



Renewal of conditioned responding to food cues in rats: Sex differences and relevance of estradiol



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HIGHLIGHTS

- Investigated context-dependent renewal of extinguished responding to food cues
- Male rats showed renewal of responding, while intact female rats were inconsistent.
- Ovariectomized females showed similar conditioned responding across conditions.
- Estradiol replacement to ovariectomized females rescued renewal of responding.

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ABSTRACT

Cues associated with food can stimulate food anticipation, procurement, and consumption, independently of hunger. These and other behaviors driven by learned cues are persistent and can reappear after extinction, because the original learned associations continue to exist. Renewal, or reinstatement, of extinguished conditioned behavior may explain the inability to change maladaptive eating habits driven by food cues, similar to the mechanisms of drug use relapse. Here, we investigated sex differences in context-induced renewal of responding to food cues, and the role of estradiol in females in a Pavlovian conditioning preparation. We compared adult male and female rats because there is evidence for sex differences in learning and memory and in the control of feeding. Context-induced renewal involves conditioning and extinction in different contexts and the renewal of conditioned behavior is induced by return to the conditioning context (“ABA renewal”; experimental groups). Control groups remain in the same context during conditioning, extinction, and test. In Experiment 1, male and female rats were trained to associate a tone with food pellets during acquisition, and after extinction with tone only presentations, were tested for renewal of responding to the tone. Learning was assessed through the expression of the conditioned response, which included approach and activity directed at food receptacle (food cup behavior). Males and females learned the acquisition and extinction of tone–food associations similarly, but there were sex differences during renewal of the conditioned responses to the food cue. Males showed robust renewal of responding, while renewal in intact females was inconsistent. Males in the experimental group had significantly higher food cup behavior compared to males in the control group, while females in both groups showed similar levels of food cup behavior during the tone. In Experiment 2, we examined a potential role of estradiol in renewal, by comparing intact females with ovariectomized females with, and without, estradiol replacement. Rats in all groups acquired and extinguished tone–food associations similarly. During the test for renewal, the ovariectomized rats with estradiol replacement in the experimental group showed renewal of responding, evidenced by significantly higher food cup behavior compared to the control group. Intact and ovariectomized rats in the experimental groups had similar rates of food cup behavior as their corresponding control groups. These results provide novel evidence for sex differences and relevance of estradiol in renewal of responding to food cues and more broadly in contextual processing and appetitive associative learning, potentially relevant to maladaptive eating habits and eating disorders.

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1. Introduction

Learned associations have an important impact on our behavior, usually beneficial but sometimes negative, especially when they become persistent. These associations are readily formed when cues from the environment are paired with biologically important events, such as

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finding food or avoiding danger, and can later drive appetitive and aversive behaviors important for survival. However, behaviors driven by learned cues can persist and reappear after extinction, because the original learned associations continue to exist, evidenced by spontaneous recovery and other forms of renewal of responding (for reviews see [7,36,41]). Persistent behaviors and thoughts, driven by learned cues, have been implicated in eating disorders and drug abuse, as well as anxiety disorders and post-traumatic stress disorder [35,45,46].

In particular, cues previously associated with food can later stimulate appetite and food consumption in the absence of hunger, which can lead to maladaptive overeating behavior and obesity (for reviews see [30,33,37]). Renewal, or reinstatement, of extinguished conditioned behaviors may help explain the mechanisms underlying persistent responding to food cues and difficulty associated with changing unhealthy eating habits [6,13,48]. Indeed, the food reinstatement model was recently introduced as a framework to study mechanisms of relapse to palatable food seeking during dieting, similar to the reinstatement model for relapse of drug use [13,46,49,50].

The current study examined context-dependent renewal of conditioned responding to Pavlovian food cues, using an adapted ABA protocol [8]. In a context-based renewal preparation a return to the context in which the initial learning occurred induces robust responding to the cues that were extinguished elsewhere. We compared behavior of adult male and female rats, because there are sex differences in learning and memory and in the control of feeding behavior and associated disorders. Women are more susceptible to severe obesity and eating disorders, and obese women show more impairments in food reward-associative learning (for reviews see [5,12,34]; [54]). Nevertheless, female subjects are underrepresented in basic and clinical research [2, 55]. Prior research on extinction and renewal has been conducted exclusively with male rats or with no comparisons between males and females [10,14]. Notably, studies that have compared males and females found sex differences in associative learning and contextual processing (e.g., [17,29,40]).

