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Carbon Dioxide Reactivity Differentially Predicts Fear Expression After Extinction and Retrieval-Extinction in Rats

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ABSTRACT

BACKGROUND: Cues present during a traumatic event may result in persistent fear responses. These responses can be attenuated through extinction learning, a core component of exposure therapy. Exposure/extinction is effective for some people, but not all. We recently demonstrated that carbon dioxide (CO_2) reactivity predicts fear extinction memory and orexin activation and that orexin activation predicts fear extinction memory, which suggests that a CO_2 challenge may enable identification of whether an individual is a good candidate for an extinction-based approach. Another method to attenuate conditioned responses, retrieval-extinction, renders the original associative memory labile via distinct neural mechanisms. The purpose of the current study was to examine whether we could replicate previous findings that retrieval-extinction is more effective than extinction at preventing the return of fear and that CO_2 reactivity predicts fear memory after extinction. We also examined whether CO_2 reactivity predicts fear memory after retrieval-extinction.

METHODS: Male rats first underwent a CO_2 challenge and fear conditioning and were assigned to receive either standard extinction ($n = 28$) or retrieval-extinction ($n = 28$). Then, they underwent a long-term memory (LTM) test and a reinstatement test.

RESULTS: We found that retrieval-extinction resulted in lower freezing during extinction, LTM, and reinstatement than standard extinction. Using the best subset approach to linear regression, we found that CO_2 reactivity predicted LTM after extinction and also predicted LTM after retrieval-extinction, although to a lesser degree.

CONCLUSIONS: CO_2 reactivity could be used as a screening tool to determine whether an individual may be a good candidate for an extinction-based therapeutic approach.

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Maladaptive associative learning underlies psychiatric disorders including posttraumatic stress disorder (PTSD) and anxiety disorders, which are characterized by excessive, persistent fear responses to previously neutral stimuli that have become associated with aversive outcomes and remain associated even when they are no longer reliably predictive of such an outcome. In the laboratory, we can model how these associative memories are formed with Pavlovian fear conditioning, wherein a neutral stimulus (conditioned stimulus [CS]) is paired with an aversive stimulus (unconditioned stimulus [US]) such that the CS comes to elicit a fear response on its own. Using this model, we can then test methods to attenuate conditioned fear responses. One such method is extinction training, wherein the CS is repeatedly presented in the absence of the US such that the animal learns that the CS is no longer predictive of the US (1). Extinction learning forms the basis for exposure therapy, a treatment in which individuals are exposed to feared cues to learn that they no longer predict an aversive outcome (2). However, in both the laboratory and the clinic, in some individuals, conditioned responses will return with the passage of time (spontaneous recovery), exposure to the US or stress (reinstatement), or a change in context (renewal) (3–7). One reason for this is that rather than directly persistently weakening the existing fear memory, extinction learning forms a new CS-noUS associative memory that may temporarily inhibit the original CS-US association. As such, it is important to continue developing strategies to ensure persistent fear attenuation in all individuals.

One such strategy is to take advantage of individual differences in extinction learning to identify responders. Multiple predictors of fear extinction have been identified (8), including the activation of orexin neurons in the hypothalamus. Orexin is a neuropeptide synthesized in the hypothalamus that has been implicated in a variety of motivated behaviors (9–11). Increased activation of orexin neurons is associated with greater fear expression during extinction training, and antagonism of orexin neurons facilitates extinction (12,13). However, in humans, orexin activation cannot be quantified in a reliable and noninvasive manner (14,15). In rats, orexin neurons are also activated by exposure to carbon dioxide (CO_2), and in humans,

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emotional reactivity to CO_2 predicts later development of PTSD symptoms (16,17). Inspired by these findings, our lab previously found that in rats, behavioral reactivity to a CO_2 challenge predicts orexin activation, and the number of CO_2-activated orexin neurons predicts fear extinction memory (18). Behavioral CO_2 reactivity might thus be used as a proxy, although an imperfect one, for orexin activation to predict which individuals will respond well to extinction.

Another avenue for improving fear attenuation is to modify the original CS-US memory instead of forming a CS-noUS memory. We have previously found that this can be achieved by delivering a retrieval trial prior to extinction (retrieval-extinction) (19). Retrieval-extinction first opens the reconsolidation window that renders the fear memory labile and then updates it with the CS-noUS association. Retrieval-extinction effectively prevents the return of fear in both rodents and humans and does so via mechanisms that are distinct from, but overlap with, extinction (19–27) [see (28) for review]. We should note that not all studies have successfully replicated this effect (29–44), and there has been relatively little research conducted on individual differences or predictors of retrieval-extinction responding (38,45–48).

