

Fear and panic in humans with bilateral amygdala damage

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Decades of research have highlighted the amygdala's influential role in fear. We found that inhalation of 35% CO₂ evoked not only fear, but also panic attacks, in three rare patients with bilateral amygdala damage. These results indicate that the amygdala is not required for fear and panic, and make an important distinction between fear triggered by external threats from the environment versus fear triggered internally by CO₂.

A substantial body of evidence has emphasized the importance of the amygdala in fear^{1,2}. In animals, amygdala-restricted manipulations interfere with the acquisition, expression and recall of conditioned fear and other forms of fear and anxiety-related behaviors¹. In humans, focal bilateral amygdala lesions are extraordinarily rare, and such cases have been crucial for understanding the role of the human amygdala in fear². The most intensively studied case is patient SM, whose amygdala damage stems from Urbach-Wiethe disease (**Supplementary Fig. 1**). Previous studies have shown that patient SM does not condition to aversive stimuli³, fails to recognize fearful faces² and demonstrates a marked absence of fear during exposure to a variety of fear-provoking stimuli, including life-threatening traumatic events⁴. Patients with similar lesions have largely yielded similar results^{5,6}.

One stimulus not previously tested in humans with amygdala damage is CO₂ inhalation. Inhaling CO₂ stimulates breathing and can provoke both air hunger and fear⁷⁻⁹. Furthermore, CO₂ can trigger panic attacks, especially in patients with panic disorder^{9,10}. Recent work in mice found that the amygdala directly detects CO₂ and acidosis to produce fear behaviors¹¹. Thus, we hypothesized that bilateral amygdala lesions would reduce CO₂-evoked fear in humans.

In contrast with our prediction, patient SM reported fear in response to a 35% CO₂ inhalation challenge. To the best of our knowledge, this was the first time patient SM experienced fear in any setting, laboratory or otherwise, since childhood⁴. To further explore this issue, we tested two additional patients (AM and BG), monozygotic twin sisters with focal bilateral amygdala lesions resulting from Urbach-Wiethe disease (**Supplementary Fig. 1**)⁶. As with patient SM, both patients also reported experiencing fear during the CO₂ challenge.

Notably, CO₂ triggered a panic attack in all three of the amygdala-lesion patients. The patients panicked on the first CO₂ trial and during a subsequent challenge (**Supplementary Table 1**), indicating that the effect was reproducible and not simply the result of a novel experience. In contrast, only 3 of the 12 matched, neurologically intact comparison participants panicked (**Fig. 1a**), a rate similar to that previously observed in adults without a personal or family history of panic disorder¹⁰. Self-reported levels of fear and panic in the amygdala-lesion patients were significantly higher ($P < 0.05$) than in non-panickers from the comparison group (**Fig. 1b,c**). In addition, the patients reported elevated levels of anxiety and found the CO₂ inhalation to be substantially more arousing and aversive than non-panickers (**Supplementary Figs. 2 and 3**). The patients denied experiencing any anger (with ratings of zero on all trials), suggesting that the emotional changes induced by CO₂ were largely confined to the fear domain. Moreover, during air trials, the patients reported absolutely no fear, panic or anxiety, indicating that the induction of these emotions were specific to CO₂. The observation that CO₂ evoked multiple emotions in the fear domain suggests that the subjective experience could not be easily defined by a single emotional term, such as fear, panic or anxiety. Notably, the bilateral amygdala lesions did not interfere with the ability to express or experience any of these fear-related emotions.

