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Unpredictable Mixed-Valence Outcomes Induce a Chronic and Reversible Generalized Anxiety-like Phenotype in Male Mice

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ABSTRACT

BACKGROUND: Clinical anxiety is a generalized state characterized by feelings of apprehensive expectation and is distinct from momentary responses such as fear or stress. In contrast, most laboratory tests of anxiety focus on acute responses to momentary stressors.

METHODS: Apprehensive expectation was induced by subjecting mice (for 18 days) to manipulations in which a running response (experiment 1) or a conditioned stimulus (experiment 2) were unpredictably paired with reward (food) or punishment (footshock). Before this treatment, the mice were tested in an open field and light/dark box to assess momentary responses that are asserted to reflect state anxiety. After treatment, the mice were assessed for state anxiety in an elevated plus maze, social interaction test, startle response test, intrusive object burying test, and stress-induced corticosterone elevations. In experiment 3, we treated mice similarly to experiment 1, but after mixed-valence training, some mice received either no additional training, additional mixed-valence training, or were shifted to consistent (predictable) reinforcement with food.

RESULTS: We consistently observed an increase in anxiety-like behaviors after the experience with mixed-valence unpredictable reinforcement. This generalized anxiety persisted for at least 4 weeks after the mixed-valence training and could be reversed if the mixed-valence training was followed by predictable reinforcement with food.

CONCLUSIONS: Results indicate that experience with unpredictable reward/punishment can induce a chronic state analogous to generalized anxiety that can be mitigated by exposure to stable, predictable conditions. This learned apprehension protocol provides a conceptually valid model for the study of the etiology and treatment of anxiety in laboratory animals.

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Anxiety is a pervasive and often debilitating mental disorder (1,2). A MEDLINE search for "anxiety" and "rat/mouse/mice" returns more than 33,000 studies, and the majority of reported behavioral tests are based on the concept that anxiety is a momentary state of conflict. For example, in an elevated plus maze (EPM), a mouse is motivated to explore, but is simultaneously nervous in the open, elevated environment (3). Conflict is reminiscent of the American Psychological Association's description of anxiety as "an emotion characterized by feelings of apprehensive expectation" or "worry" (4,5). However, the American Psychological Association specifies that acute responses to threat are not defining features of anxiety (6). Rather, anxiety is said to be a generalized/diffuse and persistent condition that does not necessarily have a proximate cause. The EPM and other common tests are not intended to induce a generalized condition; rather, they induce momentary conflict that is not distinguishable from transient fear or stress responsivity. While several methods induce a more chronic, generalized state, these procedures [e.g., social isolation (7,8), exposure to predator odor (9), forced swimming (10)] are based on an assumption that stress itself is sufficient to induce anxiety and thus only weakly resemble clinical descriptions of the condition. Moreover, these same procedures are commonly used to induce conditions analogous to depression, thus complicating the separation of distinct clinical disorders. All these concerns have led to substantive criticisms regarding the utility of current animal models (11,12).

In the 3 experiments described here, mice were exposed to conditions where a running response (experiment 1) or conditioned stimulus (experiment 2) was unpredictably reinforced with either food (reward) or footshock (punishment). These procedures (but not predictable food or punishment) supported the development of erratic goal-directed behavior [analogous to conflict (13)] and subsequent increases in anxiety-like behaviors. In a third experiment, this generalized anxiety was found to be persistent and reversible by exposing conflicted mice to predictable reinforcement with food. Taken together, these experiments describe an experience-

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dependent etiology of generalized anxiety and describe a procedure for its induction that resembles clinical descriptions of the disorder. These results are consistent with some contemporary theories of the etiology of clinical anxiety that suggest that worry (and the expression of anxiety) can arise from cognitive conflict (14,15).

METHODS AND MATERIALS

Animals

CD-1 outbred mice were 80 days old (young adults) at the start of the experiments. Because these were initial studies, only male mice were used [thus maximizing sample sizes and reducing the stress responses in males housed in close proximity to females (16–18)]. There is wide variability in this strain's expression of behavioral traits relevant to state anxiety (3,19–24). Animals were deprived of food during training (for details, see the Supplement).

Premanipulation Tests of State Anxiety

Experiments 1 and 2 began with 2 tests (an open field and light/dark box) to assess anxiety-related behaviors prior to any manipulation (see the Supplement for details).

Postmanipulation Tests of State Anxiety

Two days after the completion of training, mice were subjected to unique (compared with pretests) tests of anxiety-related behaviors, including an EPM, social interactions with a male conspecific mouse, intrusive object burying, startle responsivity, and corticosterone elevations in response to a mild stressor (see the Supplement for details).