Here, in rats, we examined whether we could replicate previous findings that retrieval-extinction is more effective than extinction at preventing the return of fear and that CO_2 reactivity predicts fear memory after extinction. We also examined whether CO_2 reactivity predicts fear memory after retrieval-extinction.

METHODS AND MATERIALS

Subjects and Experimental Timeline

The experimental timeline is summarized in Figure 1. Subjects consisted of 56 male Sprague Dawley rats obtained from Harlan. The rats were housed in pairs in temperature- and humidity-controlled transparent polyethylene cages with water freely available at all times on a 12-hour reverse light cycle (lights off at 7 AM). The order of experimental procedures was chosen to remain consistent with our previous work (18,19).

All rats first underwent a CO_2 challenge. Three days later, all rats underwent 3 trials of fear conditioning during which a tone was paired with a shock. The following day, rats were then assigned to receive 19 trials of either standard extinction (n = 28) or retrieval-extinction (n = 28) (19). The next day, they underwent a long-term memory (LTM) test (also known as an extinction retention test or between-session extinction) that consisted of 4 presentations of the tone without a shock. The next day, they underwent reinstatement with 3 unsignaled shock presentations followed by a test the following day. The context of the conditioning chambers was maintained throughout the testing phases. Details of the fear conditioning and extinction are presented in the Supplement. All sessions were recorded for offline analysis of freezing by an experimenter who was blinded to experimental conditions. Freezing was defined as the absence of all movement aside from breathing and ear twitching, not including sleeping or resting (49). CS-induced freezing was expressed as a percentage of the total time spent freezing during each 20-second CS.

A subset of rats underwent a second CO_2 challenge prior to euthanasia, and brains were harvested for later analysis. All procedures were conducted in compliance with the National Institutes of Health Guide for the Care and Use of Experimental Animals and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

CO_2 Challenge

Hereafter, CO_2 challenge will refer to the assay, and CO_2 reactivity will refer to the behavioral output. The assay took

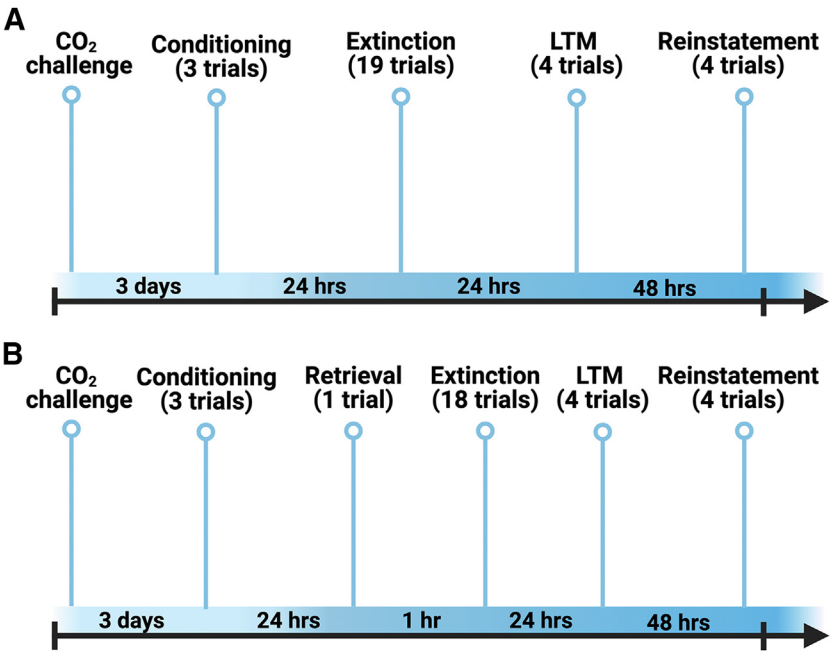

Figure 1. First, male rats underwent a CO_2 challenge. Then, they received 3 trials of fear conditioning in which a tone was paired with a shock. Next, rats were assigned to receive 19 trials of either standard extinction (n = 28) **(A)** or retrieval-extinction (n = 28) **(B)**. Then, they underwent a LTM test that consisted of 4 presentations of the tone without a shock. After reinstatement with 3 unsignaled shocks, they underwent a reinstatement test with 4 presentations of the tone without a shock. Figure created with BioRender.com. LTM, long-term memory.

