Physiology & Behavior 221 (2020) 112900

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Effects of chronic stress on reinstatement of palatable food seeking: Sex differences and relationship to trait anxiety

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ARTICLE INFO

Keywords:
Anxiety
Chronic stress
Food seeking
Reinstatement
Relapse
Sex differences

ABSTRACT

Previous research in our lab has established a causal role for chronic stress exposure in subsequent increases in relapse-like behaviors in male rats with a history of palatable food self-administration. Given that many of the neurobehavioral consequences of stress are sex dependent, we aimed to determine whether sex differences exist with regard to the effects of chronic stress on relapse. Additionally, because high trait anxiety confers vulnerability to stress-related disorders, we examined whether individual differences in trait anxiety were related to differences in relapse-like behavior after chronic stress exposure. Following elevated plus maze testing for classification into high- or low-anxiety phenotypes, male and female rats responded for highly palatable food pellets. During subsequent extinction training, stress was manipulated (0 or 90 min restraint/day for 7 days). Rats were then tested for cue- and pellet priming-induced reinstatement of palatable food seeking. Results showed that female rats displayed higher levels of responding during cue-induced reinstatement tests compared to males, and that a history of chronic stress caused an attenuation of cue-induced reinstatement in female, but not male, rats. Regarding pellet priming-induced reinstatement, there was a three-way interaction such that neither stress history nor anxiety phenotype was related to reinstatement in females, but a history of stress in males caused increased and decreased responding in low- and high-anxiety rats, respectively. These results suggest that biological sex and trait anxiety level may help to explain differences in vulnerability to relapse among individuals exposed to chronic stress. Such information may be useful in designing more personalized and effective treatments for obesity and eating disorders.

1. Introduction

Overconsumption of unhealthy foods and its related consequences, such as obesity, have become endemic in the developed, and now developing, world. Worldwide, over 2.1 billion people are classified as overweight or obese. As the fifth-leading cause of death, overweight/obesity accounts for 3.4 million deaths per year [1] and has been recognized as a public health crisis for decades [2]. Unfortunately, dietary and behavioral treatments for obesity have been associated with poor outcomes and high rates of relapse to former unhealthy eating habits [3–5]. These studies of treatment outcomes reveal that individuals undergoing treatment are likely to later regain all, or even more, weight than was lost at the outset. The development of more effective treatments requires a clearer understanding of the environmental, neural, and phenotypic determinants of craving and relapse.

Stress is a factor that has been shown to drive food craving and relapse both in the clinical [6,7] and in the pre-clinical [8] literature. Most studies, however, have focused on acute stress as a driver of relapse, even though it is recognized that chronic stress is associated with obesity in humans [9–11]. Moreover, there is evidence that food craving, which is a predictor of eating and weight gain [12,13], mediates the relationship between chronic stress and body mass index [14]. Indeed, cravings typically result in the consumption of highly palatable, unhealthy foods [15]. In our lab, we have investigated the relationship between chronic stress and relapse-like behavior in rats using the reinstatement model [8] and related models of relapse [16] that define craving and relapse as the number of unreinforced responses performed following extinction of, or abstinence from, palatable food self-administration. This responding can be triggered by response-contingent presentation of food-conditioned stimuli, non-contingent delivery of the primary reinforcer (i.e., palatable food), or an acute stressor. Our studies have shown that exposure to chronic stress after self-administration of palatable food results in increased responding during relapse tests under a variety of, but not all, conditions, and that this vulnerability endures up to 1 week following the termination of stress [17–19]. We also found that systemic blockade of dopamine D_1-like

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https://doi.org/10.1016/j.physbeh.2020.112900
Received 17 January 2020; Received in revised form 20 March 2020; Accepted 30 March 2020
Available online 04 April 2020
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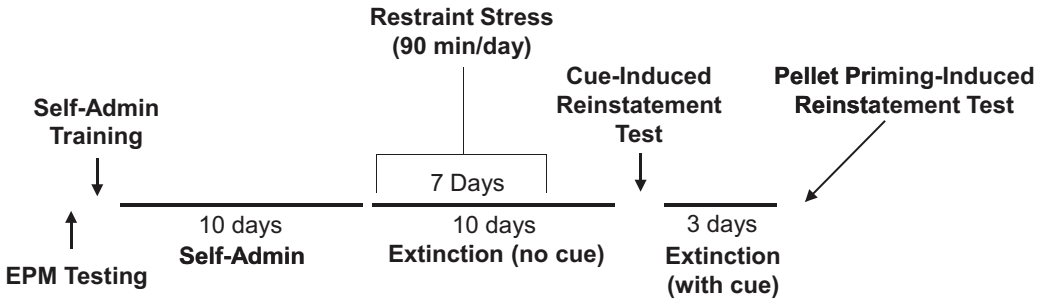

Fig. 1.. Schematic representation of the experimental design.

receptors during the stress attenuates the subsequent effects of stress on relapse in most cases, suggesting an important role for dopamine in chronic stress-potentiated relapse.