Statistical Analysis

Principal component analyses (PCAs) were conducted to reduce measurements from all behavioral tests into a single variable that captured anxiety-like behaviors. From the general anxiety factor, we computed factor scores (analogous to average *z* scores across all tests) for each animal that were interpreted as being indicative of an animal's generalized anxiety (3). One-way analyses of variance were used to assess differences in group performance across the pre- and postmanipulation anxiety tests, and Tukey's comparisons were made between individual groups.

Experiment 1: Operant Conflict Training

Following the premanipulation tests, mice were pseudorandomly assigned to one of the 3 groups (n = 26/group): conflict training (CT), appetitive control (APP), or aversive control (AVR), while balancing for body weights and pretraining factor scores for anxiety levels.

A timeline and illustration of events for this experiment are provided in the Supplement. Day 6 consisted of a 5-minute acclimation period when mice were given free access to a straight alley maze (122 cm long × 8 cm wide × 15 cm wall height; black Plexiglas) that terminated in a round (18 cm) goal area that contained a food cup and electrifiable grid floor. The cup was baited with 2 pieces of inaccessible food for the duration of this study to equilibrate for smell across trials. Initial training occurred on days 7 through 10 (6 trials/day), during which animals were allowed to traverse the alley and retrieve a 14-mg Noyes food reward from the cup.

Treatments began on day 12. The CT group received randomly scheduled footshock (0.4 mA, 400 ms) upon entering the goal area on 17 of the remaining 90 trials (1 or 2 shocks per 6-trial session; ∼18% of trials) through day 30. The remaining 73 trials proceeded as described for the initial training phase, when CT group mice received a food reward. The APP group mice had access to the maze only on trials during which CT group animals received food rewards (thus serving as a predictable reward control group). AVR group animals were only trained on those trials when CT group animals received a shock (serving as a predictable punishment control group). During these trials, AVR group animals were placed directly in the goal area of the maze and given an equivalent shock.

Beginning 2 days after the completion of training, all mice were subjected to the postmanipulation tests of state anxiety.

Experiment 2: Classical Conditioning CT

Experiment 2 was conducted to assess the ability to induce generalized anxiety in mice using a procedure that was conceptually similar to but procedurally different than that described for experiment 1. Here, a tone stimulus was paired unpredictably with the delivery of either food or footshock. A timeline and illustration of events are provided in the Supplement.

Following completion of the premanipulation tests of state anxiety-like behaviors, mice were assigned to one of the 4 groups: CT, APP, AVR, and a no-treatment control (NT) (ns = 16).

This study was conducted in chambers (32 × 28 × 28 cm) containing a food hopper that delivered 14-mg Noyes pellets, speaker, house light, and electrifiable floor. All trials were controlled using custom code (https://github.com/Dylan-W-Crawford/Apprehensive_Expectation_CC.git). Each trial began with the illumination of the chambers' house light, which was extinguished at the end of each trial.

Days 6 through 10 were identical for all animals. On day 6, animals were provided 4 pairings (360-second interevent interval) of a 60 dB (above background), 30-second tone that coterminated with delivery of a 14-mg Noyes pellet. Days 7 through 10 consisted of 40-minute sessions with 8 pairing events. Intertrial intervals were randomized and averaged 300 seconds (range 200–400 seconds). During these trials, a food pellet during the conditioned stimulus was triggered by an animal's nosepoke into the food cup portal. If an animal failed to make a response within 180 seconds of audio stimulus onset, then food was delivered to the food cup.

Beginning on day 13, animals were subjected to different treatments. On each day, the CT group received unpredictable pairings of a tone with food delivery or a 0.4 mA, 500-ms footshock. The tone ceased, and food reinforcement or shock was administered following either a nosepoke into the food cup or 180 seconds following the initiation of the tone. Footshocks were delivered on 40 of the 160 (∼25%) trials. The APP group received only the tone-food pairings, while the AVR group received only the scheduled footshock pairings. In the latter case, a nosepoke terminated in shock; if no nosepoke was made, the shock was delivered at the termination of the

30-second conditioned stimulus. NT group animals were simply placed in the conditioning chamber and received no stimulus presentations.

Postmanipulation Tests. Beginning 2 days after training, all mice were subjected to postmanipulation tests of state anxiety (as described for experiment 1).

Experiment 3: Stability and Reversal of the Effects of CT

This experiment used a procedure similar to the one used in experiment 1. We were interested in the extent to which CT produced a persistent generalized state analogous to anxiety (assessed 28 days after CT). Furthermore, we assessed whether the effects of CT could be amplified with additional exposure and whether the effects of CT could be reversed by exposure to consistent (predictable) appetitive reinforcement.

