Neurobiology of Learning and Memory 139 (2017) 11–21

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Sex specific recruitment of a medial prefrontal cortex-hippocampal-thalamic system during context-dependent renewal of responding to food cues in rats

Lauren C. Anderson, Gorica D. Petrovich *

Department of Psychology, Boston College, 140 Commonwealth Avenue, Chestnut Hill, MA 02467-3807, USA

ARTICLE INFO

Article history:
Received 8 September 2016
Revised 10 November 2016
Accepted 2 December 2016
Available online 8 December 2016

Keywords:
Conditioning
Context
Renewal
Appetitive
Medial prefrontal cortex

ABSTRACT

Renewal, or reinstatement, of responding to food cues after extinction may explain the inability to resist palatable foods and change maladaptive eating habits. Previously, we found sex differences in context-dependent renewal of extinguished Pavlovian conditioned responding to food cues. Context-induced renewal involves cue-food conditioning and extinction in different contexts and the renewal of conditioned behavior is induced by return to the conditioning context (ABA renewal). Male rats showed renewal of responding while females did not. In the current study we sought to identify recruitment of key neural systems underlying context-mediated renewal and sex differences. We examined Fos induction within the ventromedial prefrontal cortex (vmPFC), hippocampal formation, thalamus and amygdala in male and female rats during the test for renewal. We found sex differences in vmPFC recruitment during renewal. Male rats in the experimental condition showed renewal of responding and had more Fos induction within the infralimbic and prelimbic vmPFC areas compared to controls that remained in the same context throughout training and testing. Females in the experimental condition did not show renewal or an increase in Fos induction. Additionally, Fos expression differed between experimental and control groups and between the sexes in the hippocampal formation, thalamus and amygdala. Within the ventral subiculum, the experimental groups of both sexes had more Fos compared to control groups. Within the dorsal CA1 and the anterior region of the paraventricular nucleus of the thalamus, in males, the experimental group had higher Fos induction, while both females groups had similar number of Fos-positive neurons. Within the capsular part of the central amygdalar nucleus, females in the experimental group had higher Fos induction, while males groups had similar amounts. The differential recruitment corresponded to the behavioral differences between males and females and suggests the medial prefrontal cortex-hippocampal-thalamic system is a critical site of sex differences during renewal of appetitive Pavlovian responding to food cues. These findings provide evidence for novel neural mechanisms underlying sex differences in food motivation and contextual processing in associative learning and memory. The results should also inform future molecular and translational work investigating sex differences and maladaptive eating habits.

1. Introduction

Learned associations between cues from the environment and biologically important events can largely impact our behavior. Cues associated with food can stimulate appetite and food consumption independently of hunger (for review see Petrovich, 2013) and responding to food cues has been correlated with long-term weight gain (Boswell & Kober, 2016; Sun et al., 2015). Food cues can drive these behaviors even after extinction, because the original learned associations continue to exist, evidenced by spontaneous recovery and other forms of renewal of responding (Bouton, 2004; Rescorla, 2004). Renewal, or reinstatement, of responding to previously extinguished food cues may help explain the difficulty associated with changing unhealthy eating habits—persistent cravings and the inability to resist palatable foods even when eating is maladaptive (Boutelle & Bouton, 2015; Todd, Winterbauer, & Bouton, 2012). This model was recently introduced as a framework to study the relapse of palatable food seeking during dieting, based on the reinstatement model of relapse of drug use (Calu, Chen, Kawa, Nair, & Shaham, 2014).