Although our work suggests that chronic stress is an important factor modulating relapse to palatable food seeking, there are other factors related to obesity and/or dietary relapse reported in the clinical literature. One important factor, which is often overlooked in the preclinical literature is biological sex. Indeed, female biological sex doubles the chance of becoming overweight [20]. Correspondingly, the proportion of women who seek dietary treatment is twice that of men [21,22], even though women typically experience worse treatment outcomes [23,24]. These sex differences are likely attributable, in part, to craving-related differences between men and women. For example, women have greater cravings for sweet foods [25,26], have more intense and frequent cravings [26–29], and have more difficulty resisting cravings [28,30]. These craving-related sex differences correspond to increased neural activity in response to food cues in women compared to men [31,32]. Thus, a main goal in the present study was to determine whether sex differences exist with regard to the effects of chronic stress on reinstatement of palatable food seeking. This is especially relevant given evidence that chronic stress induces sex-dependent adaptations in the medial prefrontal cortex [33,34], a brain region driving relapse behavior [35,36].

Another factor that may contribute to relapse is anxiety. The α-2 adrenoceptor antagonist yohimbine, which induces high anxiety in both humans and laboratory animals [37,38], triggers reinstatement of palatable food seeking in rats [39,40]. Moreover, we found that a history of chronic exposure to yohimbine predisposes animals to greater reinstatement of food seeking primed by acute yohimbine administration or re-exposure to palatable food [17,18]. These findings are supported by the clinical literature, which suggests a positive association between anxiety disorders and obesity [41,42]. Thus, a second goal in the present study was to determine whether differences in trait anxiety are related to differences in reinstatement of palatable food seeking. Indeed, studies in humans have shown that high trait anxiety is predictive of sweet food craving [43] and emotional eating [44]. Further, because of the neurobiological links between stress and anxiety [45], and because high trait anxiety confers vulnerability to stress-related psychopathologies such as anxiety disorders and depression [46,47], we hypothesized that the effect of chronic stress on reinstatement would depend on rats' trait anxiety levels.

2. Methods

2.1. Subjects and apparatus

We used experimentally naïve male and female, Sprague–Dawley rats (Envigo; total $n = 38$ of each sex) weighing 275–330 g (age ~10 weeks) and 200–240 g (age ~11 weeks) (males and females, respectively) at the commencement of procedures. Rats were housed individually under standard laboratory conditions (12-h light cycle from 7:00 AM to 7:00 PM) with ad libitum access to standard chow and water in their home cages and were weighed every other day. All testing was conducted between 7:00 AM and 7:00 PM and occurred in standard modular operant conditioning chambers (Coulbourn Instruments, Whitehall, PA) that were housed in sound-attenuating, ventilated cubicles and connected to a PC with the Graphic State 4 software interface system (Coulbourn Instruments). Each chamber was equipped with an active and an inactive response lever. Responses on the inactive lever were recorded, but had no programmed consequences. Chambers also included a house light, a row of multicolored LED cue lamps (above active lever), a tone generator, and a food tray (between the two response levers). All procedures were in compliance with NIH guidelines and were approved by the Bloomsburg University Institutional Animal Care and Use Committee. We excluded a total of six rats due to failure to acquire self-administration ($n = 1$ of each sex), a fall off of the elevated plus maze (EPM; $n = 1$ of each sex), or extinction responding that was > 3 standard deviations from the group mean on the day before cue-induced reinstatement testing ($n = 2$ females).

2.2. EPM testing and anxiety classification

The study began with EPM testing. The EPM (San Diego Instruments, San Diego, CA) was black, made of ABS plastic, and consisted of two open arms (width x length = 110.5 × 10.2 cm with 0.35 cm lip) and two closed arms (width x length x wall height = 110.5 × 10.2 × 30.5 cm). The arms were elevated 49.5 cm from the ground. Rats were taken from their cage and placed at the junction of the four arms facing the open arm opposite the experimenter. Behavior was recorded for 5 min and analyzed using Any-Maze behavioral tracking software (Stoelting, Wood Dale, IL). The arms were cleaned with 70% ethanol and thoroughly dried between animals. *K*-means clustering [48] based on proportion of time spent on open arms was used as an unbiased method to categorize rats into low anxiety (LA) and high anxiety (HA) phenotypes. This analysis was performed separately for male and female data. See Fig. 1 for schematic of experimental design.