Animals. Forty naïve CD-1 male mice (10/group) served in this experiment.

Procedure. A timeline and illustration of events is provided in the Supplement. All animals were initially trained to traverse the straight alley for food reinforcement. Subsequently, 3 groups of 10 mice (CT/CT, CT/NT, and CT/APP) underwent phase 1 CT for 18 days in the manner described for experiment 1. A fourth group of 10 mice (APP/APP group) received consistent food reinforcement during this phase of training. Four days after the completion of this testing, all animals were tested in the open field.

Four days after the open field test, phase 2 training began and continued for 18 days. The APP/APP group continued to receive consistent food reward. The CT/CT group continued to receive mixed-valence unpredictable reinforcement, the CT/NT group received no further alley training (i.e., remained in their home cages but were handled for an amount of time comparable to animals in other groups), and the CT/APP group received consistent food reinforcement, i.e., were shifted from mixed-valence unpredictable reinforcement to predictable food reinforcement.

Following phase 2 training, all mice were tested in an EPM, the marble-burying task, and social interaction test (male conspecific). Of particular interest was the extent to which CT promoted a persistent state of generalized anxiety (CT/NT group) and whether induced generalized anxiety could be mitigated by exposing animals trained with the conflict procedure to subsequent predictable positive-valence reinforcement (CT/APP group).

RESULTS

Experiment 1: Operant CT

Premanipulation: PCA of Tests of State Anxiety. Results from individual tests of state anxiety (in the open field and light/dark box) as well as the PCA are described in the Supplement. Performance on all measures loaded in the same direction, and a single latent variable explained 54.06% of the variance in behavior in these measures, indicating a common underlying influence (i.e., general anxiety).

Premanipulation factor scores for each animal were obtained from the PCA (lower scores indicating lower anxiety-like behavior), and scores for 77 animals (1 animal in the APP group was eliminated owing to illness) ranged from −1.53 (lowest anxiety-like behavior) to +2.60 (highest anxiety-like behavior). No initial differences were observed across CT (mean = 0.01), APP (mean = −0.02), and AVR (mean = 0.01) groups ($F_{92,750}$ = 0.01, p = .99).

Manipulation: Conflict Exposure in a Straight Alley. Statistical analyses are provided in the Supplement. For the first 24 trials, all animals were reinforced with food after entering the goal box, and no differences were observed across groups in the latency to enter the goal box. Subsequently, mice were differentially treated, i.e., the APP group continued to be consistently reinforced with food, the CT group was reinforced unpredictably with either food or footshock, and the AVR group was consistently administered footshock in the goalbox. By the end of this training, the CT group exhibited longer latencies to enter the goal area than the APP group (see Figure 1 for representative path tracings and group performance). Qualitatively, these animals could be described as exhibiting what Miller (13) has described as nervousness indicative of an underlying approach-avoidance conflict.

Postmanipulation Tests of State Anxiety Responses. After training, mice were assessed on tests used to infer anxiety-like behavior, including the EPM, social interactions with a male conspecific, acoustic startle responding, social interactions with a female conspecific, intrusive marble burying, and corticosterone elevations in response to a mild stressor. Statistical analyses for individual tests are described in the Supplement. In short, compared with both control conditions, CT induced significant decreases in the time spent in the open arms of the EPM, increased startle responsivity, increased avoidance of conspecific mice, increased novel object burying, and increased stress-induced corticosterone elevations. Similar tendencies were observed in other tests.

Postmanipulation: PCA. Results from a PCA including all 6 measures of anxiety-like behaviors are provided in the Supplement. A single factor explained 33.06% of the variance in behavior across these measures, suggesting a general influence indicative of anxiety. Postmanipulation factor scores (indicative of animals' aggregate performance across all measures of anxiety) were obtained from the PCA. Factor scores for all 77 animals ranged from −2.11 (lowest anxiety-like behavior) to +2.39 (highest anxiety-like behavior). There was a significant effect of treatment on postmanipulation factor scores ($F_{2,75}$ = 11.24, $p < .01$) (see Figure 2). Tukey's post hoc analysis showed that CT ($mean_{CT}$ = 0.61) resulted in significantly higher postmanipulation scores than APP ($mean_{APP}$ = −0.33, $p < .01$) or AVR ($mean_{AVR}$ = −0.35, $p < .01$) training. There was no significant difference between the average postmanipulation scores of the APP and AVR groups (p = .99). These results indicate that mixed-valence unpredictable reinforcement induces an increase in anxiety-like behaviors compared with predictable positive-valence reinforcement or predictable negative-valence reinforcement.

