



Sex differences in fear-induced feeding cessation: Prolonged effect in female rats

Gorica D. Petrovich*, Mariel A. Lougee

Department of Psychology, Boston College, United States

ARTICLE INFO

Article history:

Received 6 January 2011

Received in revised form 23 June 2011

Accepted 24 June 2011

Keywords:

Anorexia

Anxiety

Conditioning

Eating disorders

Animal model

Learning

ABSTRACT

Fear inhibits food intake. Cessation of eating in anticipation of danger is an adaptive response that prepares an organism for an imminent threat, but it could become maladaptive when persistent. To begin to examine the underlying mechanisms, we developed an animal model for fear-cue induced inhibition of feeding. In that preparation, food-deprived rats stop eating when presented with a tone that signals a foot-shock based on prior associations. Here, we examined whether there are sex differences in adult male and female rats. We found that female rats showed sustained fear-cue induced feeding inhibition compared to males during the extinction. During the first of four extinction tests with tone presentations, both male and female rats showed similar, robust cessation of eating. Rats of both sexes that previously received tone-shock pairings ate significantly less than the control rats that received tones without shocks during training. Male rats extinguished this behavior during the second test, while females continued to show the effect during the second and third tests, and extinguished during the fourth test. The findings provide a novel framework for investigation of sex differences in the control of feeding and the underlying brain substrates. The animal model may also be informative for understanding human eating and associated disorders. In particular, the potential contribution of fear in the maintenance of low food intake in anorexia nervosa is hypothesized.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

It has long been recognized that fear inhibits food intake [1,2], but the underlying mechanisms have not been examined, in part, because behavioral models have been lacking. Recently, we developed a paradigm for fear cue-induced inhibition of feeding in rats [3,4]. Our preparation was built on well-established fear conditioning protocols (e.g., Refs. [5–8]) in which an initially neutral, environmental signal such as a tone (conditioned stimulus, CS) acquires the ability to produce fear-related behavioral responses (conditioned responses, CRs) through pairings with an aversive event such as a mild, electric, foot-shock (unconditioned stimulus, US).

We use aversive conditioning to modulate feeding. In our preparation, rats show robust cessation of intake during tests with presentations of a fear-cue, a tone (CS) that predicts foot-shocks (US). Importantly, this behavior is acquired with minimal training that consists of only four tone-footshock presentations. Additionally, rats are food-deprived prior to testing and therefore, typically consume substantial amounts of food. The fear-cue can effectively inhibit such robust feeding.

In the current study we examined whether there are sex differences in fear-cue feeding inhibition. Sex differences have been

found in the control of food intake, and eating and anxiety disorders are more prevalent in women (for reviews see Refs. [9,10]). Therefore, we tested here whether female rats are more susceptible to the effects of fear on feeding than males.

Adult male and female rats were trained in a conditioning protocol with tone presentations, each immediately followed by a foot-shock. Rats in the control condition received the same number of tones but no foot-shocks during training. In addition, all rats received appetitive training sessions during which they consumed food pellets. After training, food-deprived rats were tested for food consumption in the tests with tone (CS) presentations.

2. Material and methods

2.1. Subjects

Thirty-two experimentally naïve, male and female Long-Evans rats (Charles River Laboratories; Portage, MI) were used in the experiment. Rats were two months old when they arrived to the colony, and the body weight range for females was 200–225 g, and 250–275 g for males. Rats were individually caged, and maintained on a 12 h light/dark cycle, and given *ad libitum* access to food and water, except as otherwise noted. Female and male rats were housed in separate housing rooms. Rats were acclimated to the vivarium for a week, and to handling prior to any behavioral training. Body weights and vaginal smears were obtained every weekday. All animal procedures were approved by the Boston College Animal Care and Use Committee.

* Corresponding author at: Department of Psychology, Boston College, 344 McGuinn Hall, 140 Commonwealth Avenue, Chestnut Hill, MA 02467-3807, United States. Tel.: +1 617 552 0416; fax: +1 617 552 0523.

E-mail address: gorica.petrovich@bc.edu (G.D. Petrovich).

2.2. Apparatus

Training and testing were conducted in a set of eight behavioral chambers (30×28×30 cm; Coulbourn Instruments, Allentown, PA) located in a behavioral testing room that was different from the colony housing rooms. Each chamber was enclosed in a cubicle (79×53×53 cm; Coulbourn Instruments, Allentown, PA) composed of monolithic rigid foam walls, which isolate from ambient sound and light. A ventilation fan, located on the back of each isolation cubicle, provided masking noise (55 dB). Video cameras attached to a recording system (Coulbourn Instruments, Allentown, PA) were mounted on the back of the isolation cubicle to record behavior during training and testing.

