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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.

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 measuring 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