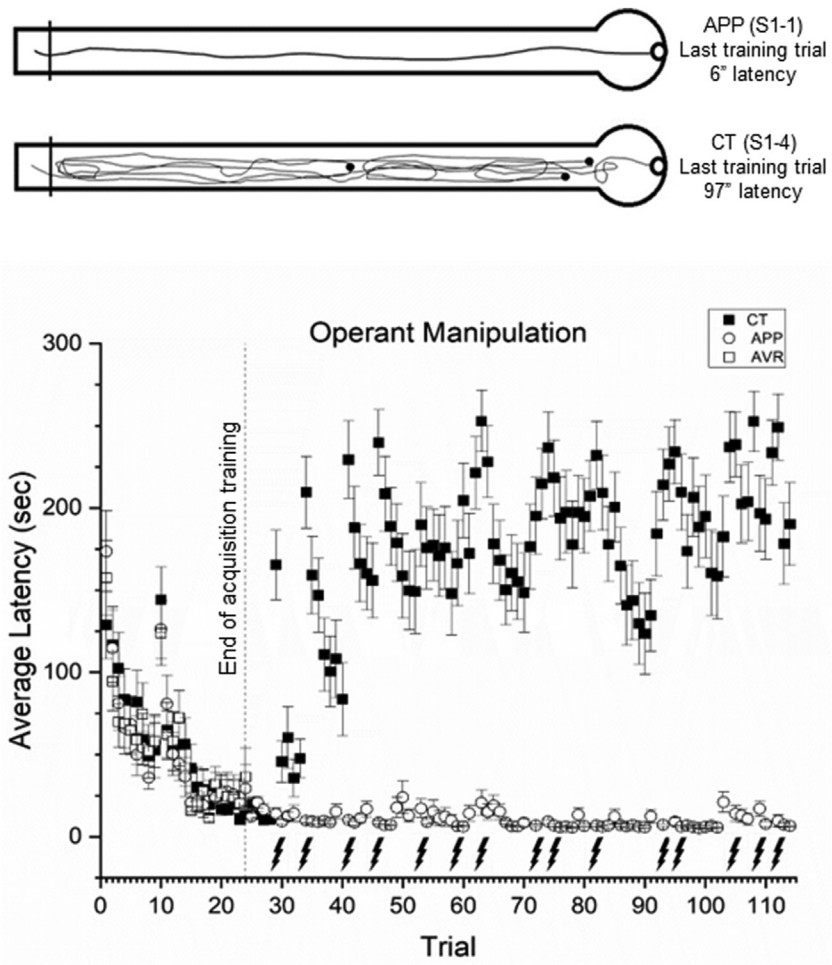

Figure 1. Tracings of running responses at the end of training from 2 individual animals are provided in the upper panel. Subject 1-1 was consistently reinforced with food (APP group), whereas subject 1-4 was randomly reinforced with either food or footshock (CT group). Dots indicate periods of immobility that lasted for 3 seconds or longer. The lower graph illustrates average latencies to enter the goal box separately by group. Trials 1 through 24 (left of dotted line) denote initial training trials. Trials 25 through 114 (differential training) are illustrated to the right of the dotted line. The APP group maintained fast and low variability running latencies, while the latencies and variability both increased in the CT group. The AVR group (which received only shock) did not traverse the alley during this phase of training. Lightning bolts above trial numbers denote trials on which footshocks were delivered to the CT and AVR animals. Brackets represent SEMs. APP, appetitive control; AVR, aversive control; CT, conflict training.

This was not simply a consequence of experience with aversive stimulation because it was not observed in animals that experienced predictable shock.

Experiment 2: Classical Conditioning CT

Premanipulation: PCA of State Anxiety-like Responses. A PCA of all 4 measures of anxiety-like behaviors from the open field and light/dark box are provided in the Supplement. A single latent variable analogous to general anxiety explained 33.87% of the variance in behavior across these measures.

Premanipulation factor scores (indicative of animals' general anxiety-like behavior) were obtained from the PCA. Scores for all 64 animals ranged from −3.75 (lowest anxiety-like behavior) to +2.48 (highest anxiety-like behavior). No initial differences in predisposition to anxiety-like behaviors existed between the CT (mean = 0.01), APP (mean = 0.02), AVR (mean = −0.06), and NT (mean = 0.04) groups ($F_{3,60}$ = 0.03, p = .99).

Manipulation: Conflict Exposure in Conditioning Chambers. Nosepoking throughout training is illustrated in Figure 3. Statistical analyses are provided in the Supplement. For the first 20 trials, all animals were exposed to pairing events where a tone signaled an opportunity to nosepoke for food, and no differences were observed across groups in the latency to nosepoke during the final training trial. Subsequently, mice were differentially treated, i.e., the APP group continued to be consistently reinforced with food following nosepokes, the CT group was reinforced unpredictably with either food or footshock, the AVR group was consistently administered footshock, and the NT group received no training. By the end of this training, the CT and AVR group mice took significantly longer to nosepoke than the APP group mice.