The chambers were modified in olfactory, visual, and tactile features to create two distinct environments—Context A and Context B. All appetitive sessions and tests were conducted in the Context A, while aversive training sessions were conducted in the Context B.

For the Context A, each chamber had aluminum top and sides, a transparent Plexiglas back and front, and a black Plexiglas panel placed on top of the grid floor so that rats could not see or feel the grids. Each chamber contained a recessed food cup (3.2×4.2 cm), and a “house light” (4 W light) that was illuminated during appetitive sessions and during tests, but not during aversive sessions. The chambers were wiped with 1% Acetic Acid (Fisher Scientific, Hanover Park, Illinois) before an animal was placed inside.

For the Context B the chambers had grid floors, and two black Plexiglas sheets were positioned to occlude the aluminum sides and angled to create a tent-like enclosure. Each chamber was equipped with a recessed food cup and a “house light”; however, the house light was turned off during aversive sessions, and instead the room lights provided illumination for the chambers. The chambers were wiped with 5% ammonium hydroxide (v/v, 28–30% stock, Acros Organics; Somerville, NJ) before each animal was placed inside for training.

2.3. Behavioral training procedure

The training protocol (Fig. 1) consisted of 9 sessions, and of those 6 were appetitive sessions (S1, S2, S4, S6, S8, S9; Fig. 1) and 3 aversive sessions (S3, S5, S7; Fig. 1). The appetitive and aversive training sessions were conducted in distinct contexts (A and B, see above). For each training session rats were transported from the housing room to the behavioral testing room in the home cages placed on a cart. A day before the behavioral protocol started rats were given ~1 g of the food pellets (formula 5TUL, 45 mg, TestDiet, Richmond, IN) in their home cages to familiarize them with the pellets.

Prior to each appetitive session rats were food deprived for 22 h. Water was available *ad libitum* throughout the behavioral protocol in the home cages. For each session rats were placed in the behavioral chamber (Context A) with 7 g of food pellets in the food cup, and

allowed to consume food for 10 min. After 10 min, rats were removed from the chambers, placed in their home cages and transported back to the housing room. Remaining food in the food cups was removed and weighed. Rats were allowed *ad libitum* access to food (lab chow) for at least 24 h between consecutive food-deprivations (S1, 2, and 8, 9; Fig. 1), or before the start of aversive sessions.

During aversive training, half of the male and female rats received tone-shock pairings (Conditioned groups), while the other half of the rats received tone presentations without any shocks (Control groups). The aversive training protocol consisted of three 10 min-long training sessions (S3, 5, 7; Fig. 1) that were conducted on separate days. Rats were allowed *ad libitum* access to food (lab chow) and water for at least 24 h prior to each aversive session. In the first session (S3) rats were placed in the experimental chamber for 10 min to habituate them to the training context (Context B, see above), which was different from the chambers used in the appetitive sessions (Context A, see above). In each of the next two aversive sessions (S5, 7; Fig. 1) rats in the Conditioned groups received 2 tone (75 dB; 2 kHz, 60 s) presentations (variable inter-trial interval, 4 min ± 50%) each immediately followed by an electric footshock (1 mA; 1 s; Precision Adjustable Shocker, Coulbourn Instruments, Allentown, PA), while rats in the Control groups received 2 presentations of the tones, and no shocks.

2.4. Food consumption tests

Rats were tested in four tests that were conducted on separate days. Prior to each test rats were deprived of food for 22 h, while water remained available *ad libitum*. For each test rats were transported from their housing room to the behavioral testing room in the home cages placed on a cart. Rats were placed in the behavioral chambers (Context A) with 7 g of food pellets in the food cup, and allowed to consume food for 10 min. During the test, the tone CS (75 dB; 2 kHz, 60 s) was presented four times, starting at 1, 3, 5, and 7 min (Fig. 1). After the test, rats were taken out of the behavioral chambers, placed into the home cages and transported back to the housing room. Remaining food was removed from the food cup, and weighed.

2.5. Vaginal smears

Following the initial week of acclimation and handling, female rats were examined by a vaginal lavage procedure daily (excluding weekends) to determine the estrous cycle stage (Supplemental Table 3). The vaginal smears were placed on glass slides, and cell types were examined under a microscope to determine the stage in the estrous cycle [11]. We applied the procedure to ensure that female rats show normal cycling, however, due to a small sample size we did not use the estrous stage as a variable in the analyses.