In the first experiment we compared behaviors of intact males and females and found sex differences. To examine whether estradiol is important in these sex differences, in the second experiment, we compared behavior of intact females, ovariectomized females (OVX) and ovariectomized females with estradiol replacement (OVX + E). Estradiol is important in the regulation of food intake and body weight in females, as well as an important modulator in learning and memory including food-associative learning and subsequent expression of learned behaviors [1, 2,18,19,21,32,42,44]. Therefore, we tested whether estradiol may be a modulator of renewed food seeking in a context-driven preparation.

2. Materials and methods

2.1. Subjects

2.1.1. Experiment 1

32 adult male and female Long–Evans rats (Charles River Laboratories; Portage, MI), which weighed 250–275 g at arrival, were individually housed and maintained on a 12 h light/dark cycle (lights on 07:00). Males and females were housed in separate colony rooms. After arrival, subjects were allowed one week to acclimate to the colony housing room before behavioral procedures began, during which they had ad libitum access to water and standard laboratory chow (18% Protein Rodent Diet #2018, Harlan Teklad Global Diets; Madison, WI), and were handled daily. All housing and testing procedures were in compliance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and approved by the Boston College Institutional Animal Care and Use Committee.

2.1.2. Experiment 2

16 ovariectomized and 8 intact adult female Long–Evans rats (Charles River Laboratories; Raleigh, NC) weighing 250–300 g at arrival

were individually housed and maintained on a 12 h light/dark cycle (lights on 07:00). Subjects were allowed one week after arrival to acclimate to the colony room before capsule implantation surgery. During acclimation rats had ad libitum access to water and standard laboratory chow (18% Protein Rodent Diet #2018, Harlan Teklad Global Diets; Madison, WI), and were handled daily. Animals were given a week to recover post-surgery before behavioral procedures began, during which they were weighed and handled daily. All housing and testing procedures were in compliance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals and approved by the Boston College Institutional Animal Care and Use Committee.

2.2. Surgical procedure and estradiol replacement for Experiment 2

Bilateral ovariectomies were performed at Charles River laboratories, and after recovery and acclimation to the Boston College colony, all rats (ovariectomized and intact) received subcutaneous placement of silastic capsules. Assembly and implantation of the silastic capsules followed the protocol outlined by Strom et al. [47]. The capsules were made using silastic tubing (1.6 mm inner diameter, 3.17 mm outer diameter; 3 cm in length; Fisher; Pittsburgh, PA) and sealed with 5 mm wooden dowels, filled either with 180 mg/ml estradiol (Sigma-Aldrich; Saint Louis, MO) in sesame oil, or vehicle (sesame oil). Half of the ovariectomized animals received estrogen (OVX + E), while the other half, along with intact, received only vehicle (OVX and Intact). Animals were anesthetized with isoflurane (2–5% in oxygen) and the capsules were inserted through an incision made caudal to the shoulders. To verify estradiol release, one day after completion of behavioral testing trunk blood was collected, and estradiol serum levels were measured using a Mouse/Rat Estradiol ELISA kit (Calbiotech, Spring Valley, CA). Serum estradiol levels were significantly higher in OVX + E rats compared to OVX rats (OVX + E: 5.14 ± 0.9 pg/ml; OVX: 1.06 ± 0.4 pg/ml; $t(28) = -4.414$, $p < 0.01$).

2.3. Apparatus

The behavioral training was conducted in identical behavioral chambers (30 × 28 × 30 cm; Coulbourn Instruments; Allentown, PA) located in a room different than the colony housing rooms. The chambers had aluminum top and sides, clear Plexiglas rear wall and front hinged door and a floor of stainless steel rods 5 mm thick spaced 15 mm apart. Chambers contained a recessed food cup (3.2 × 4.2 cm) and a 4 W house light. Each chamber was located in a sound- and light-attenuating cubicle (79 × 53 × 53 cm), which was equipped with a ventilation fan (55 dB) and video camera attached to a recording system (Coulbourn Instruments; Allentown, PA). The conditioned stimulus (CS) was a 10 second tone (75 dB, 2 kHz). The unconditioned stimulus (US) consisted of two food pellets (45 mg pellets, formula 5TUL; Test Diets, Richmond, IN, USA) delivered to the food cup. Chambers were modified in visual, tactile, and olfactory features, to create two distinct environments (Context A and Context B). In Context A, a black Plexiglas panel was placed on top of the grid floor (so that rats could not see or feel the grids), and the doors to the cubicles were closed. In Context B, a black Plexiglas panel was inserted diagonally across the side of the chamber creating a wall, and the doors to the cubicle were left open. For Context B, 1% acetic acid (Fisher Scientific; Fair Lawn, NJ) was sprayed onto the tray below the grid floor.