2.3. Palatable food self-administration

Following plus maze testing, rats were trained to press the lever for food reinforcers contingent upon a fixed-ratio (FR) − 1 schedule of reinforcement over the course of 1–2 days (this training period ended when 80 reinforcers were earned). Following initial training, rats responded on an FR-1 schedule during daily 2-h sessions for 10 days. Each lever press resulted in delivery of a 45-mg food pellet containing 12.7% fat, 66.7% carbohydrate, and 20.6% protein (Catalog # 1,811,155, TestDiet). This pellet type was chosen based on pellet preference tests conducted by Pickens et al. [49], in which it was determined to be the most preferred pellet. Delivery of the pellet was accompanied by a tone + flashing cue light conditioned stimulus (CS) presented for 5 s, which was followed by a 20-s time-out period signaled by illumination of the house light.

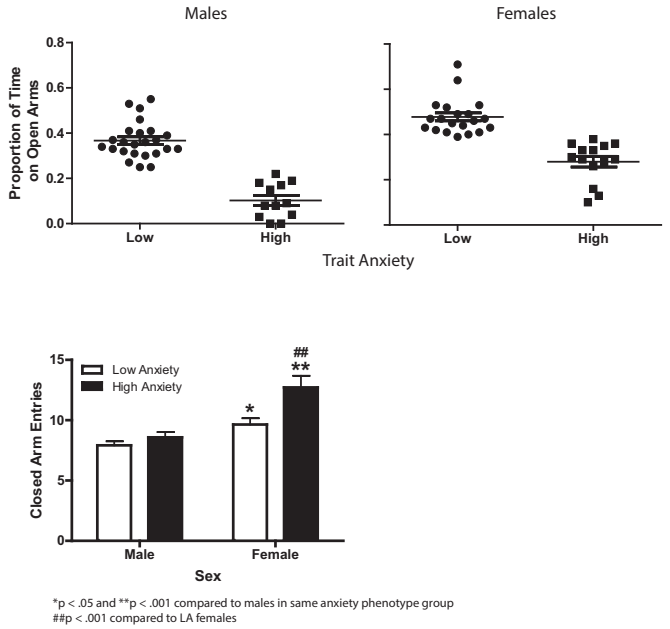

Fig. 2.. EPM behavior in male and female rats. (Top) Proportion of time spent on open arms of EPM in individual rats (± SEM) classified as LA and HA phenotype. (Bottom) Number of closed arm entries (+ SEM) in LA and HA rats.

2.4. Extinction and chronic stress treatment

On the day following the last self-administration session, daily 2-h extinction sessions began and continued for 10 days. During the extinction sessions, responses were recorded but had no programmed consequences (i.e., no CS or pellets). Beginning on the first day of extinction, half of the rats in each anxiety phenotype group were randomly assigned to receive daily stress treatment. Each day, beginning approximately 1-h after daily extinction sessions, stressed rats were placed in plastic semi-cylindrical restrainers (8.25 cm diameter x 20.32 cm length; Braintree Scientific) in an isolated room separate from other rats for 90 min for 7 consecutive days. Chronic restraint stress is a well-established procedure that produces significant increases in plasma corticosterone levels [50,51]. Unstressed rats were handled for 5 min and then returned to their home cage.

2.5. Cue-induced reinstatement testing

Following the extinction sessions, animals underwent 1 day of 2-h cue-induced reinstatement sessions. Sessions began with one non-contingent CS presentation; during the remainder of the session, conditions were identical to those of self-administration training, except that lever presses did not lead to pellet delivery.

2.6. Pellet priming-induced reinstatement testing

To extinguish lever pressing before pellet priming-induced reinstatement sessions, rats underwent three daily sessions of extinction training (2-h, with cue) that were identical to self-administration sessions, except that lever presses did not lead to pellet delivery. Next, animals underwent within-session pellet priming-induced reinstatement testing. The testing consisted of four consecutive 1-h sessions in which the conditions were identical to the previous three extinction sessions except that two and four non-contingent pellets were delivered within the first minute of sessions 3 and 4, respectively. Session 2 served as the 0-pellet baseline. Data from session 1 were not used for the pellet priming analysis. This within-session procedure is based on previous studies with cocaine and pellet priming [52–56].