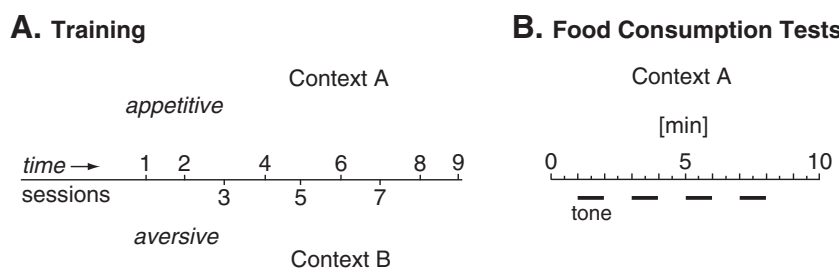


Fig. 1. Experimental design. A. Training. The behavioral protocol consisted of alternating appetitive and aversive sessions that were conducted in distinctive experimental chambers (contexts). During appetitive training sessions food-deprived rats were given free access to food pellets. During aversive training, half of the male and female rats received mild, electric foot-shocks signaled by a tone (Conditioned groups), while the other half of the rats received tones but no shocks (Control groups). That protocol consisted of three sessions with habituation to the context occurring during the first session (S3), and conditioning during the second and third sessions (S7, S9) when rats in the Conditioned groups received tone-shock pairings, and rats in the Control groups received tone presentations. B. Food consumption tests. Food-deprived rats were tested for food consumption during 10 min tests, each with 4 tone CS presentations. The tests were conducted in the appetitive context (Context A).

2.6. Behavioral observations

Freezing was assessed for each rat during the tests. Freezing behavior is a species-typical defense response that is characterized by the cessation of all movement except that required for breathing [12,13]. Observations were made every 1.25 s during the entire duration of each CS (60 s) and during the 10 s immediately preceding the CS (pre-CS), and immediately following the CS (post-CS). The observers were “blind” with respect to the training group and sex of the animals observed. The sum of all observations, which totals 5.33 min during the 10 min test, represents “total time” in the text, and the percentage of time rats spent expressing freezing behavior during that period was calculated. Due to technical malfunctioning, recordings of two rats were incomplete, and for those rats the “total time” was re-calculated accordingly.

Rats were trained in two identical replications, each with equal number of male and female rats, and equal number of rats in the conditioned and control groups (32 rats total; $n = 8$ per condition). Due to technical malfunctioning, one male rat from the conditioned group did not receive shocks during training, and was removed from the study.

2.7. Statistical analysis

Data were analyzed using paired t-tests, and ANOVA followed by Fisher's PLSD tests where appropriate. In all cases, $p < 0.05$ was considered significant.

3. Results

Rats were trained in a behavioral protocol (Fig. 1) with alternating appetitive and aversive sessions that were conducted in distinctive behavioral chambers (Context A and Context B, see [Materials and methods](#) for details). During appetitive sessions food-deprived rats were given free access to food pellets. During aversive training, half of the male and female rats received tone-shock pairings (Conditioned groups), while the other half of the rats received the same number of tone presentations, but no shocks were given (Control groups).

3.1. Training

Rats in all groups ate considerable amounts of food pellets during appetitive training sessions (Fig. 2). Overall male rats ate more than female rats, which was in accordance with the differences in body weights between male and female rats. Male rats were initially larger than females (see [Material and methods](#)) and gained weight at a much faster rate (see below). Importantly, rats of the same sex in the

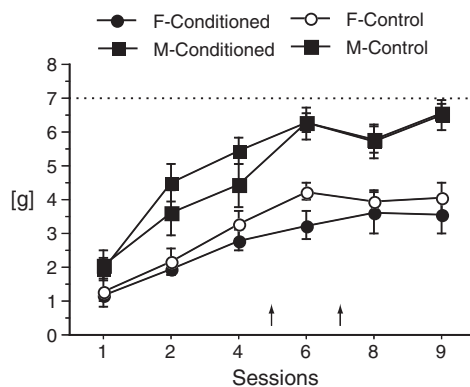


Fig. 2. Food consumption (mean \pm SEM) during appetitive training sessions that were conducted in Context A. Arrows point to the aversive sessions during which rats in the conditioned groups received mild, electric foot-shocks in Context B. Rats in the controls groups received tones, but no shocks during training. See [Results](#) for details.

Conditioned (tone-shock training) and Control (tone-only training) groups ate similar amounts of food pellets during training, and had similar body weights throughout the experiment. Thus, the experience of receiving foot-shocks during training did not produce changes in food consumption or body weight in the Conditioned group compared to the Controls that did not receive the shocks. Statistical analysis supported these observations ([Supplemental Table 1 and Table 2](#)).