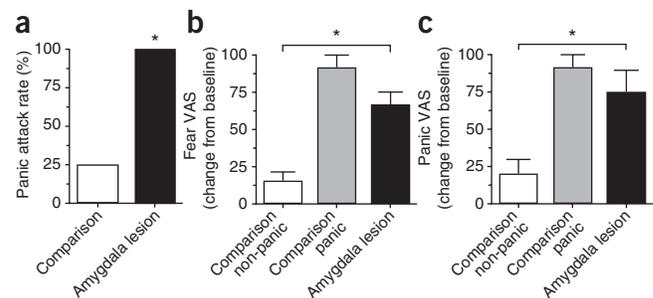


Figure 1 Panic attack rate and self-reported levels of fear and panic during the first CO₂ inhalation. **(a)** Panic attack rate (%) in amygdala-lesion patients ($n = 3$) versus neurologically intact comparison participants ($n = 12$). All of the amygdala-lesion patients had a panic attack, whereas only 3 of the 12 comparison participants panicked ($*P < 0.05$, Fisher's exact test). **(b,c)** Level of subjective fear **(b)** and level of subjective panic **(c)** reported during CO₂ relative to baseline quantified with visual analog scales (VAS). Both the amygdala-lesion patients and the comparison participants who panicked reported significantly higher levels of fear and panic relative to the comparison participants who did not panic ($*P < 0.05$, Mann-Whitney U tests). There were no significant differences between the amygdala-lesion patients and the comparison panickers. Error bars represent the s.e.m.

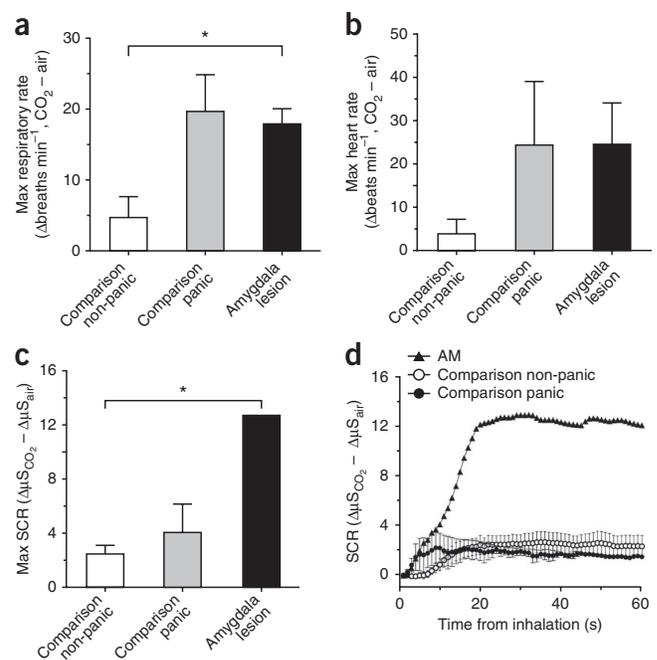
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Figure 2 CO₂-evoked physiological changes. **(a)** Change from baseline in maximum respiratory rate during the first CO₂ trial relative to the first air trial. Both the amygdala-lesion patients ($n = 3$) and the comparison participants who panicked ($n = 3$) demonstrated significantly higher increases in respiratory rate relative to the comparison participants who did not panic ($n = 9$) ($*P < 0.05$, Mann-Whitney U tests). There was no significant difference between the amygdala-lesion patients and the comparison panickers. **(b)** Change from baseline in maximum heart rate during CO₂ relative to air trials. Both the amygdala-lesion patients ($n = 2$) and the comparison participants who panicked ($n = 3$) demonstrated higher increases in heart rate relative to the comparison participants who did not panic ($n = 9$). **(c)** Change from baseline in maximum SCR during the first CO₂ trial relative to the first air trial. Patient AM demonstrated a significantly higher maximum SCR than the comparison participants who did not panic ($*P < 0.001$, modified t test). **(d)** Change from baseline in SCR during the first CO₂ trial relative to the first air trial graphed during the first minute post-inhalation. Error bars represent the s.e.m.

We examined the details of each patient's panic attack (**Supplementary Panic Descriptions**). Several observations were consistent across patients. First, all of the patients found the feelings induced by the CO₂ to be novel and described the experience as "panic." Second, all of the patients displayed similar behavioral responses to CO₂, including gasping for air, distressed facial expressions and escape behavior (for example, ripping off the inhalation mask).

