Appetite 59 (2012) 437–447

Contents lists available at SciVerse ScienceDirect

Appetite

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

Research report

Learned food-cue stimulates persistent feeding in sated rats ☆

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ARTICLE INFO

Article history:
Received 10 March 2012
Received in revised form 30 May 2012
Accepted 8 June 2012
Available online 18 June 2012

Keywords:
Appetite
Conditioning
Learning
Obesity
Obesogenic environment
Overeating
Palatable food
Priming

ABSTRACT

Cues that predict food can stimulate appetite and feeding independent of physiological hunger. How long such effects might last is currently unknown. Here we began to characterize long-term effects in a rodent model of cue-potentiated feeding. Rats were conditioned to associate a tone with food pellets distinct from their regular laboratory chow, and then were tested along with controls for food consumption following tone presentations. In Experiment 1, rats were tested under sated or food-deprived conditions to determine whether fasting would augment cue-driven feeding. Rats in the control group regulated intake based on physiological state, while conditioned rats consumed similar large amounts of food regardless. Experiment 2 tested the durability of cue-potentiated feeding to repeated testing in sated rats. We observed robust cue-potentiated feeding during the first two tests, while in the third and fourth tests both groups ate similar large amounts of pellets. In both experiments the conditioned tone-cue induced binge-like consumption of the cued food and persistent feeding for the duration of 4-h tests. Rats then failed to adjust daily chow consumption to account for their increased intake post-cue. In summary, brief cue priming stimulated substantial intake in sated states that was behaviorally uncompensated for by homeostatic mechanisms.

Introduction

Food associated cues can stimulate feeding independent of physiological hunger in animals and humans. In experimental settings both discrete cues, such as a tone previously paired with food, and contextual cues, such as a feeding environment, have been shown to potentiate feeding in sated states (Holland, Petrovich, & Gallagher, 2002; Petrovich, Ross, Gallagher, & Holland, 2007; Weingarten, 1983). In these settings food-cues drive consumption of the signaled food specifically and selectively, and in humans this is accompanied with a greater reported desire for that food (Delamater & Holland, 2008; Fedoroff, Polivy, & Herman, 1997; Ferriday & Brunstrom, 2008; Galarce, Crombag, & Holland, 2007; Petrovich, Ross, Gallagher, et al., 2007; Petrovich, Ross, Holland, & Gallagher, 2007). However, in some settings if the signaled food is absent, learned cues have also been shown to increase daily consumption of the available food option (Boggiano, Dorsey, Thomas, & Murdaugh, 2009). These circumstances enable an organism to consume a large meal when not hungry (Petrovich, Ross, Gallagher, et al., 2007; Petrovich, Ross, Holland, et al., 2007; Weingarten, 1984).

How long such appetite and feeding might persist is unknown. Non-homeostatic driven feeding is becoming an increasingly important contributor to the regulation of caloric intake and body weight, as overeating and associated obesity are becoming more prevalent in the developed world (World Health Organization, 2011). Indeed, eating dysregulation (e.g., overeating) is believed to drive the obesity epidemic to a much greater extent than metabolic deficiencies (Berthoud, 2011; Hill, Wyatt, Reed, & Peters, 2003; Kessler, 2009; Small, 2009; Volkow & Wise, 2005). To begin to characterize possible long-term effects of cue-potentiated feeding, here we tested rats during an extended period following priming with a food-cue. We used a novel behavioral preparation designed to allow for long duration testing with minimal disruptions.

In prior work, cue-potentiated feeding tests were typically carried out in behavioral chambers without access to water (for review see Holland & Petrovich, 2005; Petrovich, 2011). In the current study all tests were conducted in rats' home cages with unlimited water access. Thus, this preparation allowed us to monitor intake over extended periods and with minimal disturbance to the rats. Additionally, conducting tests in the home cage enabled us to isolate the effect of the discrete food-cue from any potential conditioned effects of the training context (see General Discussion), which alone can influence food intake (Boggiano et al., 2009; Bouton, 2011; Le Merrer & Stephens, 2006; Petrovich, Ross, Gallagher, et al., 2007; Petrovich, Ross, Holland, et al., 2007).

☆ *Acknowledgments:* We thank Pari Mody and Katherine Gildersleeve for technical assistance, and Professor Michela Gallagher for generous space and equipment support for the first experiment. This research was supported in part by NIH Grants MH67252 and DK085721 to G.D.P.

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0195-6663/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.appet.2012.06.007

We trained rats to associate a tone with food pellets that are distinct from their regular laboratory chow. Rats in the conditioned group were repeatedly presented with a tone (conditioned stimulus, CS) immediately prior to food pellet delivery (unconditioned stimulus, US). Rats in the control group were given the same number of tones and food presentations, but randomly arranged. After training we tested rats for food consumption under the influence of the CS.

Food consumption tests consisted of ten CS presentations immediately followed by *ad libitum* access to the training food pellets and standard laboratory chow. The tests were 4 h long, and we additionally monitored post-test daily chow intake. In Experiment 1 we varied physiological hunger state during testing to determine whether fasting would augment cue-driven feeding and extend the duration of the effect. Thus, we tested each rat under sated and fasted conditions in a counterbalanced manner. In Experiment 2 we tested sated rats repeatedly to determine the durability of the cue-potentiated feeding effect to repeated testing.