2.4. Behavioral training procedure

All behavioral training and testing occurred between 9:00 and 14:00. A week before start of training, rats were food deprived and their daily food allotment was restricted to gradually reach 85% of their body weight; they were maintained at this weight for the duration of the experiment. All rats received 1 g of the food pellets (US) in the home cage the day before the training started to familiarize them with

the pellets. The training consisted of three phases: conditioning (acquisition), extinction, and renewal test (Fig. 1). The training protocol followed an “ABA” design where conditioning occurred in Context A, extinction occurred in Context B and the renewal test occurred in Context A (counterbalanced across contexts) [8]. Rats in the control condition remained in the same context across all training phases (AAA or BBB). During the acquisition phase, rats were trained for five days, with one 34-minute training session per day. During each session they received eight presentations of the tone (CS), each immediately followed with delivery of food pellets (US) into the food cup. The acquisition training occurred in Context A for half of the rats, and in Context B for the other half. During the extinction phase, rats received two 34-minute sessions (one session per day), each with eight presentations of the CS alone with no USs. Rats in the experimental condition received extinction training in a context different than the training context while rats in the control condition received extinction training in the same context as acquisition. The test for renewal was one 34-minute session with eight CS presentations with no USs, conducted in the conditioning (acquisition) context. All sessions were recorded and stored on DVDs for behavioral analysis.

2.5. Behavioral observations

Trained observers, unaware of experimental condition or sex of the rats, analyzed animals' behavior from the video recordings. The primary measure of conditioning (conditioned response, CR) was the expression of ‘food cup behavior’ during the CS. The food cup behavior was defined by distinct nose pokes into the recessed food cup, or by rats standing in front of and directly facing the food cup. Behavior was scored every 1.25 s during each 10 second preCS and CS periods. At each observation only one behavior was recorded (food cup or other). The number of food cup observations were summed and converted to a percentage of the total time during each period an animal spent at the food cup.

2.6. Statistical analysis

Behavioral data (i.e., food cup behavior) were analyzed with ANOVAs, *t*-tests and Fisher's LSD post hoc tests as appropriate. In all cases, $p < 0.05$ was considered significant. SPSS software was used for all statistical analyses. Two subjects from Experiment 1 did not complete behavioral training and were removed from all statistical analyses, one due to poor health (a male in the control condition) and the other due to technical malfunction during testing (a female in the control condition). Two subjects from Experiment 2 did not complete behavioral training due to poor health (one OVX + E in the control condition and one OVX + E in the experimental condition).

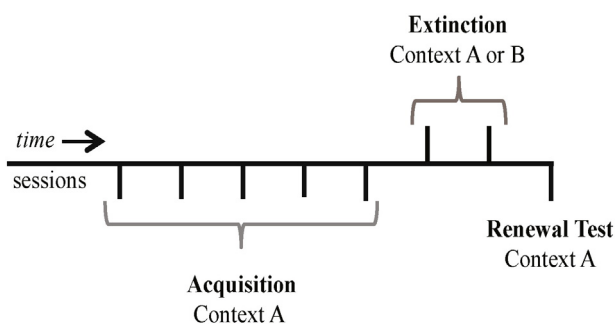


Fig. 1. Experimental design. Behavioral training consisted of three phases: conditioning (acquisition), extinction and renewal test. All rats received identical sessions throughout, except that experimental groups received extinction in a context different from the one in which the acquisition occurred, while the control groups remained in the same context throughout all training and testing (“ABA” design; counterbalanced across contexts). Rats received one session per day, and during each session there were presented with 8 tones (CSs) immediately followed by food (US) during acquisition sessions (CS–US pairings), but presented without USs during extinction and renewal.

3. Results

3.1. Experiment 1

3.1.1. Acquisition

Across training sessions during acquisition, all rats showed an increase in food cup responding (CRs) during CSs compared to their responding during preCSs which remained consistently low (Fig. 2; CRs during CS presentations $F(24) = 79.219$, $p < 0.001$; CRs during preCS $p > 0.05$). There were no sex or condition differences during acquisition ($p > 0.05$, both). During the last acquisition session (Acquisition 5), all rats showed high CRs during CSs (males: $62.60 \pm 2.9\%$; females: $61.56 \pm 3.1\%$) compared to their responding during preCSs (males: $19.79 \pm 2.9\%$; females: $17.50 \pm 4.4\%$; $t(29) = -18.613$, $p < 0.001$). There was no effect of sex, condition, or sex by condition for CRs during CSs on Acquisition 5 ($p > 0.05$, both).