2.7. Statistical analyses

Along with *K*-means clustering analysis to determine anxiety phenotype groups (see above), proportion of time spent on open arms of the EPM was analyzed with an independent-measures *t*-test with sex as the factor. A factorial ANOVA also was used to analyze closed arm entries with sex and anxiety phenotype (LA or HA) as between-subjects factors. During the self-administration phase, lever responses, pellets earned, and pellets earned/body weight (mg/kg) were used as dependent variables. These data were analyzed using mixed factorial ANOVAs with the within-subjects factors of self-administration day and lever (active and inactive), and the between-subjects factors of sex and anxiety phenotype. During the extinction phase, the dependent variable was lever responses. These data were analyzed using a mixed factorial ANOVA with the within-subjects factors of extinction day and lever, and the between-subjects factors of sex, anxiety phenotype, and chronic treatment (stressed or unstressed). Change in body weight during chronic treatment also was used as a dependent variable. These data were analyzed using a mixed factorial ANOVA with the within-subjects factor of treatment day and the between-subjects factors of sex, anxiety phenotype, and stress condition. For reinstatement testing, the dependent variable was lever responding. These data were analyzed using mixed factorial ANOVAs with the within-subjects factors of lever and either session (cue-induced reinstatement session and preceding extinction session) or number of pellets (0, 2, and 4; for pellet priming-induced reinstatement), and the between-subjects factors of sex, stress condition, and anxiety phenotype. Because the factorial ANOVAs resulted in multiple main and interaction effects, we report only significant effects that are important for interpretation. All ANOVAs were followed by Bonferroni post-tests for multiple comparisons.

3. Results

3.1. EPM behavior and anxiety phenotypes

Overall, female rats spent more time on the open arms of the EPM than males [$t(68) = 3.53$, $p = .001$, two-tailed]. Based on the *K*-means clustering analysis, the majority of both male and female rats ($n = 24$ of 36 [66.67%] and 20 of 34 [58.82%], respectively) were classified as LA phenotype. For males, the mean ± SEM percentage of time spent on the open arms was 36.71 ± 1.65% and 10.25 ± 2.23% for the LA and HA groups, respectively. For females, the mean ± SEM percentage of time spent on the open arms of the EPM was 47.80 ± 1.79% and 28.00 ± 2.38% for the LA and HA groups, respectively (see Fig. 2, Top). Regarding the number of entries into the closed arms, indicative of non-specific locomotor activity, females made more entries than males [main effect of sex; $F(1, 66) = 25.57$, $p < .001$]. There also was a sex x anxiety phenotype interaction [$F(1, 66) = 4.28$, $p = .043$] in that female, but not male, rats in the HA group made more entries compared with those in the LA group (see Fig. 2, Bottom]. Finally, mean weights of the LA and HA groups were not significantly different for either male (mean ± SEM = 312.75 ± 2.16 and 312.92 ± 2.01 for LA and HA, respectively) or female (mean ± SEM = 229.35 ± 1.90 and 225.07 ± 2.26 for LA and HA, respectively) rats.

3.2. Palatable food self-administration

As shown in Fig. 3, although the number of pellets earned was similar for males and females, both lever pressing and pellets earned per body weight were greater in females than males [main effect of sex for lever pressing and pellets earned/body weight; $F(1, 66) = 4.38$, $p = .040$, and $F(1, 66) = 43.59$, $p < .001$, respectively]. Anxiety phenotype, on the other hand, had no significant influence on any dependent measure for either sex during self-administration. Inactive lever responding was very low overall [main effect of lever; $F(1, 66) = 645.06$, $p < .001$].

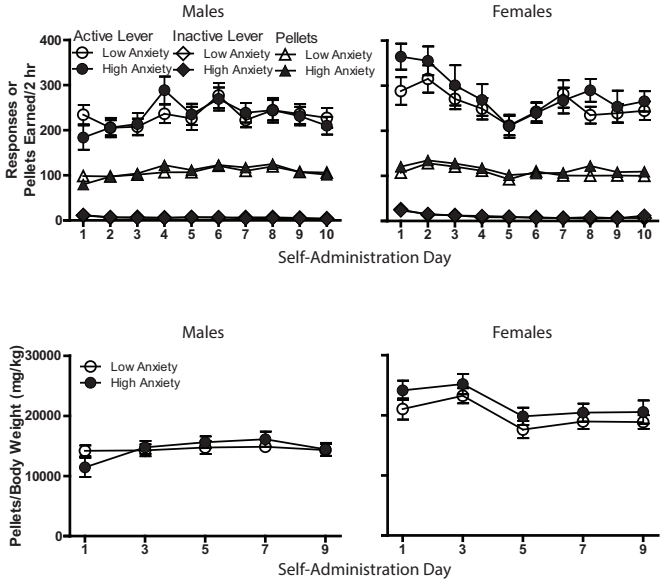

Fig. 3.. Palatable food-reinforced responding and pellets earned per body weight in male and female rats. (Top) Mean (± SEM) active and inactive lever responses and pellets earned across 10 days of 2-h food self-administration sessions in rats classified as LA and HA phenotype. Rats responded on an FR-1 schedule of reinforcement, and delivery of the pellet was accompanied by a tone + flashing cue light CS presented for 5 s, which was followed by a 20-s time-out period signaled by illumination of the house light. (Bottom) Mean (± SEM) adjusted pellets earned per body weight. Points without error bars indicate the SEM is too small to illustrate.