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

http://dx.doi.org/10.1016/j.nlm.2016.12.004
1074-7427/

Here, we sought to identify key neural systems underlying context-mediated renewal and sex differences by assessing Fos induction. We examined renewal of conditioned responding to Pavlovian food cues with an adapted ABA protocol (Bouton & King, 1983). In this preparation a return to the context in which the initial learning occurred induces robust responding to the cues that were extinguished elsewhere. Recently, we found sex differences in the ABA protocol where male rats exhibited renewal of responding, while behavior of females was inconsistent and successful renewal depended on estradiol (Anderson & Petrovich, 2015). Males and females learned the acquisition and extinction of Pavlovian cue–food associations similarly, but only males showed robust renewal of responding to the food cue. A comparison of intact females with ovariectomized females with, and without, estradiol replacement found only the group with estradiol replacement exhibited renewal of responding. These behavioral sex differences are in agreement with accumulating reports of differences between males and females during associative learning and contextual processing (e.g. Dalla, Papachristos, Whetstone, & Shors, 2009; Farrell, Sengelaub, & Wellman, 2013; Maren, De Oca, & Fanselow, 1994; Reppucci, Kuthyar, & Petrovich, 2013).

We hypothesized the ventromedial prefrontal cortex (vmPFC) is critical during renewal and would be a site of sex differences due to its well-known executive function in decision-making and behavioral guidance (Dalley, Cardinal, & Robbins, 2004; O'Doherty, 2011) and its role in associative learning, including renewal (Eddy, Todd, Bouton, & Green, 2016; Willcocks & McNally, 2013). Additionally, we examined three areas connected with the vmPFC and important for associative learning, contextual processing, and the control of food consumption: the hippocampal formation, thalamus, and amygdala. The hippocampal formation is critical for contextual processing and body weight regulation and has been implicated in context-dependent renewal of aversive and appetitive behaviors (Benoit, Davis, & Davidson, 2010; Davidson et al., 2009; Fanselow, 2000; Holland & Bouton, 1999; Marinelli, Funk, Juzytsch, Li, & Le, 2007; Orsini, Kim, Knapska, & Maren, 2011). The thalamus, specifically the paraventricular nucleus (PVT), has been implicated in context-induced renewal (Hamlin, Clemens, Choi, & McNally, 2009) and is involved in the regulation of food consumption (Bhatnagar & Dallman, 1999; Cole, Mayer, & Petrovich, 2015; Stratford & Wirtshafter, 2013). The amygdala is important for appetitive associative learning and subsequent control of behavior (Cole, Hobin, & Petrovich, 2015; Cole, Powell, & Petrovich, 2013; Crombag & Shaham, 2002; Holland & Petrovich, 2005). The current study examined Fos induction during context-mediated renewal of responding to food cues in these key brain regions, and compared the patterns in male and female rats.

2. Materials and methods

2.1. Animals

32 adult male and female Long-Evans rats weighing 250–275 g at arrival (Charles River Laboratories; Portage, MI) were individually housed and maintained on a 12 h light/dark cycle (lights on at 07:00). Males and females were housed in separate colony rooms. After arrival, subjects were allowed one week to acclimate to the colony room 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.

2.2. Apparatus

The behavioral training was conducted in identical behavioral chambers (30 × 28 × 30 cm; Coulbourn Instruments; Allentown, PA) located in a room different from 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), equipped with a ventilation fan (55 dB), and a video camera attached to a recording system (Coulbourn Instruments; Allentown, PA). The conditioned stimulus (CS) was a 10 s tone (75 dB, 2 kHz), and 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 solution (Fisher Scientific; Fair Lawn, NJ) was sprayed onto the tray below the grid floor.

2.3. 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 and extinction occurred in different contexts while renewal occurred in the same context as conditioning (Bouton & King, 1983). Rats in the control condition remained in the same context across all training phases. During the acquisition phase, rats were trained for five days, with one 34-min 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-min 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 (ABA or BAB), while rats in the control condition received extinction training in the same context as acquisition (AAA or BBB). The test for renewal was one 34-min session with eight CS presentations and no USs, conducted in the conditioning (acquisition)

	Acquisition 5 sessions	Extinction 2 sessions	Renewal Test 1 session
Experimental	A+	B-	A-
Control	A+	A-	A-

Fig. 1. Experimental design. **A** denotes training in Context A, **B** denotes training in Context B (contexts were counterbalanced). Each training session consisted of eight presentations of either CS-US (denoted as +), or CS alone (denoted as −). All animals were sacrificed 90 min after the end of Renewal test and brains were collected for Fos induction detection by immunohistochemistry.