Postmanipulation Tests of State Anxiety Responses. After treatments, mice were assessed again on tests of state anxiety. The results of individual tests are provided in the Supplement. In short, compared with the control conditions, CT induced a significant increase in behaviors indicative of anxiety in the social interaction test (female conspecific), the startle test, intrusive object burying, and stress-induced corticosterone levels. Similar tendencies were observed in other tests.

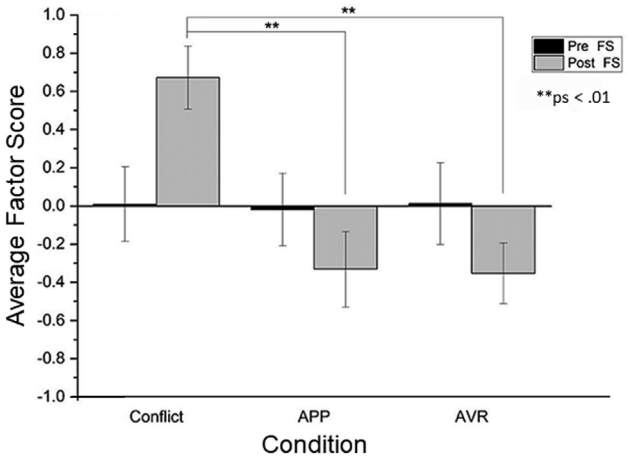

Figure 2. Average pre- and postmanipulation FSs (black and gray, respectively) for the conflict training, APP, and AVR groups. High factor scores represent increased levels of general anxiety-like behaviors. The average postmanipulation factor scores of the conflict training group increased significantly relative to their premanipulation scores and were significantly higher than the postmanipulation FSs of the APP and AVR groups. Brackets represent SEMs. Note: FSs (like *z* scores) necessarily have a mean of 0; thus, group differences represent relative rather than absolute changes. APP, appetitive control; AVR, aversive control; FS, factor score.

Postmanipulation: PCA. A PCA found that 42% of the variance across postmanipulation tests was accounted for by a single factor (indicative of general anxiety) (see the Supplement). Postmanipulation factor scores (indicative of animals' general anxiety) were obtained from the PCA. Factor scores for all 64 animals ranged from −1.13 (lowest general anxiety-like behavior) to +2.38 (highest general anxiety-like behavior). A significant effect of training on average post-manipulation factor scores was observed ($F_{3,60}$ = 15.95, $p <$ .01) (see Figure 4). A Tukey's post hoc analysis showed that CT group animals had higher average factor scores (mean = 1.60) than the APP (mean = −0.28, $p <$ .01), AVR (mean = −0.64, $p <$ .01), and NT (mean = −0.68, $p <$ .01) group animals. As in experiment 1, these results demonstrate that the experience of mixed-valence unpredictable reinforcement (food or shock) was sufficient to induce an increase in general anxiety-like behavior.

Experiment 3: Stability and Reversal of the Effects of CT

During initial training, all groups exhibited progressively shorter latencies to enter the goal box and reached similar levels of asymptotic performance. In phase 1 of differential training, the APP/APP group continued to approach the goal box with short latencies, while the CT/NT, CT/APP, and CT/CT groups (which received mixed-valence reinforcement in this phase) exhibited long and highly variable latencies (Figure 5). Upon completion of phase 1 training, all animals were assessed in an open field, where 2 measures were obtained: the number of grids crossed and the percentage of crossings in unwalled grids. A significant difference in the percentage of crossings in unwalled grids was observed across groups ($F_{3,36}$ = 20.26, $p <$ .001). Comparisons of groups indicated that the APP/APP group made significantly more crossings in open grids than the CT/CT, CT/APP, or CT/NT groups ($F \geq 3.29$, $ps <$ .05), indicating that CT training promoted a reluctance to venture into the open