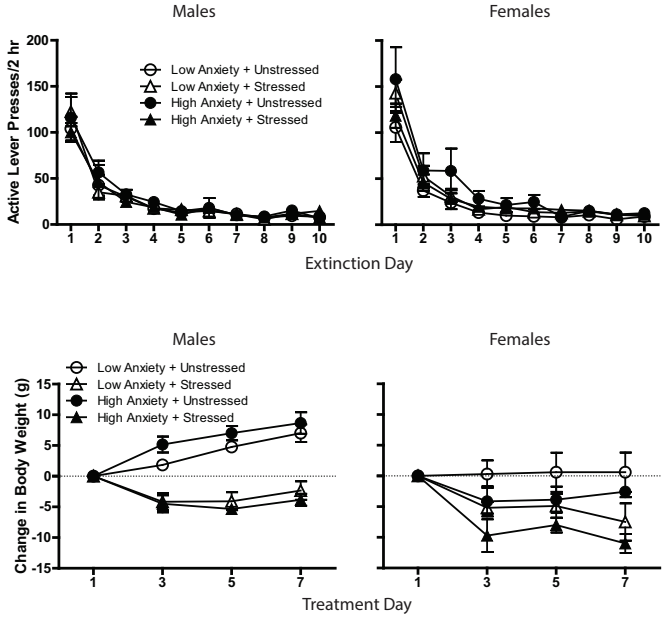

Fig. 4. Extinction of food-reinforced responding and change in body weight during chronic treatment in male and female rats. (Top) Extinction of food-reinforced responding: mean (± SEM) responses on the previously active lever. Responses had no programmed consequences (i.e., no CS or pellets). Beginning on the first day of extinction, half of the rats in each anxiety phenotype group were randomly assigned to receive daily stress treatment (~ 1-h after extinction sessions) for 7 consecutive days. Unstressed rats were handled for 5 min and then returned to their home cage. (Bottom) Mean (± SEM) weight change in chronically stressed and unstressed rats in LA and HA phenotype groups. Points without error bars indicate the SEM is too small to illustrate.

3.3. Extinction responding and body weight during chronic stress treatment

As shown in Fig. 4, Top, extinction responding was similar for males and females and decreased over days [main effect of extinction day, $F(9, 558) = 165.25$, $p < .001$]. Overall active, but not inactive, lever responding, however, was highest in the HA + unstressed groups and lowest in the LA + unstressed groups [lever x stress x anxiety phenotype interaction, $F(1, 62) = 4.22$, $p = .044$]. These group differences were largest during the earlier days of extinction training [day x lever x stress x anxiety phenotype interaction, $F(9, 558) = 3.58$, $p < .001$].

As expected, stress caused weight loss in both male and female rats [main effect of stress, $F(1, 62) = 32.50$, $p < .001$; see Fig. 4, Bottom]. However, a sex difference was evident in that female rats lost more weight and recovered less weight over days compared to male rats [main effect of sex, $F(1, 62) = 20.24$, $p < .001$ and treatment day x sex interaction, $F(2, 124) = 7.23$, $p = .001$]. Moreover, regardless of treatment, HA phenotype was related to greater weight loss in female, but not male, rats [anxiety phenotype x sex interaction, $F(1, 62) = 4.35$, $p = .041$].

3.4. Cue-induced reinstatement

Active, but not inactive, lever responding increased significantly during cue-induced reinstatement tests compared to the last extinction session for both male and female rats [main effect of session, $F(1, 62) = 109.95$, $p < .001$ and lever x session interaction, $F(1, 62) = 117.82$, $p < .001$; see Fig. 5, Top]. However, the magnitude of reinstatement was greater for females than for males [main effect of sex, $F(1, 62) = 18.52$, $p < .001$, sex x session interaction, $F(1, 62) = 19.94$, $p < .001$, and sex x session x lever interaction, $F(1, 62) = 10.87$, $p = .002$]. Moreover, although rats with a history of stress made less active, but not inactive, lever responses during reinstatement testing [stress x session interaction, $F(1, 62) = 7.35$, $p = .009$ and stress x session x lever interaction, $F(1, 62) = 8.92$, $p = .004$], this effect was significantly greater for females than for males [stress x sex interaction, $F(1, 62) = 4.40$, $p = .040$].