place in a plexiglass chamber (30.5 × 30.5 × 61 cm) that allows the control of gas flow in 4 phases. Thirty seconds after the rat was placed in the chamber (baseline), an infusion of normoxic air blended with hypercarbic gas (25% CO_2) was released into the chamber for 2 minutes (induction phase). Next, gas flow was held constant at 25% CO_2 for 2 minutes (hold phase) followed by 4 minutes of infusion with normoxic air (flush out phases 1 and 2). Each rat remained in the chamber for an additional 2 minutes before returning to their home cage. All sessions were recorded and analyzed offline for 4 behaviors: ambulation (time spent moving around, any displacement of the paws), grooming (time spent grooming), rearing (number of times rat stands on rear legs), and labored breathing (deep and long breaths noticeable from movements of the torso). The 4 CO_2 reactivity behaviors across the 4 phases yielded 16 variables or subcomponents. All videos were manually scored using BORIS (50).

Statistical Analysis

All statistical analyses were performed using R and the following packages: rstatix and beset (51). We conducted repeated-measures analyses of variance (ANOVAs) with freezing as the dependent variable, group as the between-subjects factor, and trial as the within-subjects factor separately for fear conditioning and extinction. In addition, we averaged together freezing during the first and last 4 trials of extinction and conducted a 2 × 2 ANOVA with group as the between-subjects factor and extinction phase (early and late) as the within-subjects factor. We calculated mean freezing for each of the following: the last 2 trials of extinction, first 2 trials of LTM, and first 2 trials of reinstatement and conducted two 2 × 2 ANOVAs with group (extinction and retrieval-extinction) as the between-subjects factor and session (extinction and LTM or LTM and reinstatement) as the within-subjects factor. Post hoc *t* tests (with Bonferroni correction) were conducted as warranted. We also quantified freezing during the 20 seconds prior to the first trial of extinction, LTM, and reinstatement; these analyses are reported in the Supplement. We also quantified the number of rats that had freezing below our data-driven remission criteria of 37.5% (52) at these time points and tested whether the proportions were different between groups using Fisher's exact test.

To determine which of the 16 subcomponents of CO_2 reactivity predicted the most variance in LTM, we used the best subset approach to linear regression. Linear regression allows us to take advantage of the whole range of values and minimize the amount of information lost (which occurs when variables are categorized into subgroups). The best subset approach chooses the linear model with the fewest predictors, highest predictive power, and highest degree of cross-sample replicability. This approach weighs the contribution of each variable's share of explained variance to ensure that it is worth including in the model. We also used resampling (k-fold cross-validation where k = 10) on each possible combination of predictors to estimate how well it would predict new samples. To minimize selection bias, we also ran a nested cross-validation so that test error could be evaluated on a holdout sample that was not used to fit or select the best models (53,54). This provides a fairer estimate of the model's generalizability. In other words, the selected model is the model with the fewest predictors that is best at predicting new data.

RESULTS

We quantified freezing in response to the CS in rats that underwent fear conditioning, extinction, or retrieval-extinction; an LTM test; and reinstatement and determined whether extinction or retrieval-extinction was more effective at preventing the return of fear by comparing mean freezing during LTM and reinstatement and by comparing the number of rats in each group that met our data-driven criteria for fear remission (52). We also quantified the following behaviors during a CO_2 challenge in rats: ambulation, rearing, labored breathing, and grooming. To determine whether CO_2 reactivity predicts the return of fear, we used behavioral CO_2 reactivity as predictors of long-term fear memory using the best subset approach to linear regression.