An ANOVA of pellet consumption during training with sex (male or female), and training condition (tone-shock or tone-only) as factors revealed a significant main effect of sex for each appetitive session ($p < 0.05$), but no effects of conditioning ($p > 0.05$), or sex by conditioning ($p > 0.05$) for any of the sessions (see [Supplemental Table 1](#) for details). An analysis of body weights with sex, and training condition as factors showed that male rats weighed significantly more than females at the start of training (F-Conditioned, 233 ± 4 g; F-Control 233 ± 5 g; M-Conditioned, 370 ± 16 g; M-Control, 360 ± 15 g), end of training (F-Conditioned, 250 ± 5 g; F-Control, 246 ± 5 g; M-Conditioned, 413 ± 17 g; M-Control, 393 ± 16 g), start of testing (F-Conditioned, 246 ± 5 g; F-Control, 245 ± 5 g; M-Conditioned 410 ± 16 g; M-Control, 397 ± 16), and end of testing (F-Conditioned, 251 ± 5 g; F-Control, 251 ± 5 g; M-Conditioned 420 ± 16 g, M-Control, 407 ± 16 g), while there were no effects of training condition or sex by training condition on body weights ($p > 0.05$, see [Supplemental Table 2](#) for details).

3.2. Food consumption tests

After training completion rats were tested in four identical food consumption tests that occurred on separate days. During each test food-deprived rats were provided with food pellets in the appetitive context (Context A) and given four tone (CS) presentations. The amounts of food consumed during the tests are shown in [Fig. 3](#). We show the actual amounts consumed by each group ([Fig. 3A](#)), and conditioned groups consumption relative to the controls of the same sex ([Fig. 3B](#)) because of a baseline difference in consumption of male and female rats (see [Section 3.1](#)).

During the first test both female and male rats showed CS-driven inhibition of feeding—rats that previously received tone-shock pairings consumed much less food than the Control rats (tone-only training) of the same sex (T1, [Fig. 3](#)). An ANOVA of consumption during the first test with conditioning (tone-shock or tone-only training), and sex (male or female) as factors revealed statistically significant effect of conditioning ($F(1,27) = 27.026$, $p < 0.0001$), and sex ($F(1,27) = 21.784$, $p < 0.0001$), but not conditioning by sex effect ($p > 0.05$). Subsequent analysis confirmed that the female rats in the Conditioned group ate significantly less than the female rats in the Control group ($p < 0.004$). Similarly, male rats in the Conditioned group ate significantly less than male rats in the Control group ($p < 0.0004$).

During the second and third tests female rats continued to show inhibition of feeding, while male rats in the Conditioned and Control groups ate similar amounts during each of the two tests. The analysis of consumption during the second test (T2, [Fig. 3](#)) with conditioning and sex as factors revealed a significant effect of conditioning ($F(1,27) = 5.897$, $p < 0.03$), and sex ($F(1,27) = 44.765$, $p < 0.0001$), but not conditioning by sex effect ($p > 0.05$). Subsequent analysis showed that the female rats in the Conditioned group ate significantly less than the female rats in the Control group ($p < 0.02$), while the male rats in Conditioned and Control groups ate similar amounts ($p > 0.05$).

The analysis for the third test (T3, [Fig. 3](#)) revealed the effects of sex ($F(1,27) = 139.359$, $p < 0.0001$), and sex by conditioning ($F(1,27) = 5.780$, $p < 0.03$), but not conditioning ($p > 0.05$). Subsequent analysis confirmed that the female rats in the Conditioned group ate significantly less than the female rats in the Control group ($p < 0.02$), while the male rats in Conditioned and Control groups ate similar amounts ($p > 0.05$). Finally, during the fourth test female rats extinguished the inhibition of feeding

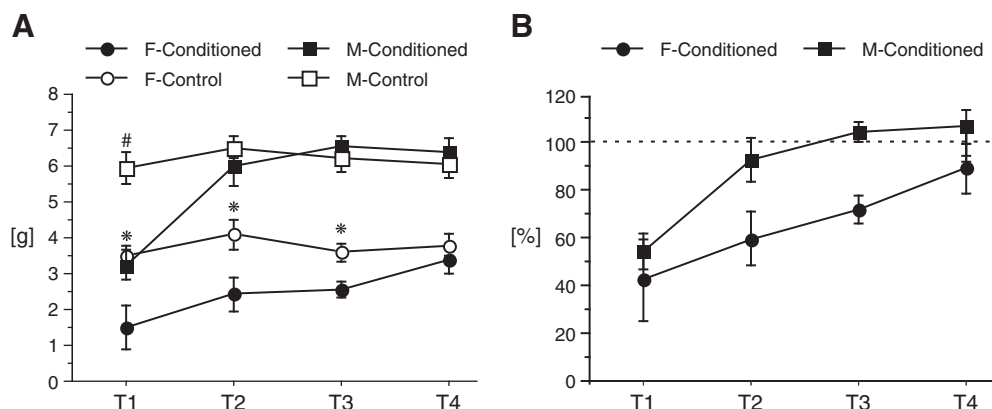


Fig. 3. Food consumption during tests with tone (CS) presentations in Context A. A. Food consumed during tests shown in grams (mean \pm SEM). An asterisk indicates significant differences in consumption between female rats in the Conditioned and Control groups ($p < 0.05$), and the pound symbol indicates significant differences in consumption between male rats in the Conditioned and Control groups ($p < 0.05$). See Results for details. B. Consumption of the male and female rats in the conditioned groups shown as percentages of the average amounts consumed by the rats of the same sex in the controls groups (shown with dotted line).