3.5. Pellet priming-induced reinstatement

Active, but not inactive, lever responding increased as a function of pellet priming for both male and female rats [main effect of pellets, $F(2, 124) = 27.41$, $p < .001$ and lever x pellets interaction, $F(2, 124) = 34.46$, $p < .001$; see Fig. 5, Bottom]. However, there was a sex difference in that females displayed similar levels of reinstatement after priming with either two- or four-pellets, whereas males displayed reinstatement only after priming with four pellets [sex x pellets interaction, $F(2, 124) = 6.68$, $p = .002$ and sex x pellets x lever interaction, $F(2, 124) = 5.89$, $p = .004$]. Moreover, there was a three-way interaction: Neither stress history nor anxiety phenotype was related to responding in females, but a history of stress in males caused increased and decreased responding in LA and HA rats, respectively [sex x stress x anxiety phenotype interaction, $F(1, 62) = 4.12$, $p = .047$]. Although this interaction was most prominent in the four-pellet condition as indicated by Bonferroni post-tests, a separate 3 (pellets) x 2 (stress) x 2 (anxiety phenotype) ANOVA in male rats only did not yield a significant 3-way interaction [$F(2, 64) = 2.45$, $p = .094$], suggesting that the interaction between anxiety phenotype and stress may not be specific to the number of pellets delivered.

4. Discussion

Using a rat model, we examined whether individual differences in trait anxiety were related to differences in reinstatement of palatable food seeking after chronic stress exposure in male and female rats. There were three main findings: (1) Overall, female rats displayed higher levels of responding during cue-induced reinstatement tests compared with males. (2) A history of chronic stress caused a reduction

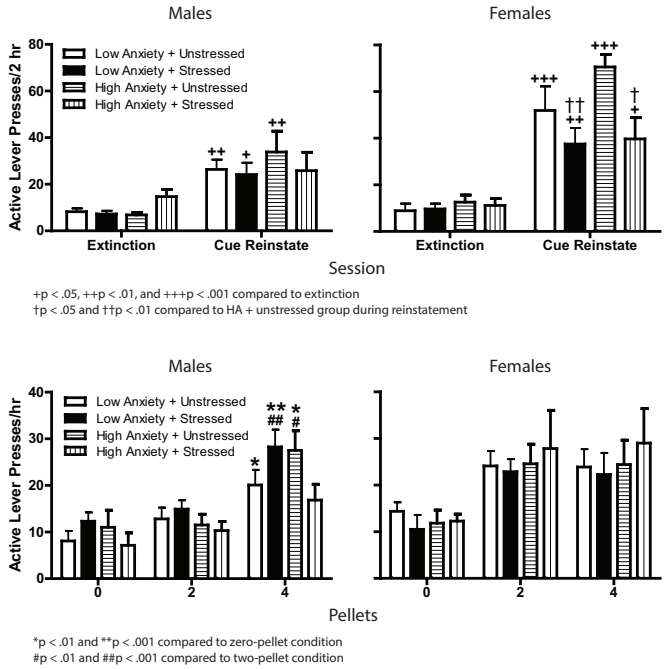

Fig. 5. Effect of chronic stress on CS- and pellet priming-induced reinstatement of palatable food seeking in male and female rats. (Top) Mean (+ SEM) active lever presses during the last extinction session and CS-induced reinstatement testing in previously stressed and unstressed rats in LA and HA phenotype groups. Reinstatement sessions began with one non-contingent CS presentation; during the remainder of the session, conditions were identical to those of self-administration training, except that lever presses did not lead to pellet delivery. (Bottom) Mean (+ SEM) active lever presses during pellet priming-induced reinstatement testing in previously stressed and unstressed rats in LA and HA phenotype groups. The testing consisted of four consecutive 1-h sessions that were identical to self-administration sessions, except that lever presses did not lead to pellet delivery, and rats received two and four non-contingent pellets within the first minute of sessions 3 and 4, respectively. Session 2 served as the 0-pellet baseline.

in cue-induced reinstatement in female, but not male, rats. (3) There was a three-way interaction such that neither stress history nor anxiety phenotype was related to pellet priming-induced reinstatement in females, but a history of stress in males caused increased and decreased responding in LA and HA rats, respectively. These results extend our previous findings, which showed that a history of chronic stress often confers increased relapse vulnerability in male subjects [17,18,19,55].

