Understanding rat emotional responses to CO₂

Lucía Améndola and Daniel M. Weary

Abstract

The aim of this review is to summarize evidence regarding rat emotional experiences during carbon dioxide (CO₂) exposure. The studies reviewed show that CO₂ exposure is aversive to rats, and that rats respond to CO₂ exposure with active and passive defense behaviors. Plasma corticosterone and bradycardia increased in rats exposed to CO₂. As with anxiogenic drugs, responses to CO₂ are counteracted by the administration of anxiolytics, SSRIs, and SSRI's. Human studies reviewed indicate that, when inhaling CO₂, humans experience feelings of anxiety, fear, and panic, and that administration of benzodiazepines, serotonin precursors, and SSRIs ameliorate these feelings. In vivo and in vitro rat studies reviewed show that brain regions, ion channels, and neurotransmitters involved in negative emotional responses are activated by hypercapnia and acidosis associated with CO₂ exposure. On the basis of the behavioral, physiological, and neurobiological evidence reviewed, we conclude that CO₂ elicits negative emotions in rats.

Introduction

Several behavioral studies indicate that carbon dioxide (CO₂) elicits negative responses and is aversive to rats, although not all studies agree. Rat exposure to CO₂ is a well-accepted translational model for the understanding of fear, anxiety, dyspnea (feeling of breathlessness), and panic in humans.

Pain, fear, panic, and anxiety are considered high arousal, negatively valenced emotional states. Here, we follow a functional working definition that identifies emotional responses as objectively observable, and feelings of emotions as the conscious awareness of emotions experienced as positive or negative. Emotions are “central states” inferred from brain arousal, and behavioral and physiological changes due to the presentation of a competent situation-dependent stimuli. Not all stimuli elicit an emotional response; the competence of the stimuli will depend on the individual’s evolutionary history (innate response), personal experience (developmental plasticity and learning), discriminative properties of the stimuli (intensity and type of stimuli), and the current situation (e.g., controllability). Induction regions in the brain are responsible for the emotional cascade (chemical and neural reaction) that lead to the execution of appropriate behavioral, physiological, and brain responses to cope with a competent stimuli.

Feelings of emotions can be described using a two-axis (arousal/valence) model, and comprise different patterns of neural, behavioral, and physiological responses. In the scientific literature, there is little consensus regarding what constitutes the feelings of emotions, and how and where these feelings are evoked in the brain. For example, some argue that this requires an internal self-representation of body and mind changes (i.e., interoception; homeostatic state, state of preparedness to cope, and motivational state) that accompany emotions, a process thought to occur in cortical areas of the brain (e.g., insula and cingulate cortex). Within this view, species that possess interoception could feel emotions. Other authors emphasize the role of neocortical working memory (i.e., temporary hold and manipulation of information while doing mental work) as a requirement for feeling emotions, an idea that may exclude some nonhuman animals. Panksepp argued that basic neurobiological subcortical areas present within all mammals are responsible for both emotion and feelings.

The assessment of felt emotions remains a major challenge, but strong inferences about felt experiences can be made through a combination of evidence from (1) central state emotions (i.e., regional and local brain arousal, and behavioral and physiological changes when a
Behavioral responses

Here, we discuss the behavioral evidence of negative emotional states in three sections: (1) studies where animals cannot escape exposure to CO₂; (2) studies where animals can avoid exposure to CO₂, and (3) other situations where behavior is used to infer negative emotional states during or after exposure to CO₂.

Inescapable exposure to CO₂

To assess emotions evoked by CO₂, one common approach is forced exposure to the gas⁶,⁹,²⁶. The working (and typically implicit) assumption is that the frequency, duration, and intensity of the response reflects the intensity of the rat’s negative emotional experience to the procedure.

Several studies have found behavioral evidence of negative states in rats exposed to CO₂. Niel and colleagues⁶⁷, using ~17% CO₂ chamber vol. min⁻¹, found that rats showed increased frequencies and intensities of several behaviors associated with distress. The onset of rearing and increased locomotion occurred at ~5% CO₂ and peaked at ~20% CO₂. Escape behaviors (i.e., pushing and scratching at the lid) were observed at between 20 and 28% CO₂. These results are consistent with others using slightly higher flow rates (18.5 and 23% CO₂ chamber vol. min⁻¹)²⁷,²⁸. Vocalizations in the range of 6–103 kHz have been reported for rats exposed to CO₂ flow rates between 17 and 30% chamber vol. min⁻¹ (refs. ²⁶). However, one other study reported that rats exposed to 10% CO₂ chamber vol. min⁻¹ did not vocalize, and that locomotion and rearing did not increase relative to baseline levels²⁹.

Rats exposed to the 20% CO₂ challenge show variable results. Some studies have reported increases in locomotion but not freezing²⁴, and others have found the reverse³⁰. These results suggest that the type of defense behavior varies (between active and passive), but that some response is usually present.

When exposed to high concentrations of static CO₂ (CO₂ > 97%), some studies have reported that rats are less active and do not show struggling, vocalizations⁸, or other signs of distress³¹, but others have reported signs of asphyxia and behavioral excitation⁴. In these studies, however, behaviors were sometimes recorded without baseline or acclimation periods⁹, and behavioral responses were not clearly defined. For example, “head rising” was described as “inquisitive or agitated movements of head,” vocalizations as “squealing and other noises,” and escape as “attempts to get out of the box”³¹, or responses were simply mentioned without description⁷. Without control animals, baseline observations, and a clear description of behaviors, interpretation of these results is challenging.

Strain differences in rat responses to CO₂ have been seldom assessed. Winter and colleagues³² showed that
exposure to 10% static CO2 elicits freezing behavior in Long Evans and Wistar Kyoto strains, but not in Sprague Dawley and Wistar strains. Sprague Dawley rats often respond to CO2 exposure by increased active defense behavioral responses2,6,24,27,28,33, but the absence of responses has also been also reported for this strain29,31. In contrast, Lister Hooded rats decrease activity during CO2 exposure26. Blackshaw et al.9. reported a decrease in activity by Wistar rats during exposure to CO2, a result that differs from the findings of Niel et al7. Fisher rats—a strain selected for lower activity6, and only half of the rats tested increased locomotion2 and increased active defense behaviors27 during CO2 forced exposure, but in another study showed no change in behavior9. Two studies recently published found no differences in behavioral responses to CO2 between male and female rats33,35.