To test whether the reports of fear and panic were accompanied by physiological changes, we also measured respiratory rate, heart rate and skin conductance response (SCR). Compared with air trials, CO₂ increased physiological responses in both the lesion and comparison groups (**Fig. 2**). Notably, physiological responses in the amygdala-lesion patients were higher than the non-panickers, including a significantly greater rate of respiration ($P < 0.05$; **Fig. 2**). In contrast, there were no significant differences ($P > 0.05$) between the amygdala-lesion patients and the comparison panickers. Together, these physiological measures paralleled the greater incidence of CO₂-evoked panic found in the amygdala-lesion patients.

Not all physiological responses were increased in the patients. In the comparison group, SCR and heart rate gradually rose before the



inhalation, as participants observed the experimenters preparing to administer the inhalation challenge (**Fig. 3**). In the lesion patients, both of these anticipatory responses were deficient (**Fig. 3**), which stands in sharp contrast with their heightened responses following CO₂ inhalation. These results are consistent with the notion that the amygdala detects potential danger in the external environment and physiologically prepares the organism to confront the threat, a process closely linked to the generation of anticipatory anxiety^{1,12}.

Contrary to our hypothesis, and adding an important clarification to the widely held belief that the amygdala is essential for fear, these results indicate that the amygdala is not required for fear and panic evoked by CO₂ inhalation. Moreover, the higher rate of panic attacks in the amygdala-lesion patients suggests that an intact amygdala may normally inhibit panic. This apparent loss of inhibition might have occurred during development, as the amygdala damage is thought to have emerged during adolescence⁴. Another possibility is that the amygdala inhibits panic acutely. Such modulation is plausible given that the output from the central nucleus of the amygdala is GABAergic¹³ and projects to a number of brainstem sites that have been implicated in producing panic-like behavior^{1,14}.

The elevated incidence of panic attacks evoked by CO₂ in the lesion patients raises the possibility that loss of amygdala function might contribute to the development of panic disorder. Supporting this possibility, patients with panic disorder have been found to have localized atrophy of the amygdala¹⁵, as well as amygdala hypoactivity^{16,17}. Anecdotal accounts from a single patient suggest that spontaneous panic can occur despite amygdala damage¹⁸. However, the absence of

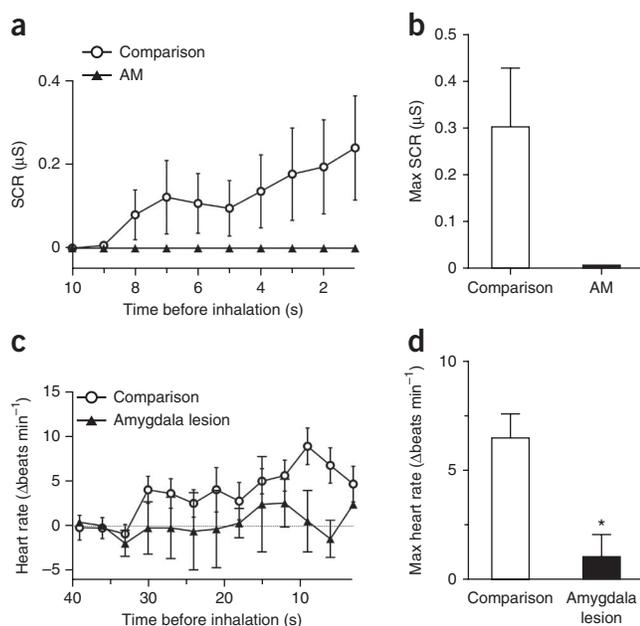


Figure 3 Anticipatory physiological responses before inhalation. **(a,b)** SCR graphed during the 10 s before inhalation **(a)** and the maximum evoked-SCR during the same time period **(b)**. Patient AM showed no anticipatory SCR on any trials. **(c,d)** Change in heart rate relative to baseline during the 40 s before inhalation **(c)** and the maximum change in heart rate during the same time period **(d)**. The amygdala-lesion patients ($n = 2$) had a significantly lower anticipatory heart rate response relative to the comparison participants ($n = 12$) ($*P < 0.05$, Mann-Whitney U test). Error bars represent the s.e.m.

prior spontaneous panic attacks in our lesion patients suggests that amygdala dysfunction alone is not sufficient to cause spontaneous panic attacks or panic disorder.