4.1. EPM behavior and anxiety phenotypes

The EPM is a widely used and validated method to measure anxiety in rodents [57–59]. We analyzed the proportion of time spent on the open arms of the EPM separately in male and female rats and found that the majority of both sexes clustered into a LA phenotype group. Consistent with previous studies, however, female rats displayed less anxiety-like behavior (open-arm avoidance) overall compared with males [e.g., [60–62]], a finding that contrasts with the clinical literature, which shows that anxiety disorders are more prevalent in females [63,64]. Given that female rats display more locomotor activity (arm entries) in the EPM compared with males [present results [65–67]], it is possible that increased open arm time is simply an artifact of increased locomotor activity. Arguing against this possibility, however, we found in the present study that female, but not male, rats in the HA group made significantly *more* arm entries compared with those in the LA group, indicating that increased locomotor activity is a sex-specific trait associated with *greater* anxiety-like behavior in the EPM. Thus, consistent with a recent study showing no relationship between distance moved and duration of time spent in the open arms of the EPM [62], our results support the argument that behaviors reflecting anxiety form the primary underlying component of behavior in the EPM for both male and female rats [66].

4.2. Palatable food self-administration

Consistent with previous studies of palatable food self-administration, we found that lever pressing and pellets earned per body weight were greater in females than males [[68–71], but see [72]]. Such findings are in line with the possibility that palatable food is more rewarding for females. Indeed, palatable food induces a more robust conditioned place preference in females than in males [73], and females work harder to obtain palatable food while on a progressive ratio schedule of reinforcement [[71,74–76], but see [69]]. Thus, our results support the bulk of the available pre-clinical literature showing that females are more motivated to self-administer palatable food compared with males.

4.3. Extinction responding and body weight during chronic stress treatment

Consistent with a previous report [49], we observed no sex difference in extinction of palatable food seeking. Overall responding, however, was highest in the HA + unstressed groups and lowest in the LA + unstressed groups, and these differences were largest during the earlier days of extinction training, indicating delayed and accelerated extinction learning in the HA + unstressed and LA + unstressed groups, respectively. Although we are not aware of any studies investigating the relationship between trait anxiety and extinction learning, there is evidence that high anxiety trait is associated with impaired spatial learning both in rats [77,78] and in humans [79,80]. The present results extend these findings by showing that extinction learning also is dependent on trait anxiety level. Interestingly, there was an interaction such that stress attenuated the effects of anxiety phenotype on extinction responding, that is, the HA + stressed and LA + stressed groups had similar levels of responding that were lower than the HA + unstressed groups and higher than the LA + unstressed groups. The mechanism of this interaction is not known, but is worthy of investigation in future studies.

There was a sex-specific effect of anxiety phenotype on weight: Regardless of treatment, HA phenotype was related to greater weight loss in female, but not male, rats. Thus, for HA females, control handling and/or extinction training alone appears to have caused weight loss, and restraint caused more weight loss in HA relative to LA females, suggesting that female biological sex and high trait anxiety have additive effects to increase sensitivity to at least one of the physiological effects of chronic stress.

4.4. Effect of biological sex on reinstatement of palatable food seeking

We found that, overall, females were more vulnerable to cue-induced reinstatement in that they responded at levels nearly twice that of males overall. In addition, our results suggest that females may also be more vulnerable to pellet priming-induced reinstatement in that they responded at levels nearly twice that of males overall during the two-pellet priming condition. Very few studies investigating reinstatement of food seeking have included both male and female subjects, and previous results are somewhat conflicting. With regard to cue-induced reinstatement, two studies reported no difference between males and females [72,81], whereas another study reported greater reinstatement in females [82], which aligns with the present results. Regarding palatable food priming-induced reinstatement, prior studies suggested little effect of biological sex [49,72,82], but these studies differed from the present study in several ways, notably in that they used only one priming dose (i.e., number of pellets) and the control and priming tests were conducted on separate days. Clearly more research in this area

utilizing both males and females is warranted, but the available data suggest that under some circumstances, female subjects are more vulnerable to craving and reinstatement of palatable food seeking relative to males. This conclusion aligns with the clinical literature showing that, compared to men, women have higher levels of food craving, greater food cue reactivity, and reduced ability to regulate craving [for review, see [83]]. Such sex differences in craving may drive women's greater chance of becoming overweight or obese compared with men [20,84].

4.5. Interaction between the effects of chronic stress, sex, and trait anxiety on reinstatement of palatable food seeking

There was an interaction such that a history of chronic stress caused a significant attenuation of cue-induced reinstatement in females, but not males. Although the neural basis of these dissociable effects in males and females is not known, it may be attributable to sex-differences in the adaptations in medial prefrontal cortex induced by chronic stress. Indeed, the medial prefrontal cortex, a critical node in the circuitry of relapse to palatable food seeking [35,36], is highly sensitive to manipulations of stress [85]—but in a highly sex-specific way. For example, in response to chronic restraint stress, male rats display dendritic retraction in medial prefrontal cortex pyramidal neurons, whereas female rats display dendritic *hypertrophy* [86]. Moreover, chronic restraint stress alters microglial cell activation patterns in the medial prefrontal cortex of females, but not males [33]. Indeed, such neural differences may contribute to sex differences in prefrontal-mediated behaviors. Notably, chronic stress is associated with deficits in spatial working memory tasks in males and improvements in females [87,88]. The present results suggest that another sex-specific behavioral adaptation induced by chronic stress is reduced cue-induced relapse vulnerability in females.