Retrieval-Extinction Results in Lower Freezing Than Standard Extinction

Freezing across trials is shown in Figure 2. To assess whether the groups comparably and successfully acquired conditioned fear responses, we conducted a repeated-measures ANOVA with group as the between-subjects factor and trial as the within-subjects factor. There was a significant main effect of

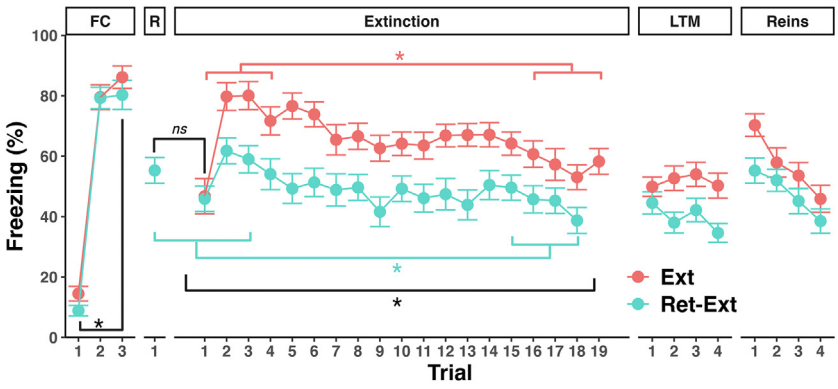

Figure 2. Mean percentage time spent freezing in response to the conditioned stimulus across FC, retrieval, extinction, LTM, and reinstatement are shown, ± SEM. Freezing increases similarly during FC in both groups. While groups show similar levels of freezing during the first trial of extinction, freezing is lower in Ret-Ext group than in Ext group during early extinction and late extinction. *$p < .05$. Ext, extinction; FC, fear conditioning; LTM, long-term memory; ns, nonsignificant; R, retrieval; Ret-Ext, retrieval-extinction.

trial ($F_{2,108}$ = 280.19, $p < .001$, η^2 = 0.76) and no significant main effect of group ($F_{1,54}$ = 1.56, p = .23, η^2 = 0.011), with significant increases in freezing between the first and last trial in both the extinction group (t_{27} = −15.70, $p < .001$) and the retrieval-extinction group (t_{27} = −14.49, $p < .001$). To assess whether the groups showed differences in extinction learning, we conducted a repeated-measures ANOVA with group as the between-subjects factor and trial as the within-subjects factor. There was a significant main effect of group, with overall reduced freezing during extinction learning in the retrieval-extinction group compared with the extinction group ($F_{1,18}$ = 13.27, $p < .001$, η^2 = 0.11). There was also a significant main effect of trial ($F_{18,972}$ = 6.26, $p < .001$, η^2 = 0.055) and a significant interaction effect ($F_{18,972}$ = 3.39, $p < .001$, η^2 = 0.031). Post hoc t tests on the first trial of extinction (that is, the retrieval trial in the retrieval-extinction group and the first extinction trial in the extinction group) showed that freezing was not different between groups (t_{54} = −1.18, p = .24), indicating that both groups retained similar levels of fear conditioning.

To account for between-trial variability, we also averaged together the first and last 4 trials of extinction to get a more stable indicator of early and late extinction. A repeated-measures ANOVA with extinction phase as the within-subjects factor and group as the between-subjects factor showed a significant main effect of group ($F_{1,54}$ = 9.27, p = .004, η^2 = 0.12) and a significant main effect of phase ($F_{1,54}$ = 26.93, $p < .001$, η^2 = 0.092) but no significant interaction ($F_{1,54}$ = 0.13, p = .72, $\eta^2 < 0.001$). Post hoc t tests showed that freezing was significantly lower in the retrieval-extinction group than the extinction group during both early extinction (t_{54} = 2.97, p = .004) and late extinction (t_{54} = 2.48, p = .016). Freezing was also significantly decreased from early extinction to late extinction in both the retrieval-extinction group (t_{27} = 3.78, $p < .001$) and the extinction group (t_{27} = 3.60, p = .001).