Individual differences in rat responses to forced exposure to CO2 are often mentioned. It has been reported that only 20% of rats climbed the cage and 20% circled (i.e., moving around the perimeter of the cage)31, some rats expressed little and others numerous escape behaviors6, and only half of the rats tested increased locomotion39. Variation in rat response to CO2 may be related to reactivity36. For example, it has been shown that individual differences in active defense behaviors are consistent between two forced exposures to CO2 (ref. 27).

In summary, there is considerable variation in responses of rats to forced CO2 exposure within and between studies. Strain, sex, and individual differences account for some of this variability, but contrasting results within the same sex and strain still exist. Within-study variation in rat responses could be reflective of individual differences in CO2 reactivity. Differences in methodology, including the use of baselines, controls, type of cages, and induction method (e.g., static versus gradual fill, variable concentrations, and flows rates), and the lack of well-defined behaviors and interpretation of behaviors limit comparability between studies. Nonetheless, considering the responses summarized in Table 1, it seems likely that rats exposed to CO2 experience negative emotional states when escape is prevented.

**Escapable exposure**

Here, we describe the behavioral responses of rats when exposure to CO2 can be avoided. One approach to assess emotions during CO2 exposure is through choice and motivational tests. This approach is based upon the “hedonic principle;” i.e., that animals are motivated to avoid undesired end states (e.g., potential harms, pain, etc.) and approach desired ones37.

Choice tests involve giving animals two or more alternative conditions (e.g., different agents or the same agent at different concentrations), and measuring the amount of time spent in each alternative as an expression of preference3. Studies have shown that rats prefer lower than higher concentrations of CO2. Rat spent between 36 and 51 s in a chamber prefilled with room air, and 2.1 and 0.7 s in chambers with 25.5% and 50.8% CO2, respectively9.

The strength of aversion can be measured by giving rats the ability to avoid exposure, with an added cost to the avoidance38. For inhalant agents, strength of aversion has been investigated through aversion- and approach-avoidance tests. In the aversion-avoidance test, the cost of avoiding the agent in a preferred dark compartment is exposure to an aversive brightly lit compartment. Using the aversion-avoidance test with a flow rate of 24% chamber vol. min−1, all rats left the dark chamber filling with CO2, escaping to the previously avoided bright chamber39.

In the approach-avoidance test, the cost of escaping to an agent free cage is the loss of a sweet reward. Rats are highly motivated to eat sweet rewards even when fed their regular diet at libitum40. When tested with different static concentrations of CO2 in the approach-avoidance apparatus, rats tolerated concentrations ranging from <1 to 10% CO2, entering the test chamber, eating the sweet rewards, and staying in the gas chamber for ~300 s. However, at 10% CO2 rats stopped eating ~20 s earlier than when tested with 5% CO2, indicating that although 10% CO2 is aversive, rats will tolerate exposure to obtain the sweet rewards. But the latency to leave the gas chamber decreased to 46 s at 15% CO2, and to 5 s with 20% CO2 (ref. 41).

Other studies using flow rates between 14 and 19% CO2 chamber vol. min−1, have shown that aversion is variable. In the aversion-avoidance test, latency to avoid CO2 ranged from 7 to 48 s between rats49. In the approach-avoidance test, the concentration avoided varied among individuals ranging from 11 to 18.6% CO2 (ref. 7). Variation among individuals in rat aversion to CO2 has shown to be consistent across exposures, within aversion27 and approach-avoidance29,32. These results support the idea that rats vary in CO2 reactivity. However, all aversion studies report that rats avoid CO2 before becoming ataxic or recumbent, even when fasted for 24 h (ref. 40).

These results (summarized in Table 2) indicate that onset of negative emotional states occurs ~10% CO2, although some rats are willing to tolerate exposure up to 18% CO2. These concentrations are consistent with the
Table 1  Forced exposure studies specifying delivery methods, concentration or flow rate used, strain and sex, whether the study contained baseline and well-defined behavioral categories, and a summary of results.

| Strain | Delivery method | Concentration/flow rate | Sex | Baseline or control | Defined behaviors | Results | Reference |
|--------|-----------------|-------------------------|-----|---------------------|-------------------|---------|-----------|
| W Gf   | ~17%            | M ✓                     | ✓   | ✓                   | ↑ line crossing (locomotor activity), ↑ nose to lid, ↑ escape behaviors, and ↑ ultrasonic vocalizations (mean range 22 ± 19 kHz) | 5       |
| W Gf   | 17%             | M ✓                     | ✓   | ✓                   | ↑ line crossing (locomotor activity), ↑ nose to lid, ↑ escape behaviors (50% of the rats) | 7       |
| W Pf   | 10%             | x ✓                     | ✓   | ✓                   | ↑ wall crossing (locomotor activity), ↑ nose to lid, ↑ escape behaviors (60% of the rats) | 32      |
| W Pf   | >97%            | M/F X                   | Vague | ↑ wall touching, ↑ wall climbing (rearing), ↑ vocalizations | 9       |
| W Pf   | 100%            | M X                     | X   | X                   | ↑ normal behavior, ↑ behavioral agitation and excitation, ↑ immobility/freeze | 3       |
| SD Gf  | 10%             | M ✓                     | ✓   | ✓                   | ↑ wall crossing (locomotor activity), ↑ rearing, ↑ escape behaviors | 29      |
| SD Gf  | ~18%            | F ✓                     | ✓   | ✓                   | ↑ line crossing (locomotor activity), ↑ rearing, ↑ escape behaviors | 27      |
| SD Gf  | 20%             | M ✓                     | ✓   | ✓                   | ↑ normal behavior, ↑ behavioral agitation and excitation, ↑ immobility/freeze | 24      |
| SD Gf  | 23%             | M ✓                     | ✓   | ✓                   | ↑ normal behavior, ↑ behavioral agitation and excitation, ↑ immobility | 30      |
| SD Gf  | 30%             | F ✓                     | ✓   | ✓                   | ↑ ultrasonic vocalizations (median range 51 kHz) | 2       |
| SD Gf  | High but undefined | M ✓ | ✓ | ✓ | ↑ wall climbing (rearing), ↑ activity, ↑ shaking (undefined), ↑ ultrasonic vocalizations | 26      |
| SD Gf  | 30%             | M/F X                   | ✓   | ✓                   | ↑ rearing, ↑ jumping, ↑ digging | 33      |
| SD Pf  | 10%             | X ✓                     | ✓   | ✓                   | ↑ freezing/immobility and ↑ rearing | 32      |
| SD Pf  | ~79%            | M X                     | Vague | ↑ signs of distress, ↑ vocalization, ↑ escape behaviors, ↑ tail lashing | 31      |
| LE Gf  | High but undefined | M ✓ | ✓ | ✓ | ↑ wall climbing (rearing, ↑ activity, ↑ shaking (undefined), ↑ ultrasonic vocalizations | 26      |
| LE Pf  | 10%             | X ✓                     | ✓   | ✓                   | ↑ freezing/immobility and ↑ rearing | 32      |
| F Gf   | 10%             | M X                     | X   | X                   | ↑ interest and curiosity, ↑ vocalizations, ↑ signs of pain | 10      |
| WK Pf  | 10%             | X ✓                     | ✓   | ✓                   | ↑ freezing/immobility, ↑ rearing | 32      |
| P-rat Gf | 10%            | M/F X                   | ✓   | ✓                   | ↑ rearing, ↑ rearing (5 in), ↑ rearing (7 in), ↑ rearing (5 in/rear) | 35      |
|        | 30%             | M/F X                   | ✓   | ✓                   | ↑ rearing, ↑ rearing (5 in), ↑ rearing (7 in), ↑ rearing (5 in/rear) | 35      |
|        | 70%             | M/F X                   | ✓   | ✓                   | ↑ rearing, ↑ rearing (5 in), ↑ rearing (7 in), ↑ rearing (5 in/rear) | 35      |