(T4, Fig. 3), and the analysis showed the effect of sex ($F(1,27) = 47.664$, $p < 0.0001$), but not conditioning ($p > 0.05$), or conditioning by sex ($p > 0.05$) effects.

Some male rats ate all the food available during testing (four rats in the Conditioned group and three Controls during T2, three rats in the Conditioned and two Controls during T3, and two rats in the Conditioned and one Control during T4). Had only the Controls, but not rats in the Conditioned group, eaten all the food available the extinction results could have been obscured, however that was not the case. Some rats in each condition ate all the food given, and fewer of those were controls.

Nevertheless, to confirm we conducted a new experiment with male rats, and provided them with more food. The results from that study are described in the Supplemental material and Supplemental Fig. 1. In brief, in the supplemental study rats had a surplus of food pellets during training and testing. As in the original study, these rats showed CS-driven inhibition of feeding during the first test, and extinction of that behavior during the second test. These results support the original study and confirm that male rats extinguish much faster than females.

3.3. Freezing behavior

In addition to food consumption measures we analyzed another conditioned response during the tests, freezing behavior. During the first test male and female rats in the Conditioned groups that previously received tone-shock pairings showed similar, substantial freezing (Fig. 4), while rats in the Control groups showed no freezing during any of the tests. Prior to the first CS presentation during the

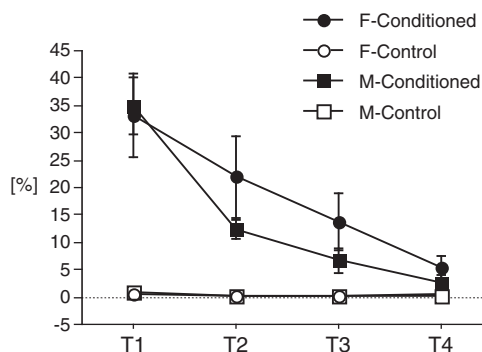


Fig. 4. Conditioned freezing behavior (mean \pm SEM) during food consumption tests. Graphs show percentages of the total time spent freezing during the tests. Observations were made during CSs, pre-CSs, and post-CSs (see Materials and methods for details).

first test, none of the rats in any of the groups expressed freezing behavior. Thus, the CS-US learning acquisition was similar for male and female rats, as evidenced by the expression of freezing behavior during the first test, and in agreement with food consumption during the same test (see Section 3.2).

The analysis of the total time spent freezing (see Materials and methods for details) during the first test (Fig. 4) revealed significant effects of conditioning ($F(1,27) = 53.986$, $p < 0.0001$), but not sex, or conditioning by sex effects ($p > 0.05$). Subsequent analysis found that male and female rats in the Conditioned group froze similarly ($p > 0.05$), and significantly more than the Controls of either sex ($p < 0.0001$). Thus, there were no differences between male and female rats in the Conditioned group, or male and female rats in the Control groups ($p > 0.05$).

During the remaining three extinction tests both male and female rats in the Conditioned groups showed extinction of freezing behavior (Fig. 4). During the second extinction test male and female rats in the Conditioned groups showed significantly less freezing compared to the amount of time they spent freezing during the first test ($t(7) = 2.884$, $p < 0.03$ for females, and $t(6) = 5.744$, $p < 0.002$ for males). Similarly, both male and female rats froze less during the third test compared to their freezing during the second test ($t(7) = 2.908$, $p < 0.03$ for females, and $t(6) = 2.480$, $p < 0.05$ for males). A small decrease in freezing between the third and fourth tests was not statistically reliable for either male or female rats ($p > 0.05$).

The average amount of freezing was slightly higher for females than males during the second and third tests, however the differences were not statistically reliable. The variability in freezing behavioral was much greater among females than males, and included a rat with the overall highest freezing observed in the current study.

The analysis of the total time spent freezing during the second test found significant effect of conditioning ($F(1,27) = 18.692$, $p < 0.0002$), but not sex or conditioning by sex effects ($p > 0.05$). Subsequent analysis showed that female rats in the Conditioned group froze significantly more than Controls of either sex ($p < 0.001$), while the small increase in freezing for females compared to males in the Conditioned groups was not statistically reliable ($p > 0.05$), and the Control groups did not differ between the sexes ($p > 0.05$).