Mean freezing to the CS during the last 2 trials of extinction, the first 2 trials of LTM, and reinstatement are shown in Figure 3. To determine whether extinction or retrieval-extinction was better at preventing the return of fear, we conducted two 2 × 2 ANOVAs with group (extinction and retrieval-extinction) as the between-subjects factor and session (extinction and LTM or LTM and reinstatement) as the within-subjects factor. For the extinction-LTM ANOVA, there was a significant main effect of group, with reduced freezing in the retrieval-extinction group compared with the extinction group, which yielded a medium effect size ($F_{1,108}$ = 11.44, p = .001, η^2 = 0.096). There was no significant main effect of session or a significant interaction. For the LTM-reinstatement ANOVA, there was a significant main effect of group, with reduced freezing in the retrieval-extinction group compared with the extinction group, which yielded a medium effect size ($F_{1,54}$ = 6.65, p = .013, η^2 = 0.08). There was also a significant main effect of session, with increased freezing during reinstatement relative to LTM with a medium-large effect size ($F_{1,54}$ = 24.20, $p < .0001$, η^2 = 0.12). There was no significant interaction ($F_{1,54}$ = 0.008, p = .93, $\eta^2 > 0.001$). Post hoc t tests showed that at reinstatement, freezing was significantly lower in the retrieval-extinction group than the extinction group (t_{54} = 2.04, p = .046). To assess whether these group effects reflect meaningful differences in fear attenuation, we looked at the proportion of each group that met our data-driven criteria for remission (Table 1) (52). We found that 5 of 28 (17.9%) of the extinction group and 13 of 28 (46.4%) of the retrieval-extinction group met criteria for remission at LTM (Fisher's exact test, p = .0437, 2-tailed). At reinstatement, 2 of 28 (7.1%) of the extinction group and 7 of 28 (25%) of the retrieval-extinction group met criteria for remission (Fisher's exact test, p = .143, 2-tailed).

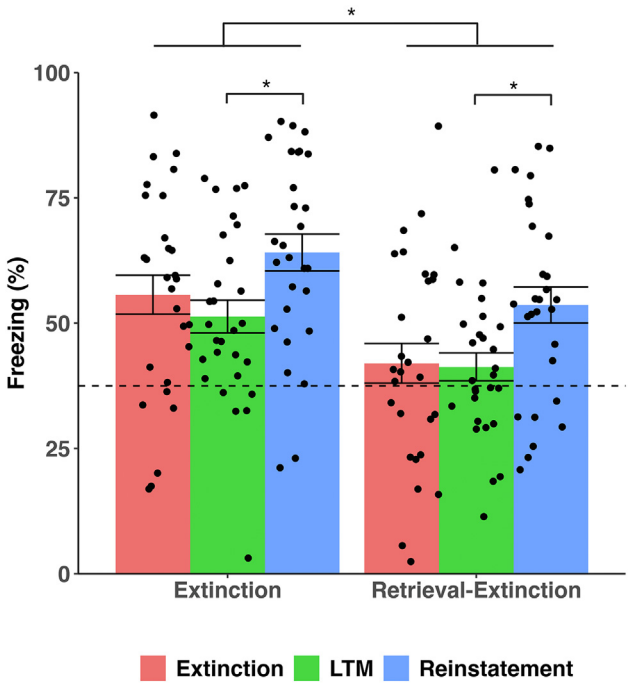

Figure 3. Mean freezing in response to the conditioned stimulus during the last 2 trials of extinction and the first 2 trials of LTM and reinstatement are shown, ± SEM. The individual data points are also shown. The dashed horizontal line depicts our data-driven criteria for remission (52). Neither group shows a significant return of freezing at LTM. Both groups show increased freezing at reinstatement compared with LTM. Freezing is lower overall in the retrieval-extinction group than the extinction group across phases. A significantly higher proportion of rats met criteria for remission at LTM in the retrieval-extinction group than in the extinction group. *$p < .05$. LTM, long-term memory.

CO_2 Reactivity

The 4 behaviors (ambulation, rearing, labored breathing, and grooming) quantified during the 4 phases of the CO_2 challenge (induction, 25% hold, flush out 1, and flush out 2) yielded 16 behavioral subcomponents, as in our previous work (18). The distribution of behavioral subcomponents during the CO_2

Table 1. Proportion of Each Group That Met Criteria for Remission

	Long-Term Memory	Reinstatement
Extinction	5/28 (17.9%)	2/28 (7.1%)
Retrieval-Extinction	13/28 (46.4%)	7/28 (25%)

challenge was visualized (Figure 4). To determine whether CO_2 reactivity predicts the return of fear after extinction or retrieval-extinction, we used behavioral CO_2 reactivity as predictors of long-term fear memory using the best subset approach to linear regression. We report 2 R^2 statistics for the best subset model: the train-sample R^2 (the unadjusted R^2 obtained by fitting the model to the full dataset) and the cross-validation–selection R^2 (the R^2 obtained by predicting random holdout examples and used to select the best model). This analysis was run separately for the extinction and retrieval-extinction groups. Our primary outcome variable was LTM. We ran a secondary exploratory analysis using reinstatement as the outcome variable, which we report in the Supplement.

