demonstrating the stability over time of interactions between CO_2 and attention to threat.

DISCUSSION

Our findings are the first to show that 7.5% CO_2 inhalation can trigger dysfunction in neurocognitive mechanisms that characterize generalized anxiety. Inhalation of 7.5% CO_2 induced erroneous eye movements toward negative stimuli on antisaccade trials, consistent with evidence that patients with GAD more readily orient toward threat stimuli in other eye-tracking paradigms (Mogg *et al*, 2000).

CO_2 challenge substantially increased self-report anxiety and autonomic arousal, consistent with previous findings (Bailey *et al*, 2005), and revealed correlations between subjective (state anxiety) response to CO_2 and increases in heart rate, but not blood pressure (likely reflecting the greater interoceptive salience/awareness of heart rate when determining subjective levels of distress).

Blood pressure response to challenge was strongly associated with CO_2-induced deficits in threat inhibition (ie, greater orienting toward and slower orienting away from threat on antisaccade trials), and was further associated with faster eye movements toward threat on prosaccade trials. These associations reflect a large effect size and CO_2-induced increases in blood pressure and selective attention to threat were of comparable magnitude (small–medium effect sizes). However their covariation could be further clarified by measuring autonomic response throughout the inhalation period (in addition to immediately afterward as in our study).

The strong relationships between induced change in blood pressure and attention to threat remained significant after controlling for CO_2 induced change in state anxiety (which itself was not correlated with induced attention to threat). Furthermore additional analysis of those participants who did not report an increase in state anxiety in response to CO_2 ($n = 5$) replicated findings from the entire group: notably a significant interaction between gas inhalation and valence on antisaccade errors characterized by greater erroneous eye-movements towards threat relative to neutral images during CO_2 relative to air [$F(1,4) = 12.78$, $p = 0.023$]. These findings demonstrate that inhalation of 7.5% CO_2 can induce hyper-vigilance towards and deficient inhibition of threat in humans independent of changes in subjective mood. Future studies should clarify the extent to which 7.5% CO_2 modulates attention to threat independent of changes in subjective mood through direct comparison of CO_2 challenge with non-pharmacological mood induction procedures that can further control for subjective anxiety

(such as Hayes *et al*, 2008; anticipatory worry period of the Trier social stress test, Kirschbaum *et al*, 1993). Future evidence that 7.5% CO_2 and non-pharmacological stressors known to trigger characteristics associated with GAD (eg, worry, nervous apprehension) produce comparable subjective response profiles that differ from the effects of 35% CO_2 challenge, would lend further support to proposals that the 7.5% CO_2 challenge models GAD, rather than acute fear and panic.

Supplementary analyses confirmed that CO_2-induced change in antisaccade performance was not affected by the duration of inhalation either before commencing or during the antisaccade task. This is consistent with evidence that the autonomic effects of 7.5% CO_2 rise early in the inhalation period and then plateau with minor fluctuations (Poma *et al*, 2005; Bailey *et al*, 2005). Studies have yet to examine variation in subjective effects during inhalation, and it would be of interest to directly assess the temporal characteristics and covariation of subjective, autonomic (including respiration rate/volume), and neurocognitive processing of threat (and positive/neutral control stimuli) throughout the CO_2 inhalation period.

Our findings complement evidence that 10% CO_2 elicits anxious behavior in rodents (Ziemann *et al*, 2009). Recent evidence that CO_2 triggers fear behavior in rats via ASC1a in amygdala suggests a mechanism through which CO_2 can increase the salience of environmental threat cues, interrupt goal-directed behavior, direct processing resources to threat through interactions with prefrontal cortex (Bishop *et al*, 2004b; Davidson, 2002), and comodulate autonomic response. Future research should clarify the extent to which CO_2-induced threat processing in humans reflects amygdalic innervation of norepinephrine via the locus coeruleus (also subjected to direct regulation by CO_2 (Pineda and Aghajanian, 1997) and implicated in both response to CO_2 challenge (Bailey *et al*, 2003) and attention (Grefkes *et al*, 2010)) and interactions with GABAergic mechanisms involved in response to CO_2 challenge and threat inhibition (Bailey and Nutt, 2008).

Our findings show clear effects of 7.5% CO_2 inhalation on selective attention to threat in healthy humans and further validate the CO_2 model as a promising unconditioned cross-species translational tool with which to challenge (and evaluate novel treatments that aim to resolve) subjective, autonomic, and neurocognitive processes that underlie anxious behavior in humans, and which characterize the generalized anxiety phenotype.

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DISCLOSURE

DB has acted as a paid consultant to a number of companies with an interest in anxiety and depressive disorders (Eli Lilly, GlaxoSmithKline, Lundbeck, Pfizer, and Servier) and holds or has held research grants (on behalf of his employer) from Lundbeck and Pfizer. He has accepted paid speaking engagements in industry-supported satellite symposia at international and national meetings organized by Lundbeck, Pfizer, and Servier. The remaining authors declare no conflict of interest.

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