Strain: W Wistar, SD Sprague Dawley, LE Long Evans, WK Wistar Kyoto, F Fisher, BN Brown Norway, P-rat alcohol preferring rats; delivery method: Pf pre-fill, Gl gradual fill, GF2 20% CO2 challenge; concentration or flow rate: static concentration (%) or flow rate (% CO2 chamber vol.min⁻¹); sex: M male, F female, X unspecified; baseline: ✓ present, X absent; defined behaviors: ✓ if a clear ethogram, vague if an unclear ethogram, X absent; results: ↑ increase, ↓ decrease, ↔ no change in behavior or absent.

aWithin the same study, two different results were found depending upon age: for young rats no change in activity but increase in stationary episodes, while old rats decreased both activity and stationary episodes. These authors concluded arrived to the same conclusion for both young and old rats.

bBehavior when exposed to CO2 in an induction chamber in comparison to exposure in the rat home cage.

cFlow rate was given as 6 l/min⁻¹ and cage size unspecified, calculations were made based on the brand and type of the cage (Makrolon type III = ~17.2).

dComparison between flow rates.
Table 2 Escapable exposure studies specifying test used, delivery methods, concentration or flow rate used, strain and sex, and summary of results. In all choice and aversion tests all rats avoided CO₂ before loss of consciousness.

| Strain | Sex | Test | Delivery method | Concentration/flow rate | Variables measured | Results | Notes | Reference |
|--------|-----|------|----------------|--------------------------|---------------------|---------|-------|-----------|
| W      | F   | Choice | Pf             | 100% air                  | Total dwelling time | > 35 s  | 4     |
|        |     |       |                | 25.5% CO₂                 |                     | 1.9 ± 0.6 s |       |
|        |     |       |                | 34.9% CO₂                 |                     | 0.8 ± 0.1 s |       |
|        |     |       |                | 50.8% CO₂                 |                     | 0.7 ± 0.1 s |       |
| W      | F   | Choice | Pf             | 100% air                  | Total dwelling time | > 36 s  | 5     |
|        |     |       |                | 25.5% CO₂                 |                     | 2.1 ± 0.5 s |       |
|        |     |       |                | 34.9% CO₂                 |                     | 1.0 ± 0.1 s |       |
|        |     |       |                | 50.8% CO₂                 |                     | 0.7 ± 0.2 s |       |
| SD     | M   | Aversion-avoidance | Gf           | 32% O₂                    | Total dwelling time | ~90 s   | 39    |
| SD     | M   | Aversion-avoidance | Gf           | 24% CO₂                   | Total dwelling time | 19 ± 5 s and 24 ± 3 s | Depending on the level of illumination of the light chamber |
| SD     | F   | Aversion-avoidance | Gf           | 19% CO₂                   | Latency to avoid CO₂ | 33 ± 6 s and 35 ± 4 s | Two consecutive exposures |
|        |     |       |                |                           | CO₂% avoided        | 14 ± 2% and 15 ± 1% |       |
| W      | M   | Approach-avoidance | Pf           | 100% air                  | Total dwelling time | ~240 s  | 41    |
|        |     |       |                | 5% CO₂                   |                     | ~230 s   |       |
|        |     |       |                | 10% CO₂                  |                     | ~225 s   |       |
|        |     |       |                | 15% CO₂                  |                     | 46 ± 6   |       |
|        |     |       |                | 20% CO₂                  |                     | 5 ± 6    |       |
| W      | M   | Approach-avoidance | Gf           | 17% CO₂                   | CO₂% avoided        | 184 ± 2% | 41    |
| W      | M   | Approach-avoidance | Gf           | 21% air                   | Latency to leave the chamber | 270 ± 6 s | 8     |
|        |     |       |                | 3–27% CO₂                | Latency to avoid CO₂ | 1 from ~95 s to ~28 s | Depending on flow rate |
|        |     |       |                | 3–27% CO₂                | CO₂% avoided        | 14–16%   |       |
| W      | M   | Approach-avoidance | Gf           | 17% air                   | Latency to leave the chamber | 288 ± 2 s | 7     |
| W      | M   | Approach-avoidance | Gf           | 17% CO₂ (Exp. 1)          | Latency to avoid CO₂ | 40 ± 2 s | 40    |
|        |     |       |                | 15% CO₂ (Exp. 2)         | CO₂% avoided        | 163 ± 0.3% | Average from three consecutive exposures |
|        |     |       |                |                           |                     | 136 ± 0.3% | Average from different levels of food deprivation |
| SD     | F   | Approach-avoidance | Gf           | 18% O₂                    | Latency to leave the chamber | 237 ± 27 s | 27    |
|        |     |       |                | 18.5% CO₂                | Latency to avoid CO₂ | 23 ± 4 s and 28 ± 4 s | Two consecutive exposures |
|        |     |       |                |                           | CO₂% avoided        | 9 ± 2 % and 11 ± 1 % |       |
| SD     | F   | Approach-avoidance | Gf           | 20% air                   | Latency to leave the chamber | 420 ± 27 s | 42    |
|        |     |       |                | Midazolam + 20% air       | Latency to avoid CO₂ | 391 ± 28 s | Average from three exposures |
|        |     |       |                | 20% CO₂                  | CO₂% avoided        | 25 ± 2 s   | Average from three exposures |
|        |     |       |                |                           |                     | 107 ± 1.14% |       |
| SD     | F   | Approach-avoidance | Gf           | Midazolam + 20% CO₂      | Latency to avoid CO₂ | 40 ± 3 s  | 42    |
|        |     |       |                |                           | CO₂% avoided        | 155 ± 1.41% |       |

Strain: W Wistar, SD Sprague Dawley; delivery method: Pf pre-fill, Gl gradual fill; concentration or flow rate: static concentration (%) or flow rate (% gas chamber vol min⁻¹); sex: M male, F female; s seconds; results mean ± standard error (unless specified otherwise); Exp. experiment.