3.1.2. Extinction

All rats showed a decrease in conditioned responding due to extinction training (Fig. 2). Rats in all groups (both sexes and conditions) had lower CRs to the CSs during extinction sessions compared to the last acquisition session (Table 1). This decrease was statistically significant between the total responding during last acquisition session (Acquisition 5) and second extinction session (Extinction 2) for males and females in both conditions ($t(29) = -12.767$, $p < 0.001$). We also compared responding during the first and last CSs of each extinction session. During the first CS of the first extinction session there was a significant effect of condition in CRs ($F(26,1) = 25.564$, $p < 0.01$). Experimental male and female rats expressed significantly lower CRs compared to control male and female rats ($p < 0.05$). During the last CS of Extinction Session 1, as well as the first and last CS of Extinction Session 2, all groups had similar responding and there was no effect of condition, sex, or condition by sex ($p > 0.05$). CRs during preCSs remained consistently low across all sessions and did not differ between groups ($p < 0.05$).

3.1.3. Renewal

During the test for renewal, only male groups showed differential conditioned responding (Fig. 3). An ANOVA (sex and condition) revealed a significant effect of condition on CRs during CSs ($F(1,26) = 10.097$, $p < 0.01$). There was no effect of sex ($p > 0.05$), however there

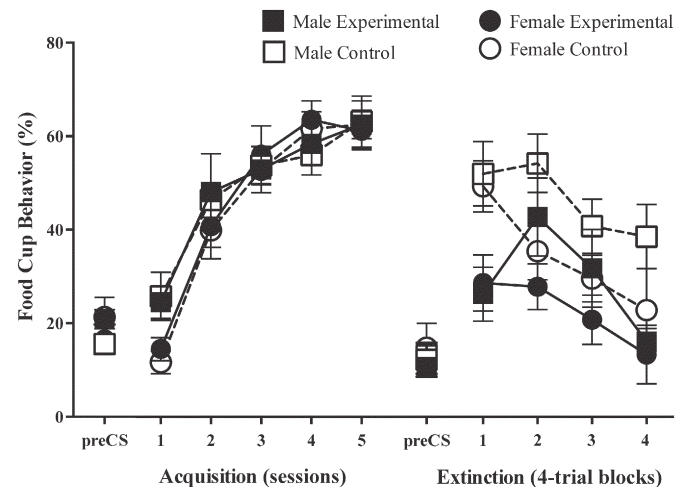


Fig. 2. Conditioned responses during acquisition and extinction in Experiment 1. Percentage of time rats expressed food cup behavior (mean \pm SEM) during the preCS and CS periods during training sessions. PreCS values are the average across all sessions for acquisition and extinction, respectively. Acquisition is shown as the average responding during each session. Extinction is shown as the average responding in 4-trial blocks (2 blocks per session; blocks 1 & 2 were trials during Session 1 and blocks 3 & 4 during Session 2).

Table 1

Conditioned responses during extinction in Experiment 1. Percentage of time rats expressed food cup behavior (mean \pm SEM) during the CSs on the final day of acquisition (Session 5) and during each extinction session.

Group	Acquisition 5	Extinction 1	Extinction 2
Male control	62.90 \pm 5.4	52.90 \pm 5.7	39.51 \pm 5.5
Male experimental	62.30 \pm 3.0	34.38 \pm 5.8	22.46 \pm 4.2
Female control	62.28 \pm 5.1	42.19 \pm 5.4	26.12 \pm 6.6
Female experimental	60.94 \pm 4.0	28.13 \pm 4.2	17.00 \pm 5.4

was a trend towards significance for condition by sex interaction ($p = 0.062$). Post hoc tests confirmed sex differences. Experimental males spent significantly more time expressing CRs during CSs compared to control males (male experimental: 42.77 \pm 3.2%, male control: 17.19 \pm 4.2%; $p < 0.01$). Experimental and control females showed similar rates of responding, with no significant difference between conditions (female experimental: 33.59 \pm 5.3%, female control: 27.46 \pm 6.9%; $p > 0.05$). CRs during preCSs remained low and did not differ between groups ($p > 0.05$). We found similar results when preCS responding was subtracted from CS (elevation scores) to assess learning independent of individual variability in responding. There was a significant effect of condition ($F(1,26) = 14.630$, $p < 0.01$), but no sex, or sex by condition effects ($p > 0.05$). Experimental males had significantly higher responding than control males (male experimental: 31.84 \pm 3.5%, male control: 5.80 \pm 4.9%; $p < 0.01$) while there was no significant difference between experimental and control females (female experimental: 23.83 \pm 4.8%, female control: 14.29 \pm 5.5%; $p > 0.05$). To further assess sex differences and lack of differential responding in females, we compared CRs during CS and preCS within each group during renewal tests. Paired t -tests confirmed the male experimental group had higher CRs during the CS compared to the preCS ($t(7) = -9.147$, $p < 0.001$), while CRs in male control group remained low during preCS and CS periods ($p > 0.05$). Both female groups had higher CRs during the CS period compared to the preCS (female control: $t(6) = -2.611$, $p < 0.05$; female experimental: $t(7) = -5.007$, $p < 0.01$).