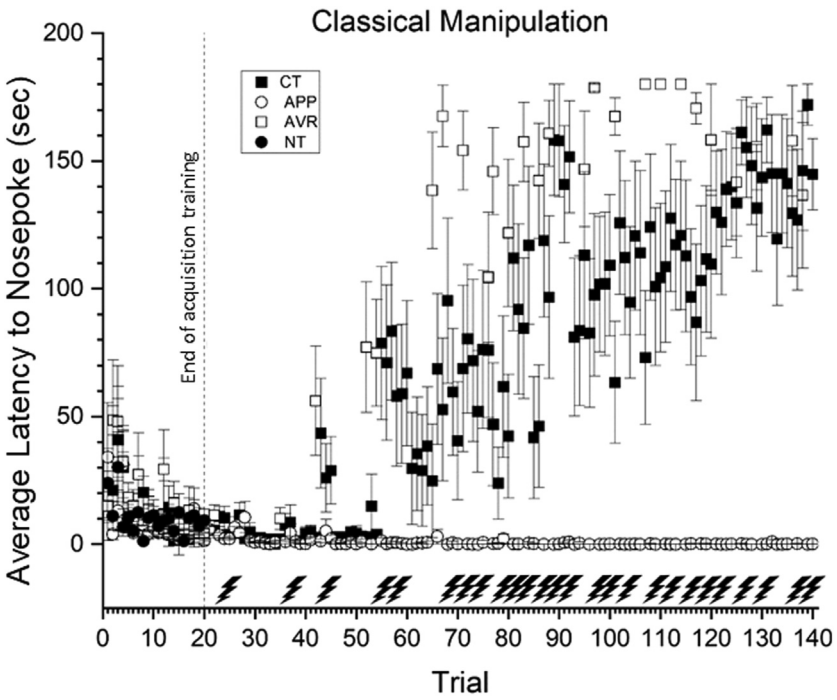

Figure 3. Average latencies, by group, to nosepoke in the food dispenser during the acquisition and differential manipulation phase of experiment 2. Trials 1 through 20 (left of dotted line) denote initial training trials, and trials 20 through 140 (right of dotted line) are the latencies during differential training. Notably, the APP group (consistently reinforced) developed short latencies that persisted across the 140 trials, while the AVR group began to exhibit long latencies (often not responding) when the nosepoke response was consistently reinforced with footshock. The CT group (which received unpredictable reinforcement with food or shock) developed long and highly variable nosepoke responding after the initial acquisition trials. The NT group did not receive any training after the completion of initial acquisition. Lightning bolts denote trials on which footshocks were delivered to the CT and AVR groups. Brackets represent SEM. APP, appetitive control; AVR, aversive control; CT, conflict training; NT, no treatment.

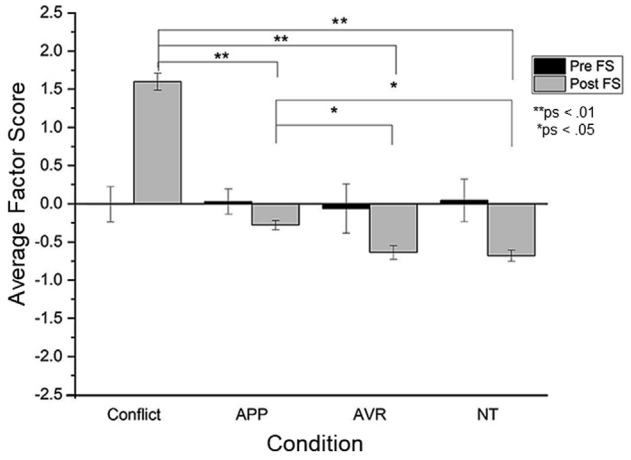

Figure 4. Average pre- and postmanipulation factor scores (black and gray, respectively) for the conflict training, APP, AVR, and NT groups. High FSs represent increased levels of anxiety-like behaviors. A significant increase in the average FSs in the conflict training group between pre- and postmanipulation time points was observed. There was also a significant increase in anxiety-like behaviors between the conflict training group and all controls at the postmanipulation time point. Brackets represent standard error. Note: FSs (like *z* scores) necessarily have a mean of 0; thus, group differences represent relative rather than absolute changes. APP, appetitive control; AVR, aversive control; FS, factor score; NT, no treatment.

areas of the field. Although the APP/APP group tended to exhibit more total activity, this tendency did not reach significance ($F_{3,36}$ = 2.43, $p < .09$). An illustration of all results is provided in the Supplement.

After the open field, the mice began 18 days of phase 2 training. Mice that received consistent appetitive reinforcement (APP/APP group) continued to exhibit fast, low variability running responses. In contrast, mice that were exposed to unpredictable mixed-valence reinforcement in both phases of training (CT/CT group) exhibited long and highly variable latencies. Importantly, mice that were exposed to mixed-valence reinforcement in phase 1 and predictable appetitive reinforcement in phase 2 (CT/APP group) exhibited a recovery of responding in phase 2 such that their performance was similar to that of animals that had been consistently/predictably reinforced with food. Thus, at this initial behavioral level, the direct effect of CT on operant responding was in fact reversed if conditions were shifted from unpredictable mixed-valence reinforcement to predictable appetitive reinforcement. All results are illustrated in Figure 5.