Average values estimated from a figure.

Median values.
onset and peak of active behaviors during forced exposure\(^6\), and freezing behaviors reported using the 20% CO\(_2\) challenge\(^30\).

Human feelings of immobility (freezing), feeling paralyzed, desire to flee, and wanting to leave the room following inhalation of similar concentrations of CO\(_2\) (20–35% CO\(_2\))\(^33,34\), seem consistent with the rat defense behaviors reported above. Humans report feelings of anxiety at lower CO\(_2\) concentrations, and fear and panic at higher concentrations. For example, prolonged inhalation (20 min) of low CO\(_2\) concentrations (7.5%) elicits anxiety and induces hypervigilance in healthy volunteers\(^41\). This emotional experience is likely related to hypercapnia (rather than to hypoxia), since CO\(_2\) was administered to provide a normoxic gas mixture (containing \(~21%\) O\(_2\))\(^46\). Increasing doses (e.g., double inhalation of 35% CO\(_2\)), often induce panic attacks in healthy people\(^47\). These results provide some basis for suggesting that rat behavioral responses to CO\(_2\) may also be associated with the onset of negative emotional states related to feelings of anxiety, fear, or panic.

As reviewed above, the behavioral responses of rats vary between individuals. This variation has also been reported for human subjects. Human sensitivity to CO\(_2\) falls on a continuum panic disorder (PD) patients being the most sensitive\(^48\). In healthy humans, increases in fear and anxiety were reported in only 50% of the participants after a double inhalation of CO\(_2\) concentrations between 9 and 35% (ref. \(^49\)). When healthy humans inhale 20% CO\(_2\) for 20 s, 13% and 20% of the individuals experience modest or severe feelings of immobility and desire to flee, respectively\(^68\). With a double inhalation of 35% CO\(_2\), often induce panic attacks in healthy people\(^47\). These parallels in the human and rat literature are consistent with the idea that individual variation in rat behavioral responses to CO\(_2\) are associated with differences in the emotions elicited by this agent.

**Exposure in other situations**

CO\(_2\) is often used as an unconditioned stimulus in studies designed to induce negative emotional states in rodents. These studies support the conclusion that CO\(_2\) exposure is anxiogenic. In the Vogel test, two opposing motivations—gaining a reward versus avoiding a punishment—are used to assess the anxiolytic and anxiogenic effects of drugs. Food or water-deprived rats can choose to receive a reward (water or food) at the cost of receiving punishments (shocks); anxiogenic drugs suppress reward consumption and anxiolytics increase reward consumption\(^50\). Using the Vogel test, rats previously exposed for 60 s to CO\(_2\);O\(_2\) (35:65%) showed a similar response to that of rats treated with the anxiogenic benzodiazepine receptor ligand FG 7142; rats exposed to CO\(_2\) suppressed water licking by 40% relative to control rats\(^51\), indicating that CO\(_2\) exposure has an anxiogenic effect.

In the open field test, the tendency to avoid the central area and display thigmotaxis (locomotion close to the walls of the apparatus) is enhanced by anxiogenic drugs, while anxiolytics increase locomotion in the central areas of the arena\(^32\). In the social interaction test, anxiogenic drugs decrease the frequency of social interactions (e.g., sniffing, following, and grooming) while anxiolytics have the opposite effect\(^53\). After exposure to the 20% CO\(_2\) challenge, rats showed a 15% increase in thigmotaxis and a 50% decline in social interactions compared to rats exposed to air\(^24\).

Conditioning tests are also used to assess the aversiveness of a stimulus, and different variants of this test can be found. Potentially aversive stimuli can be paired with a neutral stimulus (Pavlovian conditioning), such as a neutral environment (place conditioning). Rats are then later exposed only to the paired stimulus, in absence of the aversive stimulus. Avoidance of the stimulus is an indicator of aversion, but if avoidance is restricted then immobility can be used as a measure of aversion\(^64\). Rats exposed to vanilla scent before 30 s of forced inhalation of different concentrations of CO\(_2\) (<1, 5, 35, or 100%), showed a conditioned response to vanilla 24 h later. Rats that inhaled <1% CO\(_2\) froze less than rats that inhaled higher concentrations. Rats exposed to 100% CO\(_2\) froze more and this conditioning resisted extinction relative to rats exposed to 5% CO\(_2\). These results indicate that CO\(_2\) can be used to condition anxiety in rats, and that the degree of behavioral response (freezing) and extinction reflect the severity of the experience\(^39,35\).

In summary, rat emotional states induced by CO\(_2\) inhalation are sustained after exposure, and CO\(_2\) acts as an unconditioned negative stimulus especially at higher concentrations (Table 3).

**Physiological responses**

Sympathetic responses in rats exposed to CO\(_2\) include increased blood pressure\(^31\) and bradycardia before the loss of righting reflex (LORR)\(^33\). In rats, arterial blood pressure increases during the 20% CO\(_2\) challenge\(^24\). Bradycardia has been reported for rats exposed to flow rates between 10 and 20% CO\(_2\) chamber vol. min\(^{-1}\) (refs. 1,29), and to the 20% CO\(_2\) challenge\(^24\). The cardiovascular response also correlates with changes in PaCO\(_2\) and pH (ref. 34). Altholtz et al.\(^56\) found that rats anesthetized with 70% CO\(_2\) showed increased plasma corticosterone levels after 30 min (but see ref. 35); similar results were found for rats exposed to 35% CO\(_2\) (ref. 57). In humans, inhalation of CO\(_2\) concentrations between 7 and 14% for 10–20 min induces an increase in minute ventilation, blood pressure, heart rate, plasma noradrenaline, and cortisol\(^58\). A single
inhalation of 35% CO₂ activates the HPA axis, and cortisol increases for ~30 min after exposure. In addition, blood pressure increases and heart rate decreases with exposure⁴³,⁵⁹. The sympathetic and neuroendocrine responses to CO₂ exposure indicate arousal likely reflective of negative valence. When taken together with the behavioral responses described in the previous section, we suggest that these physiological responses to CO₂ exposure are associated with negative emotional states.