3.2. Experiment 2

3.2.1. Acquisition

Across training sessions during acquisition, all rats showed an increase in CRs during CSs compared to their responding during preCSs, which remained consistently low (Fig. 4; CRs during CS presentations $F(4,172) = 72.702$, $p < 0.001$; CRs during preCS $p > 0.05$). There were no condition (control, experimental) or treatment (Intact, OVX, OVX + E) differences during acquisition ($p > 0.05$ for all). During the last acquisition session (Acquisition 5), all rats showed significantly

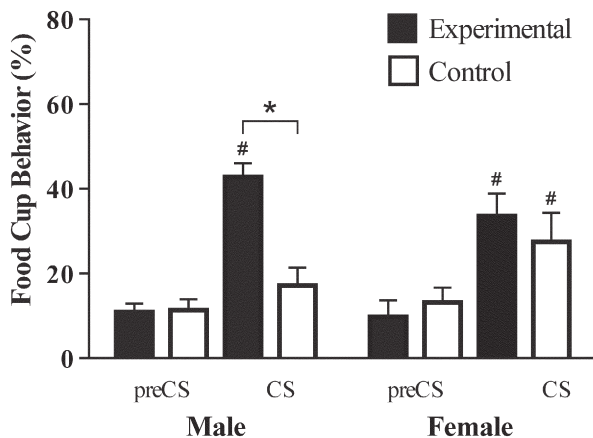


Fig. 3. Conditioned responses during the test for renewal in Experiment 1. Percentage of time rats expressed food cup behavior (mean \pm SEM) during preCS and CS periods; * indicates $p < .001$ and # indicates within-group preCS vs CS difference $p < 0.05$.

higher CRs during CSs (Intact females: 56.34 \pm 3.4%; OVX: 58.89 \pm 6.9%; OVX + E: 65.40 \pm 3.4%) compared to their responding during preCSs (Intact females: 17.77 \pm 2.9%; OVX: 19.34 \pm 4.3%; OVX + E: 16.4 \pm 3.7%; $F(1,43) = 462.026$, $p < 0.001$). Rats in all groups had similar, low CRs during preCSs on Acquisition 5 ($p > 0.05$, both).

3.2.2. Extinction

All rats showed a decrease in conditioned responding due to extinction training (Fig. 4). Rats in all groups had lower CRs to the CSs during extinction sessions compared to the last acquisition session (Table 2). This decrease was statistically significant between total responding during the last acquisition session and second extinction session (Extinction 2) for all groups ($F(1,40) = 154.189$, $p < 0.001$). During the first CS of the first extinction session there was a significant effect of condition in CRs during CSs ($F(1,40) = 20.489$, $p < 0.001$), but no effect of treatment, or treatment by condition interaction ($p > 0.05$). During the last CS of Extinction Session 1, as well as the first and last CSs of Extinction Session 2, all groups had similar responding ($p > 0.05$). CRs during preCSs remained consistently low across extinction sessions and did not differ between groups ($p > 0.05$).

3.2.3. Renewal

During the test, OVX + E rats in the experimental group showed renewal of conditioned responding compared to OVX + E rats in the control group, while there were no differences between experimental and control groups in OVX rats. The pattern of Intact rats was more complex: the experimental group showed renewal when baseline responding was accounted for (elevation scores [CS–preCS] and CS vs baseline [preCS]) even though their CRs were similar to that of intact controls during the CS (Fig. 5). An ANOVA (condition and treatment) revealed a significant effect of condition on CRs during CSs ($F(1,40) = 5.337$, $p < 0.05$), and no effect of treatment, or condition by treatment effect ($p > 0.05$). Post hoc tests confirmed that OVX + E rats showed renewal. Experimental OVX + E rats had significantly more CRs compared to control OVX + E rats (OVX + E experimental: 41.74 \pm 8.5%, OVX + E control: 20.98 \pm 8.5%; $p < 0.05$). Intact and OVX rats had similar patterns, and within each, the experimental and control groups showed similar responding, with no significant differences (Intact experimental: 32.23 \pm 5.6%, Intact control: 21.29 \pm 4.1%; OVX experimental: 24.02 \pm