Similarly, during the third test there was a significant effect of conditioning ($F(1,27) = 12.501$, $p < 0.002$), but not sex, or conditioning by sex effects ($p > 0.05$). Subsequent analysis showed that female rats in the Conditioned group froze significantly more than male or female Controls ($p < 0.003$), but not significantly more than males in the Conditioned group ($p > 0.05$), and the Control groups did not differ between the sexes ($p > 0.05$). Finally, freezing was minimal for all groups during the fourth test, and there were no effects of conditioning, sex, or conditioning by sex ($p > 0.05$).

4. Discussion

The main finding of the current study was that female rats showed prolonged fear-cue inhibition of feeding compared to males. Rats were tested in our recently developed preparation in which food-deprived rats inhibit feeding during tests with presentations of a tone (CS) that signals foot-shocks (US) [3,4]. We found no sex differences during the first test, which showed that male and female rats acquired and expressed conditioned responses similarly. Sex differences emerged during the extinction of CS-induced feeding cessation. Female rats extinguished this behavior at a much slower rate than males. Male rats in the conditioned and control groups ate similar amounts during the second extinction test, while it took two additional tests for females in the conditioned group to reach the consumption levels of the controls.

Cognitive and behavioral sex differences have been found in a variety of tasks that relate to facets of our paradigm ranging from differences in associative learning and control of feeding and energy homeostasis to regulation of motivated behaviors, stress responses, and anxiety (for reviews, see Refs. [9,14–17]). Nevertheless, this is the first study that showed sex differences in learned, fear-cue driven cessation of feeding.

In a previous study, differences were shown in a task that used emotional stress (communication box) to inhibit food intake. Consistent with our findings here, that study found greater inhibition of feeding in female rats compared to males following emotional stress [18]. Interestingly, the effect was dependent on estradiol and corticotropin-releasing hormone/factor (CRH/CRF) type 1 receptor [18], both of which could act on the brain substrates underlying fear conditioning and extinction—the amygdala, prefrontal cortex and associated circuitry [19,20].

Notably, CRH neurons and estrogen receptors are present in the central nucleus of the amygdala (CEA) [21–23], which we recently showed is critical for fear-cue-driven inhibition of feeding [3]. Furthermore, the CEA shares substantial connections with the brain areas related to anxiety and initiation of feeding, the bed nuclei of the stria terminalis and the lateral hypothalamus, respectively [19,24–26]. There is also evidence that the CEA participates in the critical forebrain network underlying dehydration-induced anorexia [27,28]. Thus, the CEA might be an important site where estradiol, which inhibits intake (for reviews see Refs. [29,30]) could contribute to the effects observed here.

Cessation of eating in anticipation of danger is an adaptive response that prepares an organism for an imminent threat, but it could become maladaptive when persistent. In that regard, prolonged cessation of feeding in female rats might be informative for understanding human eating and associated disorders. Anorexia nervosa disproportionately affects women, and intense fear of weight gain despite being underweight, is one of its key symptoms and a diagnostic criterion [31] [10,32,33]. Anorexia is characterized by relentless maintenance of extremely low body weight through restricted eating often combined with exercise and purging [31] [10,32,33]. Another feature of this complex disease is high comorbidity with anxiety disorders (reviewed in Ref. [10]). The disease persists despite serious health consequences including death, and restricted eating is maintained in spite of emaciation, and in conflict with physiological hunger signals [10,34]. The exact brain mechanisms underlying this paradoxical behavior are currently unknown. We hypothesize that persistent fear and associated anxiety could facilitate the maintenance of restricted eating in anorexia, and the current data suggest that females might be more susceptible in that setting.

In that regard, abnormalities in the fear network regions have been found in anorexia nervosa patients, including abnormal amygdalar functioning [35], and a decrease in its volume [36]. Another study found enhanced recruitment of the medial prefrontal cortex in

anorexia patients in response to food images, which the patients found threatening and disgusting [37]. Similarly, greater amygdala activation was found among anorexia patients when confronted with a threatening, symptom-provoking cue—their distorted body image [38]. Finally, amygdala recruitment was correlated with increased anxiety in young women (without eating disorders) when viewing pictures of slim, idealized female bodies [39].

Clearly, future studies are necessary to examine how fear overrides homeostatic signals triggered by food-deprivation to inhibit feeding, and whether sustained fear could produce changes in body weight. Likewise, the mechanisms and plasticity underlying fear-cue integration with the feeding regulators remain to be determined. That integration will ultimately involve complex network of communications between the telencephalon, hypothalamus, and brainstem [19,20,26,40–44]. The current findings provide a novel framework for future behavioral and brain analyses.