Neurobiological responses

In this section, we review the effects of CO₂ on activation of brain regions, and nuclei within regions, involved in fear and anxiety. Chemoreceptive areas of the brain implicated in the ventilatory response to hypercapnia (e.g., medullary raphe, retrotrapezoid nucleus, caudal medulla, nucleus tractus solitaries, etc.) have been previously reviewed⁶⁰,⁶¹.

The amygdala is implicated in emotional responses to sensory inputs, and in generating the behavioral and physiological adaptive responses⁶². In rats exposed to 10% CO₂, the medial and central amygdala show increased c-Fos expression. This increase is associated with augmented minute ventilation, breathing frequency, and tidal volume⁶³. Johnson et al.⁶⁴ found that rats exposed to the 20% CO₂ challenge tended to increase c-Fos expression in the central amygdala, and this increase was related to increased fecal boli production (indicative of fear and anxiety) and thigmotaxis in the open field tests, but no effect of hypercapnia on c-Fos expression was detected in the medial and basolateral amygdala. Increased c-Fos expression in the central and basolateral amygdala has been found in high CO₂-sensitive mice (i.e., mice that

| Strain | Sex | Test | Delivery method | Concentration/flow rate | Treatment | Results | Reference |
|--------|-----|------|----------------|-------------------------|-----------|---------|-----------|
| SD     | M   | Vogel| Pf             | 35.65% (CO₂O₂)          | CO₂       | ↓ liking (40%)⁶¹ | ⁵¹        |
|        |     |      |                |                         | Alprazolam (0.5 mg/kg i.p.) + CO₂ | ↑ liking⁶¹ |
| SD     | M   | Pavlovian conditioning | Pf | From 1 to 100% CO₂ | CO₂ + vanilla odor | ↑ of freezing episodes with CO₂ concentration | ⁵⁵        |
| SD     | M   | Open field | Gf⁴ | 20% | CO₂ | ↑ 15% thigmotaxis⁶¹,↑ fecal boli production⁶¹ | ³⁴        |
| SD     | M   | Open field | Gf⁴ | 20% | CO₂ | ↑ 15% thigmotaxis⁶¹,↑ fecal boli production⁶¹ | ³⁴        |
| SD     | M   | Social interaction | Gf⁴ | 20% | CO₂ | ↓ 50% social interactions⁶¹ | ²⁴        |
| SD     | M   | Open field | Gf⁴ | 20% | CO₂ | ↔ thigmotaxis and line crossing⁶¹ | ⁷⁵        |
|        |     |      |                |                         | Lorazepam + CO₂ | ↓ line crossing⁶¹ | ²⁴        |
|        |     |      |                |                         | 1 mg/kg i.p. | ↔ line crossing⁶¹ | ²⁴        |
|        |     |      |                |                         | 0.5 mg/kg i.p. | ↔ line crossing⁶¹ | ²⁴        |
|        |     |      |                |                         | 0.3 mg/kg i.p. | ↔ line crossing⁶¹ | ²⁴        |
|        |     |      |                |                         | 0.1 mg/kg i.p. | ↔ line crossing⁶¹ | ²⁴        |
| SD     | M   | Social interaction | Gf⁴ | 20% | CO₂ | ↑ social interactions⁶¹ | ⁷⁵        |
|        |     |      |                |                         | Lorazepam + CO₂ | ↓ social interactions⁶¹ | ²⁴        |
|        |     |      |                |                         | 1 mg/kg i.p. | ↔ social interactions⁶¹ | ²⁴        |
|        |     |      |                |                         | 0.5 mg/kg i.p. | ↑ social interactions⁶¹ | ²⁴        |
|        |     |      |                |                         | 0.3 mg/kg i.p. | ↑ social interactions⁶¹ | ²⁴        |
|        |     |      |                |                         | 0.1 mg/kg i.p. | ↑ social interactions⁶¹ | ²⁴        |

Table 3  Studies assessing the effects of CO₂ post exposure, specifying test used, delivery methods, concentration or flow rate used, strain and sex, and a summary of results.

Strain: SD Sprague Dawley; delivery method: Pf pre-fill, Gf⁴ 20% CO₂ challenge; concentration or flow rate: static concentration (%) or flow rate (% CO₂ chamber vol. min⁻¹); sex: M male, F female; results: ↑ increase, ↓ decrease, ↔ no change in behavior or absent; i.p. intraperitoneal.

⁶¹Compared to control rats exposed to air.

⁶¹Compared to rats treated with vehicle.
show a higher freezing responses to 5% CO2 than low responders) due to CO2 inhalation compared to that of mice exposed to air.65 Asic1a+/+ mice (mice with intact acid sensing ion channels 1a in the amygdala and in other brain regions) froze when exposed to 10% static CO2, increased thigmotaxis when exposed to 20% CO2 in an open field test and preferred an air chamber, to a chamber prefilled with 20% CO2 in a choice test. These responses were attenuated in Asic1a−/− mice (mice without intact acid sensing ion channels). In vitro amygdala neurons of Asic1a+/+, but not Asic1a−/− mice, responded to a reduction in pH. In addition, CO2 inhalation decreased pH in the basolateral amygdala of Asic1a−/− mice. Focal acidification of this region elicited a strong freezing response, while the administration of HCO3− (to counteract acidosis) reduced freezing due to CO2 inhalation.66 Overall, these results suggest that the amygdala acts as a chemoreceptor for changes in PaCO2/H+, and its activation is involved in the behavioral response to CO2.

The bed nucleus of the stria terminalis (BNST), frequently referred as the extended amygdala, is associated with modulation of behavioral responses to threatening stimuli.67 Taugh and colleagues68 have shown that the BNST is also involved in the behavioral response to hypercapnia. These authors found that lesions in the BNST decreased freezing responses of mice during 10% CO2 exposure. When compared air exposure, inhalation of 5% CO2 increased c-Fos expression in the BNST of high CO2-sensitive mice.65 In rats, c-Fos expression in the BNST did not differ when exposed to the 20% CO2 challenge versus air.64

Activation of the hypothalamus is related to the execution of different behavioral and physiological responses; the most commonly identified is the activation of the HPA axis and modulation of autonomic responses. During the HPA axis cascade, the paraventricular nucleus (PVN) in the hypothalamus secretes corticotropin-releasing factor.69 Several studies indicate that when rats are exposed to CO2 concentrations of between 5 and 20%, the PVN shows a high density of positive c-Fos expression, indicating PVN activation during hypercapnia.64,70–72 One other study reports no significant increase in c-Fos expression in the PVN of rats exposed to 10% CO2 (ref. 63).