Upon completion of phase 2 training, mice were tested in an EPM, for intrusive object (marble) burying, and for social interactions. An illustration of all results is provided in the Supplement.

Three measures were recorded in the EPM: total arm entries, percentage of entries into open arms, and percentage of time in open arms. Total arm entries and number of open arm entries were added to this analysis to provide for additional resolution of the principal component derived from the factor analysis. Although a tendency for a group difference was observed in the total arm entries, this did not reach statistical significance ($F_{3,35}$ = 2.32, $p < .10$). However, the groups did differ in the percentage of entries into open arms ($F_{3,35}$ = 6.98, $p < .001$) and the percentage of time spent in open arms ($F_{3,35}$ = 5.11, $p < .01$). For the percentage of open arm entries, the APP/APP and CT/APP groups did not differ (p = .97), but each of these groups differed from the CT/CT and CT/NT groups ($ps < .05$). Percentage of time spent in open arms followed a similar pattern, where the APP/APP and CT/APP groups did not differ (p = .82), but each of these groups differed from the CT/CT and CT/NT groups ($ps < .02$).

For marble burying, each mouse received a score ranging from 0 to 12, where a score of 0 indicated no attempt to bury

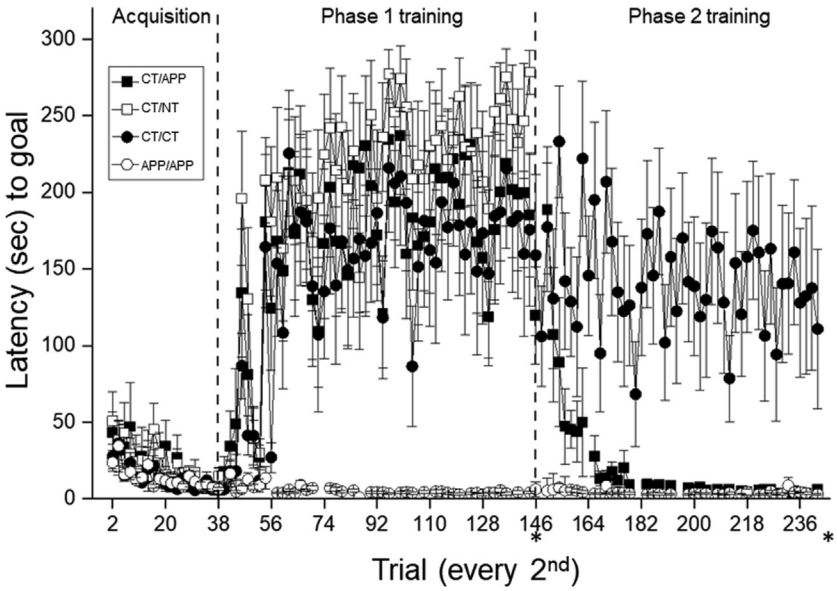

Figure 5. All animals were trained to approach the goal box to obtain food (appetitive) reinforcement during the acquisition phase of training. Subsequently (phase 1), 3 groups (CT/APP, CT/NT, and CT/CT) received mixed-valence unpredictable (food or shock) reinforcement, while the APP/APP group continued to be reinforced with food. In phase 2, the APP/APP group continued to receive consistent reinforcement with food, the CT/NT group received no treatment, and the CT/APP group were shifted to consistent (predictable) reinforcement with food. Notably, the shift from unpredictable mixed-valence reinforcement to predictable reinforcement with food reversed the effects (long and variable running latencies) of the previous mixed-valence unpredictable reinforcement. All animals were administered tests of state anxiety at the end of phase 1 training* and again after phase 2 training*. For better visualization, only every second trial is illustrated. Brackets indicate SEMs. APP, appetitive control; CT, conflict training; NT, no treatment.

the intrusive marbles, whereas a score of 12 indicated complete hiding of all marbles. Analysis of variance revealed a significant difference between groups ($F_{3,35}$ = 4.03, $p < .02$). Comparisons of groups found that the APP/APP and CT/APP groups did not differ (p = .97). Likewise, the CT/NT and CT/CT groups did not differ (p = .82). The CT/APP group differed from the CT/CT group (p = .02), but other nominal differences did not reach significance.

Finally, all mice were assessed for social interactions with a male conspecific. The groups differed in the amount of time spent in proximity to the conspecific ($F_{3,34}$ = 13.94, $p < .001$). The APP/APP and CT/APP groups did not differ (p = .84), and the CT/NT and CT/CT groups did not differ (p = .98), but each of the latter 2 groups differed from each of the former 2 groups (ps < .001).