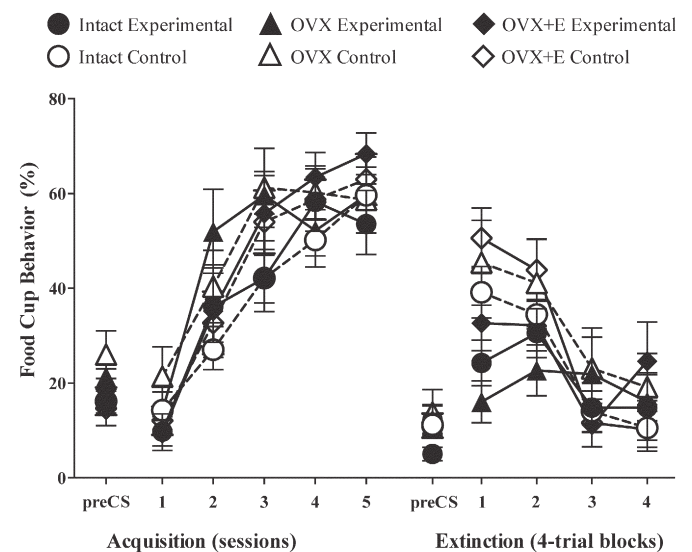


Fig. 4. Conditioned responses during acquisition and extinction in Experiment 2. Percentage of time rats expressed food cup behavior (mean \pm SEM) during the preCS and CS periods during training sessions. PreCS values are the average across all sessions for acquisition and extinction, respectively. Acquisition is shown as the average responding during each session. Extinction is shown in 4-trial blocks (2 blocks per session; blocks 1 & 2 were trials during Session 1 and blocks 3 & 4 during Session 2).

Table 2

Conditioned responses during extinction in Experiment 2. Percentage of time rats expressed food cup behavior (mean \pm SEM) during the CSs on the final day of acquisition and during each extinction session.

Group	Acquisition 5	Extinction 1	Extinction 2
Intact control	59.38 \pm 2.0	36.72 \pm 3.7	28.32 \pm 6.4
Intact experimental	53.32 \pm 6.7	27.34 \pm 3.7	17.38 \pm 4.9
OVX control	58.40 \pm 6.9	43.16 \pm 6.5	34.77 \pm 8.5
OVX experimental	59.38 \pm 8.0	19.34 \pm 2.9	22.46 \pm 6.1
OVX + E control	62.72 \pm 5.3	47.10 \pm 5.4	18.08 \pm 5.0
OVX + E experimental	68.08 \pm 4.4	32.37 \pm 2.6	29.24 \pm 7.4

6.3%, OVX control: 19.14 \pm 5.6%; $p > 0.05$). Conditioned responding during preCSs remained low and did not differ between groups ($p < 0.05$). To assess learning independent of individual variability in non-specific responding, we analyzed elevation scores (baseline, or preCS, responding subtracted from CRs during CS). Similar to behavior during CSs above, there was a significant effect of condition ($F(1,40) = 9.588$, $p < 0.01$), but no treatment, or condition by treatment effects ($p > 0.05$). Post hoc tests confirmed again that OVX + E experimental rats responded significantly higher than OVX + E control rats (OVX + E experimental: 34.82 \pm 7.9%, OVX + E control: 15.63 \pm 6.9%; $p < 0.05$). In addition, responding of Intact experimental rats was significantly higher than responding of Intact control rats (Intact experimental: 25.00 \pm 4.6%, Intact control: 8.01 \pm 5.1%; $p < 0.05$), while responding of OVX rats in both conditions remained similar (OVX experimental: 20.51 \pm 4.7%, OVX control: 14.06 \pm 4.6%; $p > 0.05$). As in Experiment 1, we compared CRs during CS and preCS within each group during renewal tests. Paired t -tests showed the OVX + E experimental group had higher CRs during the CS compared to the preCS ($t(6) = -4.416$, $p < 0.01$), while CRs in the OVX + E control group remained low during preCS and CS periods ($p > 0.05$). Both OVX groups had higher CRs during the CS period compared to preCS (OVX experimental: $t(7) = -4.320$, $p < 0.01$; OVX control: $t(7) = -3.081$, $p < 0.05$). Intact rats showed a pattern similar to OVX + E: the experimental group had higher CRs during the CS compared to the preCS ($t(7) = -5.420$, $p < 0.01$), while the control group expressed similar, low CRs during preCS and CS periods ($p > 0.05$).