The dorsomedial region of hypothalamus (DMH) is involved in the execution of behavior responses to aversive stimuli.73,74 One study reported that rats exposed to 10% CO2 did not differ from controls in the number of labeled cells on the DMH.65 However, other studies have found that the DMH shows high c-Fos expression in rats exposed to 5% CO2 and the 20% CO2 challenge, particularly in orexin neurons found only in the DMH and perifornical nucleus of the hypothalamus75. Thigmotaxis in the open field was attenuated in rats treated with an orexin receptor antagonist before the 20% CO2 challenge.34 Orexin neurons are chemosensitive; the firing rate of in vitro orexin neurons increases with fluctuations in CO2 and pH (ref. 76). Furthermore, individual differences in rat behavioral responses to the CO2 challenge were related to variation in orexin activity in the lateral hypothalamus.36 These results show that the DMH and the perifornical nucleus of the hypothalamus are involved in the behavioral response to hypercapnia, and act as chemoreceptors.

Another region relevant in the modulation of behavioral responses to threatening stimuli is the periaqueductal gray (PAG). The ventrolateral PAG (VL-PAG) is involved in immobility responses,77 while the dorsal PAG (dorsolateral DL-PAG and dorsomedial DMP-PAG) is related to flight behaviors.78,79 The VL, DL, and DMP of rats exposed to CO2 show a dose-dependent increased c-Fos expression.5,80 Rats exposed to CO2 concentrations between 8 and 13% showed increased immobility and flight behaviors associated with PAG electrical stimulation.81 Lesions in the DL and DMP of rats exposed to low concentrations of CO2 (7% CO2) decreased the ventilatory response compared to controls, without altering the cardiovascular response.82 These results indicate that the PAG is activated during CO2 inhalation and involved in the behavioral and ventilatory responses to hypercapnia.

Consistent with this literature on rats (summarized in Table 4), research on human subjects shows that CO2 inhalation is associated with the activation of the amygdala, PAG, hypothalamus, and anterior insula. Moreover, this work on humans shows that activation of these brain regions is correlated with feelings of dyspnea.83 Interestingly, patients with bilateral amygdala lesions still experienced fear and panic when inhaling 35% CO2 (ref. 84), but not when exposed to external life-threatening stimuli.85

A key central chemoreceptor region is the medullary raphe; local acidification of the medullary raphe produces an increase in the ventilatory response.86 Serotonin (5HT) is originated in the medullary raphe by tryptophan hydroxylation. Serotonin is implicated in mediating emotional states, perception, cognition, and sympathetic arousal.87 Administration of serotonin reuptake inhibitors (SRIs), which increase synaptic serotonin, decreases anxiety in humans, and reduces respiratory rate of rats exposed to 6% CO2 (ref. 88). Mice treated with selective serotonin reuptake inhibitors (SSRIs) showed a decrease in freezing responses and ventilatory response during 5% CO2 exposure, and showed less context conditioning after exposure, than did control mice.68 These results indicate that serotonin is involved in the ventilatory and behavioral response to hypercapnia. In humans, treatment with serotonin antagonists, or tryptophan depletion before one or two inhalations of 35% CO2, enhances the experience of anxiety, fear, dyspnea,
and panic. Treatment with serotonin precursors and SSRIs have the opposite effect.

The role of the γ-aminobutyric acid (GABA)—a mammalian neurotransmitter that mediates synaptic inhibitions and is associated with anxiety—is also involved in the response to hypercapnia. In rats, stressful events like acute handling, chronic restraint, and social isolation decrease GABA<sub>A</sub> receptor function. The administration of compounds that bind to benzodiazepine recognition sites and that decrease the function of GABA<sub>A</sub> receptors (i.e., anxiogenic drugs) induce anxiety-like behavior in rats. Likewise, exposure to 35% CO<sub>2</sub> decreases GABA<sub>A</sub> function in the rat cerebral cortex, cerebellum, and hippocampus. The administration of benzodiazepines—which

### Table 4 Neurobiological responses to CO<sub>2</sub> specifying brain region, delivery method, concentration or flow rate used, and a summary of results. In all studies only male Sprague Dawley rats were used. All rats were forced exposed to CO<sub>2</sub>.

| Brain region        | Delivery method | Concentration/flow rate | c-Fos expression | Reference |
|---------------------|-----------------|-------------------------|------------------|-----------|
| Amygdala            | Pf              | 10%                     | ↑<sup>a</sup>     | 63        |
| Medial              |                 |                         |                  |           |
| Central             |                 |                         |                  |           |
| Basolateral         |                 |                         |                  |           |
| Amygdala            | Gf<sub>a</sub>  | 20%                     |                  | 64        |
| Medial              |                 |                         |                  |           |
| Central             |                 |                         |                  |           |
| Basolateral         |                 |                         |                  |           |
| BNST                | Gf<sub>a</sub>  | 20%                     | ↓                 | 64        |
| Hypothalamus        | Pf              | 5%                      | ↑                 | 70        |
| PVN                 |                 |                         |                  |           |
| DMH                 |                 |                         |                  |           |
| Hypothalamus        | Gf<sub>a</sub>  | 15%                     | ↑                 | 71        |
| PVN                 |                 |                         |                  |           |
| Hypothalamus        | Pf              | 10%                     | ↑                 | 63        |
| PVN                 |                 |                         |                  |           |
| DMH                 |                 |                         |                  |           |
| Hypothalamus        | Gf<sub>a</sub>  | 20%                     | ↑                 | 64        |
| PVN                 |                 |                         |                  |           |
| DMH                 |                 |                         |                  |           |
| Hypothalamus        | Pf              | 5 and 12%               | ↑                 | 72        |
| PVN                 |                 |                         |                  |           |
| Hypothalamus        | Gf<sub>a</sub>  | 20%                     | ↑                 | 34        |
| DMH                 |                 |                         |                  |           |
| Periaqueductal gray | Gf<sub>a</sub>  | 20%                     | ↑                 | 36        |
| VLPAG               |                 |                         |                  |           |
| DLPAG               |                 |                         |                  |           |
| DMPAG               |                 |                         |                  |           |
| Periaqueductal gray | Pf              | 8, 10, and 15%          | ↑                 | 81<sup>b</sup> |
| VLPAG               |                 |                         |                  |           |

<sup>a</sup>Rats were ventilated.
<sup>b</sup>In this study, the stain and sex of the rats used are unspecified.