In addition to food consumption, we analyzed another conditioned response (CR), freezing behavior, in response to the same fear-cue (CS). Freezing is a species-typical defense response that is a commonly analyzed CR measure in aversive learning paradigms. Here we found that male and female rats spent equal time expressing freezing behavior during the first test, in agreement with prior work ([45,46], but see other strains [47]). Thus, both the freezing behavior and food consumption patterns during the first test show that male and female rats acquired CS–US relationship equally well. Additionally, we found similar extinction of freezing behavior in male and female rats in agreement with prior work with auditory cues ([46], in this study also see estrous cycle effects on extinction).

Importantly, we have shown previously that the CS-induced feeding cessation and CS-induced freezing are dissociable behavioral responses induced by the same CS [3]. In other words, the CS-inhibition of feeding is not merely a consequence of immobilization due to CS-induced conditioned freezing. Rather, the CS's influence on feeding is independent of CS-induced freezing, and engages somewhat dissociable amygdalar and brainstem subsystems. Brain lesions that abolished conditioned freezing left inhibition of eating intact—lesions of the ventrolateral region of the periaqueductal gray [48], an area critical for conditioned freezing [49], or lesions of the basolateral amygdala [3].

In conclusion, here we found prolonged fear-cue inhibition of intake in food-deprived female rats compared to males. These findings provide a novel framework for examination of the brain mechanisms and sex differences. The animal model may be also relevant for understanding regulation of eating in humans, and in particular to the maintenance of low intake in anorexia nervosa. Speculatively, female biological susceptibility would be especially relevant in environments abundant in cues that the anorexic population perceives as threatening, notably, in Western societies with excessive images of idealized bodies and relentless food advertisements.

Acknowledgments

We thank Kate Gildersleeve and Brandon Davenport for technical assistance, and Professor Michael Numan for instructions on estrous cycle detection.

Appendix A. Supplementary data

Supplementary data to this article can be found online at [doi:10.1016/j.physbeh.2011.06.020](https://doi.org/10.1016/j.physbeh.2011.06.020).

References

- [1] Cannon WB. *Bodily changes in pain, hunger, fear and rage*. 2nd ed. New York: Appleton; 1915.
- [2] Schachter S, Goldman R, Gordon R. Effects of fear, food deprivation, and obesity on eating. *J Personality Soc Psychol* 1968;10:91–7.