CO_2 Reactivity Differentially Predicts Long-Term Fear Memory After Extinction and Retrieval-Extinction

We used the best subset approach to linear regression to estimate the best model within 1 standard error, with the lowest cross-validation error, and with the fewest number of predictors. Using these parameters in the extinction group, the best predictive effect of CO_2 reactivity came from a model with 1 predictor, rearing during flush out 1, which accounted for 32% of the variability in the total sample and is expected to account for up to 17% of the variability in future samples. Our nested cross-validation approach also allowed us to analyze how sensitive the selection outcome was to randomized assignments of observations to cross-validation folds. We found that this 1-predictor model was selected for 41% of the random subsamples. The relationship of the most important predictor to LTM is shown in Figure 4A. The secondmost selected model was the null (intercept-only) model, which was selected 16% of the time.

Using the best subset parameters in the retrieval-extinction group, the best predictive effect of CO_2 reactivity came from a model with 1 predictor: labored breathing during 25% hold, which accounted for 19% of the variability in the total sample and is expected to account for up to 9% of the variability in future samples. This single-predictor model was selected for 56% of random subsamples. The relationship of this predictor to LTM is shown in Figure 4B. The secondmost selected model was the null model, which was selected 27% of the time.

DISCUSSION

Here, we sought to replicate previous findings that retrieval-extinction is more effective than extinction at preventing the return of fear and that CO_2 reactivity predicts fear memory after extinction. We also sought to determine whether CO_2 reactivity predicts fear memory after retrieval-extinction. We partially replicated previous findings that retrieval-extinction is more effective than extinction at preventing the return of fear. We found an effect of retrieval-extinction on within-session extinction compared with standard extinction. In addition, freezing was lower overall in the retrieval-extinction group than in the standard extinction group in the LTM and reinstatement tests. Neither group showed a return of fear in the LTM test,

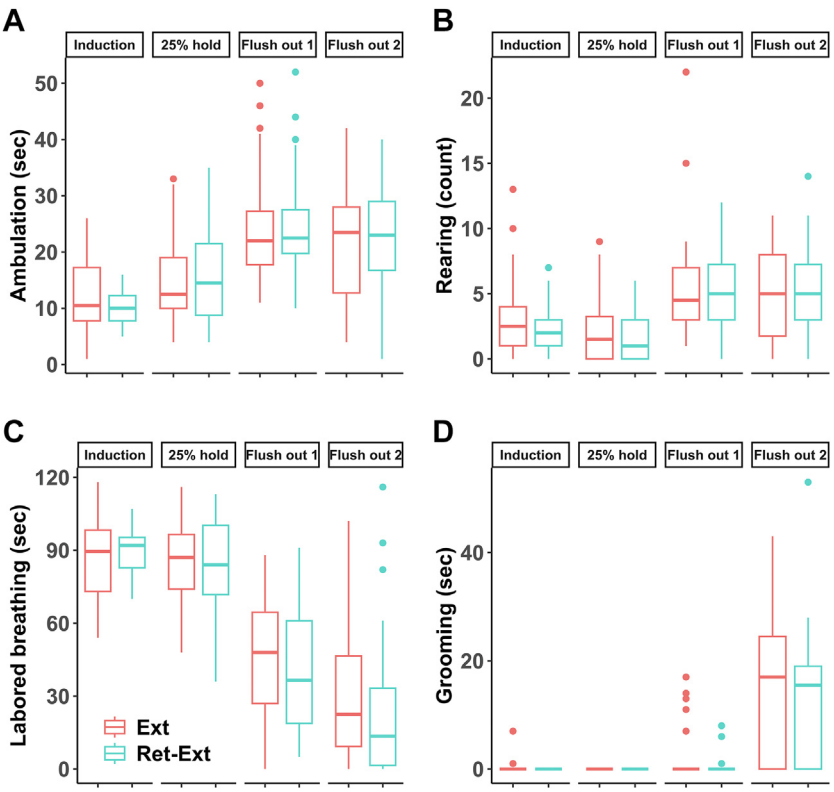

Figure 4. Distribution of CO_2 reactivity behaviors. Boxplots of the behaviors that were analyzed [ambulation **(A)**, rearing **(B)**, labored breathing **(C)**, grooming **(D)**] during each phase (induction, 25% hold, flush out 1, flush out 2) of the CO_2 challenge. The quantity of each of the 4 behaviors that were observed during the 4 phases yielded 16 behavioral subcomponents that were entered as possible predictors of long-term fear extinction memory in our model. Ext, extinction; Ret-Ext, retrieval-extinction.