Delivery method: Pf pre-fill, Gf<sub>a</sub> 20% CO<sub>2</sub> challenge; concentration or flow rate: static concentration (%) or flow rate (% CO<sub>2</sub> chamber vol. min<sup>-1</sup>); results: ↑ increase, ↓ decrease, ↔ no change, ~↑ tendency.
bind to the benzodiazepine receptor sites enhancing GABAergic transmission—before exposure to CO2, counteracts the effects of CO2 on GABA_A receptor functioning199,100, increase reward consumption in a Vogel conflict test51, increase tolerance to CO2 in approach-avoidance82, and enhance social interactions in the social test (at doses that do not impair locomotor activity)56. These results indicate that CO2 acts as a negative stimulus on GABA_A functioning, and that following drug treatment with known anxiolytics the behavioral effect of CO2 is diminished. Similarly, healthy people pretreated with alprazolam before 5, 7.5, and 35% CO2 inhalation, show diminished experiences of fear, feeling like leaving the room, dyspnea, panic, and distress101,102.

In summary, brain areas, ion channels, and neurotransmitters involved in fear and anxiety are activated by hypercapnia. These regions include the amygdala, BNST, hypothalamus, and PAG. This body of evidence is consistent with the conclusion that rats experience negative emotions when inhaling CO2. Since these responses can, to some extent, be counteracted by the administration of anxiolytics and SSRIs, and similarly to behavioral changes in rats, benzodiazepines, serotonin precursors, and SSRIs ameliorate feelings of anxiety, fear, distress, dyspnea, and panic due to hypercapnia in humans.

Conclusions

Concentrations <10% CO2 are tolerated, do not elicit intense behavioral responses, and cause mild conditioning in rats. Rats respond with active and passive defense behaviors to concentrations >10%. If escape is possible all rats avoid CO2, even to concentrations <10%, but when motivated some rats may tolerate higher concentrations (up to 18% CO2). The behavioral responses in the open field and social tests show that negative emotions, which resemble those associated with exposure to anxiogenic drugs, were sustained when CO2 was no longer present. Exposure to concentrations over 35% has anxiogenic effects and produces strong conditioning. The level of conditioning and extinction resistance depend upon CO2 concentration, implying that the magnitude of the emotional response increased with the intensity of the stimuli. The behavioral responses to CO2 are accompanied with neuroendocrine and sympathetic activation. Brain responses related to fear and anxiety are detected when rats inhale CO2. Overall, these studies indicate that CO2 elicits negative emotional states in rats.

Humans report feelings of fear, anxiety, dyspnea, distress, and panic during CO2 inhalation, and the evidence reviewed above suggests that rats experience similar emotions. Caution is required when functional homology is used to draw inferences regarding felt experiences19, but ventilatory and cardiovascular changes due to CO2 inhalation are similar between rats and humans, behavioral responses of rats when exposed to CO2 correspond well with feelings reported by humans following CO2 inhalation, and human feelings of anxiety, fear, dyspnea, and panic in response to hypercapnia can be attenuated by benzodiazepines and SSRIs (drugs that also diminish the defense behaviors seen in rats exposed to CO2). In addition, variation in rat and human CO2 reactivity is linked to the emotional experience during inhalation.

Throughout this review, we used functional homology to link rat and human felt emotions during CO2 inhalation. Although such inferences require caution, there is considerable research in human felt emotions due to CO2 inhalation. One example of a study comparing rodents and humans using the same CO2 concentrations is that by Leibold and colleagues103. These authors used static 9% CO2 concentrations to compare behavioral, respiratory, and cardiovascular responses of mice and humans. This study found that mice avoided the central areas of an open field, showed immobility responses and produced fecal boli during 9% CO2 exposure, and humans reported an increase in feelings of fear and panic due to 9% CO2 inhalation. Inhalation of 9% CO2 induced bradycardia in both species. The authors concluded that mice reactivity to CO2 can be used as model for humans. We suggest also that the opposite is also true; i.e., that human reports can be used to better understand the emotional experience of rodents during CO2 exposure.

Taken together, this evidence supports our conclusion that rats experience negative emotions during and after CO2 exposure, and that these emotions likely correspond to fear, anxiety, dyspnea, distress, and panic. This work contributes to the study of translational models of anxiety and panic, and is also relevant to ongoing debate regarding the use CO2 for killing animals. In addition, we suggest that this literature will be of interest to all who study felt emotions in animals.

Acknowledgements

We thank Catherine Schuppil, Marina Von Keyserlingk, Becca Franks, Huw Gollidge, and anonymous referees for their helpful comments and suggestions. This research was funded by an NSERC Discovery grant to D.M.W. L.A. was supported by the CONACyT PhD. scholarship (no. 381124), the James A. Shelford Memorial Scholarship, and the Charles River Scholarship in Animal Welfare.

Author contributions

Contributed to the writing of the manuscript (L.A. and D.W.).

Conflict of interest

The authors declare that they have no conflict of interest.

Publisher’s note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
References

1. Chisholm, J. M. & Pang, D. S. Assessment of carbon dioxide, carbon dioxide/ oxygen, isoflurane and pentobarbital killing methods in adult female Sprague-Dawley rats. PLoS ONE 11, e0162639 (2016).

2. Chisholm, J. I., Ranter, D., Fernandez, N. J., Krapac, A. & Pang, D. S. Carbon dioxide, but not isoflurane, elicits ultrasonic vocalizations in female rats. Lab. Anim. 47, 324–327 (2013).

3. Cotten, A. M. L., Drinkenburg, W. H. I. M., Hoenderken, R. & Van Luijtelaar, E. L. J. M. Carbon dioxide euthanasia in rats: oxygen supplementation minimums signs of agitation and asphyxia. Lab. Anim. 29, 262–268 (1995).

4. Leach, M. C., Bowell, V. A., Allan, T. F. & Morton, D. B. Aversion to gaseous receptors determine the respiratory sensitivity of central chemoreceptors and ether. Lab. Anim. 22, 67–75 (1988).

5. Hackforth, H., Kuppers, N. & Bohnet, W. Euthanasia of rats with carbon dioxide-animal welfare aspects. Lab. Anim. 34, 91–96 (2000).