4. Discussion

The current study found sex differences in renewal of conditioned responding to food cues and established an important role of estradiol in this behavior in females. To accomplish this, we tested males and females in context-dependent renewal of appetitive Pavlovian conditioned responding. Much of recent work on appetitive renewal used instrumental conditioning, drugs as reinforcers, or examined only males (e.g., [10,16]). The current study complements prior work with comparisons of responding to food cues in both sexes.

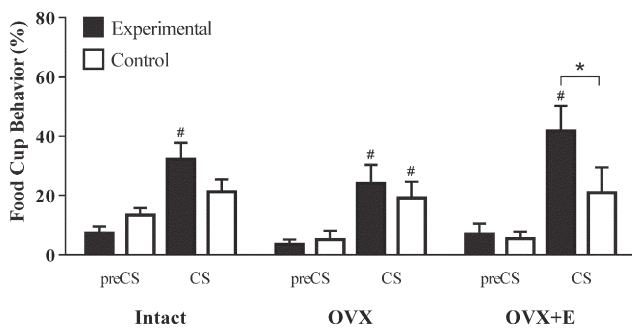


Fig. 5. Conditioned responses during the test for renewal in Experiment 2. Percentage of time rats expressed food cup behavior (mean \pm SEM) during preCS and CS periods; * indicates $p < 0.05$ and # indicates within-group preCS vs CS difference $p < 0.05$.

Here, we found sex differences specifically during the test for renewal, but not during learning acquisition or extinction of conditioned responses. The observed sex difference in renewal, therefore, was not due to differential learning acquisition or extinction of the tone–food association, or females' inability to distinguish between contexts. In Experiment 1, male rats displayed renewed food seeking after extinction when tested in the acquisition context, while renewal in females was ambiguous. Males in the experimental groups had higher food cup behavior to the tone (CS) than the males in the control group, whose responding remained low during preCS and CS. Both female groups responded similarly and each expressed higher food cup behavior during the tone compared to the baseline (preCS) period. This high expression of conditioned responses in the control condition suggests poor extinction retention in females. Therefore it cannot be disambiguated whether food cup behavior in the experimental group, which was similar to controls, is due to specific context-induced renewal or poor extinction retention.

In Experiment 2, we examined the potential role of estradiol by comparing behavior of females with no gonadal steroid hormones (OVX) to those with estradiol replacement (OVX + E), along with intact females. The acquisition and extinction of conditioned responses was similar across the three groups. Interestingly, we found renewal of food seeking in rats with estradiol replacement, but not in ovariectomized females. OVX + E rats in the experimental groups had higher conditioned responding to the food cue than the OVX + E rats in the control group, whose responding remained low during preCS and CS. In contrast, both OVX groups responded similarly and each had higher responses during the tone (CS) compared to the baseline (preCS) period. This pattern was similar to that of intact rats in Experiment 1, and suggests poor extinction retention in OVX control group. Consequently, the responding in the experimental OVX group could reflect context-specific renewal and/or poor extinction retention. Renewal in intact rats was again inconsistent. Similar to findings in Experiment 1, there were no differences in conditioned responses between the two groups of intact females; however, when responding was expressed as an elevation score (preCS subtracted from CS), it was significantly higher in the experimental compared to the control group. Furthermore, only the experimental group had higher food cup behavior during the tone (CS) compared to the baseline (preCS) confirming that this renewal was context-induced and not due to impaired extinction memory. It should be noted, during behavioral training and testing the amount of estradiol was constant in OVX + E, but was likely variable and insufficient in intact rats depending on the stage of estrous cycle, which may explain different the behavioral outcomes in Experiments 1 and 2.

This is the first evidence there are sex differences in renewal of extinguished food cue responding. Indeed, prior work has demonstrated successful context-induced renewal in female rats [10,11,14, 48,53]. There were important procedural differences between the current and prior studies with Pavlovian conditioning that likely account for differential findings. In the current study, there was no pre-exposure to the behavioral apparatus (contexts) prior to conditioning, and there were fewer extinction sessions than in the prior work [9,11, 53]. Therefore, lack of context pre-exposure and shorter extinction is likely why females were impaired in our preparation.