and both groups showed a return of fear after reinstatement, although fear levels remained lower in the group that received retrieval-extinction. Differences between the 2 groups tended to reflect moderate effect sizes, although the effect size for group differences in within-session extinction approached a large effect size. Our lab had not reported facilitation of fear attenuation following retrieval in rats before, although we have seen it in a phobia sample, and others have seen it in rats (40,55); so, it remains consistent with the reported literature. Notably, a significantly greater proportion of rats in the retrieval-extinction group met criteria for remission at LTM than the extinction group. Retrieval-extinction more than doubled the chance of meeting criteria for remission compared with extinction.

We also replicated our previous findings that CO_2 reactivity predicts fear memory after extinction and found that this extends to retrieval-extinction. That is, we found that CO_2 reactivity predicted fear memory after extinction and predicted fear memory after retrieval-extinction, although to a lesser degree. We had previously found that the best predictive effect of CO_2 reactivity for long-term fear memory after extinction came from a model with 3 predictors: rearing during flush out 1, grooming during flush out 1, and labored breathing during CO_2 hold (18). In our current study, the best predictive effect of CO_2 reactivity for long-term fear memory after extinction came from rearing during flush out 1, and after retrieval-extinction, it came from labored breathing during CO_2 hold. One important caveat is that an *n* of 28 is too small to reliably capture generalizable relationships, as evidenced by the fact that they were only present in approximately one half of random subsamples, likely the ones that include the examples of very high rearing and very low labored breathing, which appear to drive the linear relationships depicted in Figure 5. This effect indicates that much larger samples will be required to observe enough examples of the full range of CO_2 reactivity behaviors such that the predictive relationships become less variable across samples.

In both our previous and current study, we found a negative relationship between our predictors and long-term fear memory (whether after extinction or retrieval-extinction), with higher levels of CO_2 reactivity behaviors being associated with lower freezing (18). In our previous study, these same behaviors were negatively associated with the number of CO_2-activated orexin neurons, and the number of CO_2-activated orexin neurons was positively associated with long-term fear memory after extinction (18). As such, higher behavioral CO_2 reactivity may be reflective of lower orexin activity that allows for adaptive responding to threatening stimuli, whether that be CO_2 or a fear CS. In a rat model of panic disorder, activation of orexin neurons is necessary for panic-like responses and is prevented by orexin antagonism (56). Future work will examine whether the relationship between CO_2-activated orexin neurons, CO_2 reactivity, and long-term fear memory is replicated in cross-samples.

We also asked whether the predictive effect of CO_2 reactivity for long-term fear memory was specific to extinction or whether it generalizes to retrieval-extinction. In our sample, CO_2 reactivity predicted 32% of the variability in long-term fear memory after extinction and 19% after retrieval-extinction. The fact that CO_2 reactivity more reliably predicted the return of fear in the extinction group than in the retrieval-extinction group may be due to their distinct underlying neural mechanisms. Extinction engages the medial prefrontal cortex and lateral amygdala (57), as does retrieval-extinction, although to a lesser degree (24). Furthermore, retrieval-extinction leads to increased expression of Zif268 and rpS6P (2 molecular markers of reconsolidation) in these same areas above either retrieval or extinction alone (23). Thus, it appears that retrieval-extinction engages both extinction and reconsolidation mechanisms, and while there is overlap in the areas that are engaged, the underlying mechanisms are distinct. Exposure to CO_2 activates orexin neurons (17). There are orexinergic projections to the prefrontal cortex and amygdala (58,59), and both structures innervate the lateral hypothalamus, where orexinergic neurons are exclusively found. In addition, individual differences in orexin activation predict fear extinction (12), and antagonism of orexin neurons facilitates fear extinction through increased activation of basolateral amygdala neurons projecting to the infralimbic cortex (13,60). In addition, our previous work found that CO_2 reactivity predicted fear extinction memory and orexin activation, and orexin activation predicted fear extinction memory (18). If behavioral CO_2 reactivity is a proxy for orexin activation, its ability to predict extinction memory could reflect the degree of influence of orexinergic projections on the areas that are implicated in fear