6. Niell, L. & Weary, D. M. Behavioural responses of rats to gradual-fall carbon dioxide euthanasia and reduced oxygen concentrations. Appl. Anim. Behav. Sci. 100, 295–309 (2006).

7. Niell, L., Kirkden, R. D. & Weary, D. M. Effects of novelty on rats' responses to CO2 exposure. Appl. Anim. Behav. Sci. 111, 183–194 (2008).

8. Niell, L., Stewart, S. & Weary, D. M. Effect of flow rate on gradual-fall carbon dioxide exposure in rats. Appl. Anim. Behav. Sci. 109, 77–84 (2008).

9. Blackshaw, J. K., Fenwick, D. C., Beattey, A. W. & Allan, D. J. The behavior of chickens, mice and rats during euthanasia with chloroform, carbon dioxide and ether. Lab. Anim. 22, 67–75 (1988).

10. Parkes, J. The Affective Brain and Core Consciousness: How does Neural Activity Generate Emotional Feelings? (eds Lewis, M., Haviland-Jones, J. M. & Barrett, L. F.) (Guilford Press 2008).

11. Mendl, M., Burman, O. H. & Paul, E. S. An integrative and functional framework for the study of animal emotion and mood. Proc. R. Soc. B 277, 2895–2904 (2010).

12. Adolphs, R. The biology of fear. Curr. Biol. 23, R79–R93 (2013).

13. Adolphs, R. Emotion. Curr. Biol. 20, R549–R552 (2010).

14. Craig, A. D. Interoception and Emotion: a Neuroanatomical Perspective (eds Lewis, M., Haviland-Jones, J. M. & Barrett, L. F.) (Guilford Press 2008).

15. LeDoux, J. E. & Pine, D. S. Using neuroscience to help understand fear and anxiety: a two-system framework. Annu. Rev. Psychol. 57, 1083–1093 (2016).

16. Weary, D. M., Droege, P. & Braithwaite, V. A. Evidence of felt emotions: approaches, inferringes, and refinements. Adv. Stud. Behav. 49, 27–48 (2017).

17. Mellor, D. Updating animal welfare thinking: moving beyond the “Five Freedoms” towards “a Life Worth Living”. Anim. A2, 61 (2016).

18. Beausoleil, N. J. & Mellor, D. J. Introducing breathlessness as a significant animal welfare issue. N. Z. Vet. J. 63, 44–51 (2015).

19. Prange, H. Respiratory physiology: understanding gas exchange (Springer Science & Business Media 2012).

20. Blain, G. M., Smith, C. A., Henderson, K. S. & Dempsey, J. A. Peripheral chemoreceptors determine the respiratory sensitivity of central chemoreceptors. J. Physiol. 588, 2455–2471 (2010).

21. Hickman, D. L. et al. Evaluation of low versus high volume per minute displacement CO2 methods of euthanasia in the induction and duration of panic-associated behavior and physiology. Animals 6, 45 (2016).

22. Gorman, J. M. et al. Physiological changes during carbon dioxide inhalation in patients with panic disorder, major depression, and premenstrual dysphoric disorder: evidence for a central fear mechanism. Arch. Gen. Psychiatry 58, 125–131 (2001).

23. Bret, D. P. The Humaneness of Carbon Dioxide as an Agent of Euthanasia for Laboratory Rats., 19–31 (Potter's Bar, UK, 1987).

24. Améndola, L. & Weary, D. M. Evidence for consistent individual differences in rat sensitivity to carbon dioxide. PLoS ONE 14, e0215808 (2019).

25. Makowska, I. J. & Weary, D. M. Using rat behavior to assess aversion to euthanasia agents. AniTech 1, 465–467 (2012).

26. Burkholder, T. H. et al. Comparison of carbon dioxide and argon euthanasia: effects on behavior, heart rate, and respiratory lesions in rats. J. Am. Assoc. Lab. Anim. Sci. 49, 448–453 (2010).

27. Johnson, P. L., Hollis, J. H., Mostallia, R., Lightman, S. L. & Lovny, C. A. Acute hypercarbic gas exposure reveals functionally distinct subpopulations of serotonergic neurons in rats. J. Psychopharmacol. 19, 327–341 (2005).

28. Smith, W. & Harrap, S. B. Behavioral and cardiovascular responses of rats to euthanasia using carbon dioxide gas. Lab. Anim. 31, 337–346 (1997).

29. Winter, A., Ahlbbrand, R., Naik, D. & Shah, R. Differential behavioral sensitivity to carbon dioxide (CO2) inhalation in rats. Neuroscience 346, 423–433 (2017).

30. Holtman, D. L. Home cage compared with induction chamber for euthanasia of laboratory rats. J. Am. Assoc. Lab. Anim. Sci. 57, 729–733 (2018).

31. Johnson, P. L. et al. Activation of the orexin 1 receptor is a critical component of CO2-mediated anxiety and hypertension but not bradycardia. Neuropsychopharmacology 37, 1911–1922 (2012).

32. Hickman, D. L. Wellbeing of alcohol-prefering rats euthanized with carbon dioxide at very low and low volume displacement rates. J. Am. Assoc. Lab. Anim. Sci. 58, 78–82 (2019).

33. Monfils, M. H. et al. Predicting extinction phenotype to optimize fear reduction. Psychopharmacol. 236, 99–110 (2019).

34. Fraser, D. & Duncan, I. J. ‘Pleasures,’ ‘pains’ and animal welfare: toward a natural history of affect. Anim. Welf. 7, 383–396 (1998).

35. Kirkden, R. D. & Pajor, E. A. Using preference, motivation and aversion tests to ask scientific questions about animals’ feelings. Appl. Anim. Behav. Sci. 100, 29–47 (2006).

36. Wong, D., Makowska, I. J. & Weary, D. M. Rat aversion to isoflurane versus carbon dioxide. Biol. Lett. 9, 20121000 (2013).

37. Kirkden, R. D. et al. The validity of using an approach-avoidance test to measure the strength of aversion to carbon dioxide in rats. Appl. Anim. Behav. Sci. 114, 216–234 (2008).

38. Niell, L. & Weary, D. M. Rats avoid exposure to carbon dioxide and argon. Appl. Anim. Behav. Sci. 107, 100–109 (2007).

39. Améndola, L., Ratuski, A. & Weary, D. M. Variation in the onset of CO2-induced respiratory signs of agitation and asphyxia. Animals 11, 346 (2021).

40. Améndola and Weary Translational Psychiatry (2020) 10:253

Page 11 of 12