Experiment 1. The effect of physiological hunger state on cue-potentiated feeding

Materials & methods

Subjects

Sixteen experimentally naïve, male Long–Evans rats approximately 2 months of age (Charles River Laboratories; Raleigh, NC), were individually housed, and maintained on a 12 h light/dark cycle (lights on at 6:00). All training and testing was conducted during the light phase, approximately between 9:00 and 14:00. Upon arrival, subjects were allowed one week to acclimate to the colony room, during which time they had *ad libitum* access to standard laboratory chow (18% Protein Rodent Diet #2018, Harlan Teklad Global Diets; Madison, WI; 3.1 kcal/g; 20% protein, 18% fat, 58% carbohydrate) and water, 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 Johns Hopkins University Institutional Animal Care and Use Committee.

Apparatus

Behavioral training was conducted in a set of four identical chambers ($30 \times 24 \times 30$ cm; Colbourn Instruments; Allentown, PA), with aluminum top and sides, a transparent Plexiglas back and front, and a grid floor. Each chamber also contained a recessed food cup (3.2×4.2 cm). Dim background illumination was provided by two 25 W red bulbs, each placed 1.5 m from the chambers. Masking noise (60 dB) was provided by ventilation fans located outside the conditioning chambers. A tone (1.5 kHz, 75 dB), served as the CS, and 45 mg food pellets (5TUL; Test Diets; Richmond, Indiana; 3.4 kcal/g; 20% protein, 13% fat, 67% carbohydrate) were used as the US. These food pellets have a similar caloric density and macronutrient energy composition to standard laboratory chow, with the exception that the carbohydrates are from starch in the chow and sucrose in the pellets. Video cameras attached to videocassette recorders were placed in the back of the test chambers to record behavior for 10 s periods both before and during stimulus presentation. Stimulus presentation and videocassette recorders were controlled by LabView software (National Instruments; Austin, TX) run on Macintosh computers (Apple Computers; Cupertino, CA).

Behavioral training procedure

Before behavioral training, rats were gradually reduced to 85% of their *ad libitum* weight. After a shaping procedure in which rats learned to eat from the food cup, rats received 10 training sessions (one session per day, excluding weekends) each approximately 32 min in length. For half of the rats (conditioned group, Paired), these sessions consisted of eight presentations of the CS, a 10 s tone, immediately followed by delivery of the US, two food pellets, into the food cup. For the other half of the rats (control group, Unpaired), the sessions consisted of the same number of tone and food presentations as the Paired group, but delivered in a non-conditional random order. After the last training session, rats were given *ad libitum* access to standard laboratory chow for 8–10 days to allow them to reach at least 110% of their pre-training body weight. During this time, rats were habituated to a new testing room and to glass dishes ($107 \times 87 \times 70$ mm) that would be used for food presentation during testing.

Rats completed two consumption tests each, which occurred three days apart in a counterbalanced design. For one of the tests, rats were deprived of food for 24-h prior to testing (“food-deprived condition”) and for the other test rats remained under *ad libitum* access to standard laboratory chow (“sated condition”). For each test rats were transported to the testing room, and for the sated condition all chow was removed from the cage just prior to transport. Rats remained in their home cages and were given 10 presentations of the CS (10 s tone) over 5 min. Rats were then immediately given 20 g of chow in one glass dish and 20 g of food pellets in a second identical glass dish, and returned to the colony room. After 30 min all uneaten chow and pellets were removed and replaced with fresh chow (20 g) and food pellets (20 g). This process was repeated at 1 h and 2 h after the tone test. At the 4-h time point pellets were removed and only chow was replaced (100 g); chow consumption 20 h later was measured in order to calculate 24 h post-test chow consumption. For all time points, remaining chow and food pellets were weighed and the amount consumed, during the interval as well as cumulative total, was calculated.

Rats were trained in two replications ($n = 4$ per condition for each) that were identical except that the period from the end of training and start of testing was 8 days in the first replication, and 10 days in the second replication.

Behavioral observations

To confirm that the rats in the Paired group had learned the tone-food association, conditioning was assessed during the last training session (S10). The expression of “food cup behavior” was the primary measure of conditioning, the conditioned response (CR). Food cup behavior included nose pokes into the recessed food cup, and standing in front of and facing the food cup. Observations were made every 1.25 s and were paced by auditory signals recorded onto the tapes. Observers were “blind” with respect to the training group of the rats observed. At each observation, only one behavior was recorded (food cup or other). Food cup behavior during the10 s tone (CS) and for the 10 s immediately preceding the CS (Pre-CS) was scored. The percentage of time rats spent expressing food cup behavior during these two periods was calculated by dividing the number of positive observations of food cup behavior by the total number of observations made.

Statistics

Behavioral data were analyzed using appropriate ANOVAs and *t*-tests in SPSS. In all cases, $p < 0.05$ was considered significant.

Results

Training

Learning was assessed during the last training session (Fig. 1). Conditioning of the rats in the Paired group was clearly evident from observations of the conditioned responses (CRs) directed