All post-phase 2 measures of state anxiety were subjected to a PCA in which a principal factor accounted for 52% of the variance across tests. All test scores loaded in a similar direction on the principal factor, indicating that they were all representative of a common underlying influence (see the Supplement). A factor score was computed for every animal, and these scores ranged from −1.95 to +1.80, where higher values were representative of higher general anxiety. These scores were separated by treatment group (see Figure 6) and were found to differ significantly ($F_{3,34}$ = 26.41, $p < .001$). Tukey's comparisons determined that the APP/APP and CT/APP groups did not differ (p = .97), and the CT/NT and CT/CT groups did not differ (p = .99). However, the CT/NT and CT/CT groups differed from both the APP/APP and CT/APP groups (ps < .01).

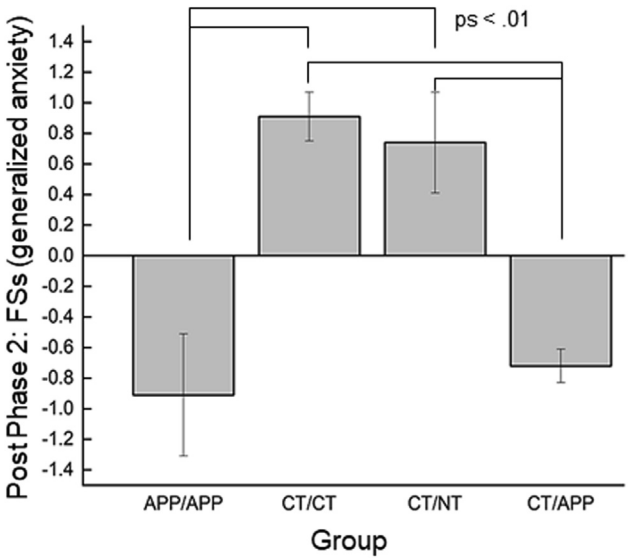

Figure 6. Average postmanipulation (phase 2) FSs for the APP/APP, CT/CT, CT/NT, and CT/APP groups. Higher FSs represent increased levels of anxiety-like behaviors. Brackets indicate SEMs. Note: FSs (like z scores) necessarily have a mean score of 0; thus, group differences represent relative rather than absolute changes. APP, appetitive control; CT, conflict training; FS, factor score; NT, no treatment.

DISCUSSION

In 2 experiments, mice were exposed to unpredictable delivery of either appetitive (food) or aversive (footshock) reinforcement, promoting an approach-avoidance conflict (13,25) or what descriptions of clinical anxiety describe as "apprehensive expectation." This CT induced long-latency and highly variable approaches to the goal areas and promoted significant increases in anxiety-like behaviors (including stress-induced corticosterone elevations) compared with controls that received predictable appetitive or aversive reinforcement. These results suggest that exposure to mixed-valence unpredictable reinforcement (but not simply predictable aversive reinforcement) was sufficient to induce a generalized anxiety-like state.

In a third experiment, we observed that the generalized anxiety resulting from mixed-valence unpredictable reinforcement was chronic in nature (lasting at least several weeks) and could be reversed by exposing animals to consistent (predictable) positive-valence reinforcement. As with experiments 1 and 2, this experiment indicates that a distrust of the environment induced by unpredictable delivery of reward or punishment induces a condition analogous to anxiety. The results of experiment 3 further indicate that this distrust can be overcome with experience with a more stable and predictable environment, suggesting possible treatment strategies to mitigate the anxiety that can arise from prior experience.

Anxiety disorders have a complex etiology (26–29). However, learning theories suggest that some anxiety disorders can develop through experience (30–32). The current results are consistent with these theories because conflict involves the formation of associations between a stimulus or response and unpredictable reward or punishment, inducing a state of apprehension. Over time, this cognitive burden promoted a chronic state of uncertainty that was subsequently expressed in novel contexts weeks after the exposure.

Conclusions

The current conclusions are limited by the use of only male mice given that among humans, females exhibit a higher incidence of anxiety disorders than males (33). As an initial assessment, to maximize sample sizes, it was reasonable to use only male mice in the current study. In ongoing studies, we have begun to assess any differential susceptibility of males and females to conflict-induced induction of generalized anxiety.

We have reported (34) that long-term memory of conflict favors the expression of fear memories, i.e., when tested 20 days after the conclusion of mixed-valence reinforcement, mice demonstrated an increase in the expression of fear when approaching the goal area (with its mixed-valence history of reinforcement). This increase in fear could reflect the impact of negativity biases on the expression of long-term memories (35–37). In combination with the current work, these results provide good evidence for the role of learned apprehensive expectation in the etiology of anxiety and possibly post-traumatic stress disorders.

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ARTICLE INFORMATION

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