Finally, the patients reported being surprised by their reaction to CO₂ and found the induced feelings of fear and panic to be completely novel. This suggests that the high concentration of inhaled CO₂ activated a pathway that had remained mostly dormant up until the point of the experiment. These observations raise the question of what is different about CO₂ compared with previous stimuli that failed to evoke fear or panic⁴, as well as the stimuli in this study that failed to evoke anticipatory responses. One possibility is that all of these other stimuli were exteroceptive in nature, mainly processed through visual and auditory pathways that project to the amygdala. In contrast, CO₂ acts internally at acid-activated chemoreceptors and causes an array of physiological changes^{7,9,11}. Thus, CO₂ might engage interoceptive afferent sensory pathways that project to the brainstem, diencephalon and insular cortex^{19,20}. In addition, many brain areas outside the amygdala possess CO₂ and pH-sensitive chemoreceptors, including acid-sensing ion channels⁷. Thus, CO₂ may directly activate extra-amygdalar brain structures that underlie fear and panic, which may help to explain the apparent discrepancy between these findings and previous work in mice¹¹. In either case, our results indicate that, in humans, the internal threat signaled by CO₂ is detected and interpreted as fear and panic despite the absence of an intact amygdala.

METHODS

Methods and any associated references are available in the [online version of the paper](#).

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.A.W., M.J.W., J.S.F., R.L.F., D.T., W.H.C. and N.S.D. conceived and planned the experiments. J.A.W., D.T., R.H. and W.H.C. provided financial support, equipment and supplies. J.A.W., C.B., J.S.F., R.L.F., R.H. and N.S.D. recruited participants and performed the experiments. J.A.W., C.B., M.J.W., J.S.F., R.H., D.T., R.L.F., W.H.C. and N.S.D. wrote and edited the manuscript and figures.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. We tested three female patients with bilateral amygdala damage resulting from Urbach-Wiethe disease (mean age = 39.33 years, s.d. = 4.04; mean years of education = 13.33, s.d. = 1.15) and 12 healthy, neurologically intact females of comparable age (mean = 43.08, s.d. = 5.65) and education (mean = 14.33, s.d. = 1.87). All subjects were free of psychiatric diagnoses and medications and reported no personal or family history of panic attacks. All subjects gave written informed consent, and all procedures were approved by the University of Iowa Institutional Review Board.

Data collection. Before the procedures, all subjects completed the Beck Anxiety Inventory as a measure of baseline anxiety, and both groups reported experiencing low levels of anxiety that were not significantly different (amygdala lesion mean raw score = 4, s.d. = 2; comparison group mean raw score = 3.4, s.d. = 3.8; $P = 0.365$). During each inhalation challenge, subjects were in a supine position while seated in a reclining chair. A plastic inhalation mask was comfortably placed over their nose and mouth and then strapped to the reclining chair to ensure that it would remain in place during the inhalation. Respiratory rate, heart rate and skin conductance were recorded throughout each trial using a BIOPAC MP150 data acquisition system (BioPac Systems). Baseline recordings were taken during a 2-min rest period before each inhalation and recordings continued for 2 min after each inhalation. The volume of each inhalation was recorded using an RSS 100 Research Pneumotach System (KORR Medical Technologies). Forced inspiratory vital capacity (FIVC) was calculated from height and weight as described previously²¹. During all challenges, subjects were required to inhale a minimum of 75% of their FIVC for the challenge to be considered valid. All subjects completed four single-breath FIVC challenges, two with compressed air and two with 35% CO₂ mixed with 21% oxygen (balanced with nitrogen). All of the bilateral amygdala lesion patients returned for a second visit to complete an additional set of challenges. The challenge order for each subject was air first followed by CO₂, and was repeated at least once. Subjects were blinded to trial order. Each trial was separated by an interval of at least 20 min.