Context-induced renewal involves complex processing across acquisition, extinction, and test, which depend on accurate encoding, memory, and use of different contexts. According to the role of context in 'setting the occasion' for reinforcement, or non-reinforcement, of the primary conditioned cue, one context is aiding retrieval of the CS–US association (the acquisition context) while the other context is aiding retrieval of the CS–no US association (the extinction context) [7,23]. Therefore, behavioral renewal during tests in the acquisition context illustrates an ability to accurately retrieve and use context-dependent memory. It requires evaluation of the memory of each context and CS experience in it, and the current context interpretation is then used to guide responding—a function akin to decision-making. Sex differences

in the conditioned responding during context-dependent renewal in the current study therefore might reflect differences in context interpretation (including extinction memory), or its use (context-induced renewal) to guide responding in males and females.

The current findings are in agreement with prior evidence for sex differences in associative learning and contextual processing (for review see [18]). For example, male rats showed greater contextual conditioned fear, and faster acquisition of this learning, compared to female rats ([29]; but see other strains: [38]). Another study using aversive conditioning and contextual cues found that male rats demonstrated context discrimination, as they avoided the fear conditioned context but not a novel context, up to seven days post conditioning, while female rats properly expressed discrimination one day after training but later expressed fear avoidance in both contexts. This context generalization by the females was, in part, dependent on estrogen [28]. Similarly, a study that compared food consumption in neutral and aversive contexts found that males showed appropriate fear–anorexia, they inhibited feeding only in an aversive conditioned context, while females inhibited feeding in both contexts [40]. The lack of renewal of conditioned behavior in females in the current study might also be due to generalization in the use of contextual cues during testing, which might be improved with additional training, including context pre-exposure, as mentioned above.

These findings also agree with prior evidence that estradiol is an important modulator of learning and memory. The loss of estrogen due to menopause can have significant effects on executive functions in women (for review see [43]). Furthermore, working memory impairments due to the decline in estrogen during aging in monkeys was improved by treatment with cyclic estradiol [39]. Estrogen is also important in Pavlovian conditioning and extinction ([3,22]; for a review of gonadal hormones effect on fear extinction see [25]).

Interestingly, estrogen enhances some forms of learning but impairs others, and this modulation in part depends on the amounts of circulating estrogen and whether it is continuous or cycling. For example, what memory strategy females use to solve a maze depends on estradiol levels; when high, female rats are biased to use place memory but when low, they are biased to use response memory [24]. Fear extinction during proestrus (high estradiol and progesterone) was enhanced, compared to metestrus (low estradiol and progesterone) phase, and an estrogen receptor-beta agonist facilitated extinction recall in intact female rats [25,31]. High levels of estradiol impaired performance on a long delayed alternation T-maze (non-spatial, working memory task) but low levels facilitated performance on the maze at a shorter delay [51]. Furthermore, cyclic, but not continuous, estradiol replacement facilitated acquisition of spatial place-learning in an open-field tower maze [26]. In the current study, estradiol treatment to ovariectomized rats was continuous and that was the only group that showed consistent renewal. These results suggest that females' responding to previously extinguished food cues depends on constant estradiol levels, either because it is needed during all stages of the task or because it was available during the critical stage. It might also be important that the estradiol treatment here continued for 22 days prior to testing. Chronic treatment improved radial arm maze performance in ovariectomized rats, while short-term estradiol treatment prior to testing did not alter performance [27].

In addition to estradiol, progesterone is also secreted by the ovaries and fluctuates during estrous cycle, and has been shown to facilitate extinction recall and enhance spatial learning [4,20,31]. Therefore, progesterone may also be an important modulator of renewal of conditioned food seeking, and its insufficient amounts may have contributed to inconsistencies in renewal in intact females observed here. Nevertheless, the current results demonstrated that after ovariectomy, estradiol treatment alone is sufficient to induce renewal after extinction. Future work could address if progesterone, or combined estradiol and progesterone, treatment could further enhance renewal in females, and whether normal cycling of these hormones modulates motivation to respond to food cues.

5. Conclusions

We found sex differences in context-dependent renewal of extinguished responding to food cues, using Pavlovian conditioned procedures. We also demonstrated estradiol is an important mediator of renewal processing in female rats. Mechanisms underlying renewal of suppressed responding to food cues are informative to our inability to resist palatable foods and change maladaptive eating habits, similar to the mechanisms of drug use relapse [13]. The results presented here show that in addition to its well-known effects on consumption, estradiol is also important for renewed food seeking driven by food cues (for reviews see [2,19]).

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