- [3] Petrovich GD, Ross CA, Mody P, Holland PC, Gallagher M. Central but not basolateral amygdala is critical for control of feeding by aversive conditioned cues. *J Neurosci* 2009;29:15205–12.
- [4] Petrovich GD. Forebrain circuits and control of feeding by learned cues. *Neurobiol Learn Mem* 2010 Epub.
- [5] Davis M. The role of the amygdala in fear and anxiety. *Annu Rev Neurosci* 1992;15:353–75.
- [6] Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev* 1999;23:743–60.
- [7] LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci* 2000;23:155–84.
- [8] Maren S. Neurobiology of Pavlovian fear conditioning. *Annu Rev Neurosci* 2001;24:897–931.
- [9] Geary N, Lovejoy J. Sex differences in energy metabolism, obesity, and eating behavior. Sex differences in the brain from genes to behavior. New York: Oxford University Press; 2008. p. 253–74.
- [10] Kaye W. Neurobiology of anorexia and bulimia nervosa. *Physiol Behav* 2008;94:121–35.
- [11] Becker JB, Arnold AP, Berkley KJ, Blaustein JD, Eckel LA, Hampson E, et al. Strategies and methods for research on sex differences in brain and behavior. *Endocrinology* 2005;146:1650–73.
- [12] Blanchard RJ, Blanchard DC. Crouching as an index of fear. *J Comp Physiol Psychol* 1969;67:370–5.
- [13] Fanselow MS. What is conditioned fear? *Trends Neurosci* 1984;7:400–2.
- [14] Dalla C, Shors TJ. Sex differences in learning processes of classical and operant conditioning. *Physiol Behav* 2009;97:229–38.
- [15] Becker JB, Taylor JR. Sex differences in motivation. In: Becker JB, Berkley KJ, Geary N, Hampson E, Herman JP, Young EA, editors. Sex differences in the brain from genes to behavior. New York: Oxford University Press; 2008. p. 177–99.
- [16] Solomon MB, Herman JP. Sex differences in psychopathology: of gonads, adrenals and mental illness. *Physiol Behav* 2009;97:250–8.
- [17] McEwen BS. Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. *Ann N Y Acad Sci* 2010;1204:E38–59 Suppl.
- [18] Kuriyama HTS. Sexual differentiation of the effects of emotional stress on food intake in rats. *Neuroscience* 2004;124:459–65.
- [19] Swanson LW, Petrovich GD. What is the amygdala? *Trends Neurosci* 1998;21:323–31.
- [20] Quirk GJ, Garcia R, Gonzalez-Lima F. Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry* 2006;60:337–43.
- [21] Veening JG, Swanson LW, Sawchenko PE. The organization of projections from the central nucleus of the amygdala to brainstem sites involved in central autonomic regulation: a combined retrograde transport-immunohistochemical study. *Brain Res* 1984;303:337–57.
- [22] Cassell MD, Gray TS, Kiss JZ. Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. *J Comp Neurol* 1986;246:478–99.
- [23] Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen receptors mRNA-containing cells in the rat brain: an in situ hybridization study. *J Comp Neurol* 1990;294:76–95.
- [24] Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron* 1999;22:221–32.
- [25] Dong HW, Petrovich GD, Swanson LW. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res Rev* 2001;38:192–246.
- [26] Walker DL, Toufexis DJ, Davis M. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *Eur J Pharmacol* 2003;463:199–216.
- [27] Watts AG. Neuropeptides and the integration of motor responses to dehydration. *Annu Rev Neurosci* 2001;24:357–84.
- [28] Watts AG, Salter DS, Neuner CM. Neural network interactions and ingestive behavior control during anorexia. *Physiol Behav* 2007;91.
- [29] Eckel LA. Estradiol: a rhythmic, inhibitory, indirect control of meal size. *Physiol Behav* 2004;82:35–41.
- [30] Asarian L, Geary N. Modulation of appetite by gonadal steroid hormones. *Phil Trans R Soc B* 2006;361:1251–63.
- [31] Association AP. Diagnostic and statistical manual of mental disorders. 4 ed. Washington, DC: American Psychiatric Press; 1994.
- [32] Klein DA, Walsh BT. Eating disorders: clinical features and pathophysiology. *Physiol Behav* 2004;81:359–74.
- [33] Treasure J, Claudino AM, Zucker N. Eating disorders. *Lancet* 2010;375:583–93.
- [34] Jimerson DC, Wolfe BE. Psychobiology of eating disorders. In: Wonderlich A, Mitchell JE, de Zwaan M, Steiger H, editors. Annual review of eating disorders part 2–2006. Oxford: Radcliffe Publishing; 2006. p. 1–15.
- [35] Takano A, Shiga T, Kitagawa N, Koyama T, Katoh C, Tsukamoto E, et al. Abnormal neuronal network in anorexia nervosa studied with I-123-IMP SPECT. *Psychiatry Res* 2001;107:45–50.
- [36] Giordano GD, Renzetti P, Parodi RC, Foppiani L, Zandrino F, Giordano G, et al. Volume measurement with magnetic resonance imaging of hippocampus-amygdala formation in patients with anorexia nervosa. *J Endocrinol Invest* 2001;24:510–4.
- [37] Uher R, Murphy T, Brammer MJ, Dalgleish T, Phillips ML, Ng VW, et al. Medial prefrontal cortex activity associated with symptom provocation in eating disorders. *Am J Psychiatry* 2004;161:1238–46.
- [38] Seeger G, Braus DF, Ruf M, Goldberger U, Schmidt MH. Body image distortion reveals amygdala activation in patients with anorexia nervosa—a functional magnetic resonance imaging study. *Neurosci Lett* 2002;326:25–8.
- [39] Friederich HC, Uher R, Brooks S, Giampietro V, Brammer M, Williams SC, et al. I'm not as slim as that girl: neural bases of body shape self-comparison to media images. *Neuroimage* 2007;37:674–81.
- [40] Swanson LW. Cerebral hemisphere regulation of motivated behavior. *Brain Res* 2000;886:113–64.
- [41] Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661–71.
- [42] Grill HJ, Kaplan JM. The neuroanatomical axis for control of energy balance. *Front Neuroendocrinol* 2002;23:2–40.
- [43] Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. *Neuron* 2002;36:199–211.
- [44] Berthoud HR. Interactions between the “cognitive” and “metabolic” brain in the control of food intake. *Physiol Behav* 2007;91:486–98.
- [45] Maren S, De Oca B, Fanselow MS. Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning. *Brain Res* 1994;661:25–34.
- [46] Milad MR, Igoe SA, Lebron-Milad K, Novales JE. Estrous cycle phase and gonadal hormones influence conditioned fear extinction. *Neuroscience* 2009;164:887–95.
- [47] Pryce CR, Lehmann J, Feldon J. Effect of sex on fear conditioning is similar for context and discrete CS in Wistar, Lewis and Fischer rat strains. *Pharmacol Biochem Behav* 1999;64:753–9.
- [48] Petrovich GD, Ross CA, Holland PC, Gallagher M. Central but not basolateral amygdala is critical for control of feeding by aversive, conditioned cues. *Soc Neurosci* 2006;32 (Abst.).
- [49] Amorapanth P, Nader K, LeDoux JE. Lesions of periaqueductal gray dissociate-conditioned freezing from conditioned suppression behavior in rats. *Learn Mem* 1999;6:491–9.