At the end of each inhalation, subjects completed a number of different self-report questionnaires, including an inhalation symptom checklist containing all of the DSM-IV symptoms of a panic attack, four separate VAS asking them to rate their level of fear, panic, anxiety and anger from 0 (not at all) to 100 (extremely), a bipolar valence scale asking them to rate the inhalation from 0 (extremely unpleasant) to 8 (extremely pleasant), an arousal scale asking them to rate the overall intensity of the inhalation from 0 (not at all) to 8 (extremely), and the state portion of the Spielberger State-Trait Anxiety Inventory. When completing the self-report questionnaires, subjects were instructed to rate how they felt during and immediately following the inhalation when symptoms were at their peak. The same measures were also completed before each inhalation (baseline), during which subjects were instructed to rate how they currently felt. After each trial, subjects were interviewed by a clinician or trained researcher and were asked to describe any symptoms they experienced before, during and after the inhalation.

Data analysis. The threshold for a panic attack was based on conservative criteria for differentiating panic attacks from the strong respiratory and physiological responses that many people have to CO₂ challenges¹⁰. This threshold required

that the subject endorse at least four DSM-IV symptoms of panic, either express or enact a desire to escape or flee, and report at least a 25% increase in panic as measured by the panic VAS. Of note, VAS panic scores did not differ significantly between the first and second panic attacks described in **Supplementary Table 1** (paired *t* test, $P = 0.13$). Data from the comparison group was statistically compared with the amygdala-lesion group using two-tailed Mann-Whitney *U* tests with Bonferroni-Holm correction for multiple comparisons (when appropriate) and a significance threshold of $P < 0.05$. The self-report ratings were converted to POMP scores (standardized units representing the percent of maximum possible for each scale, ranging from 0–100)²², and the valence scale was reverse scored. Several of the self-report measures were not collected in one of the non-panicking comparison participants. Evoked increases in heart rate were calculated by subtracting the baseline rate from the maximum rate during the minute following each inhalation. Baseline was calculated as the average heart rate during the 20 beats preceding the minute before inhalation, whereas maximum heart rate following inhalation was found by assessing each beat-to-beat interval averaged over 3 s. Evoked increases in respiratory rate were similarly calculated by subtracting the baseline rate from the maximum rate during the minute following inhalation. Baseline was calculated as the average respiratory rate during the 2 min before inhalation, whereas maximum respiratory rate was found by assessing each breath-to-breath interval during the minute following inhalation. Evoked SCR were calculated by subtracting the average skin conductance level during the first second of inhalation from the peak skin conductance level during the minute following the start of inhalation. Differential increases in respiration, heart rate and SCR were calculated in each subject by subtracting their maximum evoked response during the air trial from their maximum evoked response during the CO₂ trial.

Several factors affected the analysis of the physiological data in the lesion patients. We were unable to obtain skin conductance from anywhere on the palm of the hands or the fingers in both patients SM and BG (likely as a result of epithelial pathology caused by Urbach-Wiethe disease); thus, patient AM was the only lesion patient included in the SCR analysis and was compared to the comparison participants using a modified *t* test²³. Patient SM was excluded from the heart rate analysis because she was taking propranolol for treatment of hypertension. In addition, the heart rate data for AM and BG during the first CO₂ inhalation could not be analyzed because of contamination by motion artifacts secondary to the patients' escape behavior; thus, heart rate could only be analyzed during later trials.

Anticipatory physiological responses were also calculated. An anticipatory SCR was considered to be any upward deflection in skin conductance during the 10 s before inhalation. The magnitude of the response was calculated by subtracting the skin conductance level at the beginning of this deflection from the level at its peak during the 10 s before inhalation. Anticipatory heart rate was similarly calculated by subtracting the previously described baseline heart rate from the average heart rate calculated during each 3-s interval in the minute before inhalation.

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