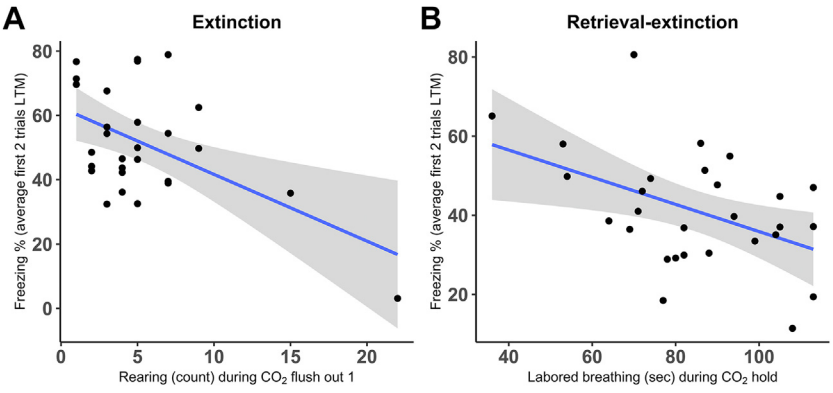

Figure 5. Relationship of the strongest predictor for the extinction group **(A)** and the retrieval-extinction group **(B)**. We used the best subset approach to linear regression to determine which subcomponents of CO_2 reactivity predict the most variance in long-term fear memory. This approach enabled us to choose a model that has the fewest predictors, the highest predictive power, and the highest degree of cross-sample replicability. The model suggests that the best predictive effect of CO_2 reactivity for extinction is from rearing during the first half of flush out, whereas for retrieval-extinction, it is from labored breathing during 25% hold. LTM, long-term memory.

expression after extinction. Our results show that CO_2 reactivity predicted a smaller portion of the variance after retrieval-extinction; as such, it is possible that the predictive effect of CO_2 reactivity is specific to extinction mechanisms. This idea is supported by our previous work showing that CO_2 reactivity did not predict variability in elevated plus maze or light/dark box behavior but did predict appetitive extinction memory (18,61). To date, no work has specifically examined the role of orexin in retrieval-extinction or reconsolidation, although CO_2 inhalation during retrieval enhances fear memory lability (62). However, this occurs only when CO_2 is inhaled during retrieval and is due to transient acidosis at amygdala neurons, so it may not be related to the mechanism of CO_2 reactivity.

One limitation of the current study is the fact that it was only conducted with male rats and did not include females. There are sex differences in fear conditioning and extinction, and females are disproportionately affected by anxiety disorders (63,64). In addition, there are sex differences in the orexin system (65,66). For the current experiment, resources precluded running an adequate number of males and females. Having now established feasibility and potential for our model, future experiments will test whether CO_2 reactivity can predict fear extinction memory in females in a large sample. In addition, we are currently conducting a clinical trial to assess whether CO_2 reactivity can predict nonresponse to exposure therapy in individuals with anxiety disorders, so we will know soon whether our findings extend to women (67).

The CO_2 challenge is safe and inexpensive to administer in humans (16,68). In humans, individuals with PTSD show impaired extinction learning, and emotional reactivity to a CO_2 challenge predicts later development of PTSD symptoms (16,69). Together with our previous work, the current study supports the idea that CO_2 reactivity could be used as a screening tool to identify patients who are likely to benefit from an extinction-based therapeutic approach and that a retrieval-extinction–based approach may be an effective alternative for those who are not (18,61,67). Ideally, such a screening tool could be used to assign patients to one treatment or another. However, our study was designed to remain consistent with our previous work and did not enable us to determine whether a CO_2 challenge administered after conditioning could differentially predict responding to extinction and retrieval-extinction. Future studies should examine whether a priori CO_2 screening for treatment assignment can be used to prevent the return of fear in a greater number of individuals.

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MR and JS were responsible for software. MR was responsible for formal analysis, writing the original draft of the manuscript, and visualization. NEK, LAA, HJL, and M-HM were responsible for investigation. NEK, LAA, JS, JAJS, MJT, HJL, and M-HM were responsible for reviewing and editing the manuscript. JS and M-HM were responsible for methodology. HJL and M-HM were responsible for conceptualization. M-HM supervised the study.

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Our data and analysis codes are made available in a University of Texas–maintained repository (https://dataverse.tdl.org/dataverse/MonfilsFearMemoryLab).

JS is employed by Aiberry. NEK is employed by Exponent. All other authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

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