We reported previously that a history of chronic stress has no effect on cue-induced reinstatement of cocaine seeking in male rats [55]. The present results show that this lack of effect of stress generalizes to cue-induced reinstatement of palatable food seeking. It is noteworthy, however, that we reported an increase in responding for palatable food cues during forced abstinence in male rats with a history of chronic restraint stress exposure [19]. Specifically, chronically, but not acutely, stressed rats displayed increased food seeking 7 days, but not 1 day, after the last restraint. We may have observed dissociable effects of chronic stress on food seeking during abstinence vs. after extinction training because different neural mechanisms mediate food seeking under these different circumstances. In support of this possibility, there are partially dissociable neural mechanisms underlying drug seeking following extinction training vs. forced abstinence [89,90].

The majority of both male and female rats in the present study were classified as LA phenotype. Male rats within this group displayed higher levels of pellet priming-induced reinstatement if previously exposed to chronic restraint stress. This result extends our previous findings in two ways. First, we previously reported that repeated exposure to the pharmacological stressor yohimbine caused an increase in subsequent pellet priming-induced reinstatement in male rats [18]. The present results show that this effect of yohimbine can be generalized to a different type of stressor, restraint. Second, we previously found that exposure to chronic restraint stress during withdrawal from cocaine self-administration increased vulnerability to subsequent cocaine priming-induced reinstatement of cocaine seeking in male rats [55]. The present results show that this effect of restraint stress can be generalized to pellet priming-induced reinstatement of food seeking. Importantly, we also found that a much smaller subgroup of male rats classified as HA phenotype displayed a trend opposite of that seen in LA rats—that is, relatively high levels of responding were reduced in HA rats with a history of chronic stress exposure. Thus, trait anxiety appears to be a critical factor determining the effect of chronic stress on some types of reinstatement in male rats. The relatively small proportion of rats in the HA phenotype category also explains why we have observed a potentiating effect of chronic stress on reinstatement at the population level in previous studies.

To our knowledge, the present results are the first evidence that HA phenotype confers vulnerability to relapse in an animal model. In clinical studies, however, high trait anxiety levels during abstinence in individuals with alcohol use disorder predicted subsequent alcohol relapse [91,92]. In such studies, however, it is not known what individuals' levels of trait anxiety were before alcohol use disorder developed or before detoxification. The present results suggest that measurement of trait anxiety before exposure to the primary reinforcer (in the present case, highly palatable food) may allow for *a priori* identification of individuals at risk for relapse depending on their sex and subsequent exposure to environmental stressors.

It is noteworthy that the factors investigated in the present study (i.e., stress, sex, trait anxiety) generally had dissociable effects on cue- vs. pellet priming-induced reinstatement. Such findings are not without precedent. For example, we found that chronic restraint stress had no effect on cue-induced reinstatement of cocaine seeking in male rats, but that it caused an increase in reinstatement primed by cocaine [55]. Recently, we found that male rats prone to obesity while on a junk-food diet displayed greater pellet priming-, but not cue-, induced reinstatement relative to obesity-resistant rats [93]. These results support evidence that distinct neural mechanisms underlie cue- vs. pellet priming- and drug priming-induced reinstatement [35,94]. Clearly, the likelihood of exposure to specific relapse triggers is an important consideration in the development of personalized relapse prevention treatment plans.

4.6. Conclusion

Our findings suggest that the effects of chronic stress on reinstatement of food seeking depend on stress' interaction with several other factors, including biological sex, trait anxiety, and type of reinstatement manipulation. Consistent with the clinical literature, our findings suggest that women who are on a dietary treatment plan may be more vulnerable to diet recidivism under some conditions. In addition, our results suggest that prior chronic stress may, in fact, help to reduce relapse vulnerability in certain situations, particularly in women and in men with high trait anxiety. Finally, our results suggest that although high trait anxiety and chronic stress combined may confer *decreased* vulnerability to relapse in men, each of these factors in isolation confers *increased* vulnerability. Determining the mechanism by which chronic stress increases or decreases relapse vulnerability in some individuals will be an important topic for future research. Such research should aid in the development of more personalized and effective treatments for obesity and eating disorders.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgments

This work was supported by the National Institutes of Health (NIDA R15 DA035432 to KTB).

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