IMPACT OF HUNGER STATE ON PALATABLE FOOD-CUE ASSOCIATIVE LEARNING AND CONSUMPTION IN ADULT AND ADOLESCENT MALE AND FEMALE RATS

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Hunger and palatability modulate food intake through homeostatic and hedonic mechanisms that can work either independently, or in tandem. Both also influence foodseeking and learning about cues for food. Our prior work suggested that hunger and satiety impact motivation for palatable food differently in males and females. Sensitivity to food rewards also differs between adolescence and adulthood. Adolescents exhibit heightened motivation to consume and work for palatable food compared to adults. However, sex and age differences in palatable food motivation under sated and hungry conditions have not been explored. Here, we examined how rats consume and learn about palatable food. To assess cue-food associative learning and memory, rats underwent Pavlovian conditioning, extinction, and testing to assess the renewal of conditioned behavior. Male and female adult and adolescent Sprague Dawley rats were either food restricted (85% ad libitum body weight) or had *ad libitum* access to regular chow (n=8 per group). Rats learned palatable food-cue associations across 8 acquisition sessions, followed by cue-only presentations for 4 extinction sessions in a different context. They were then tested for renewal of conditioned responding (time spent at the food cup) to the food cue in the acquisition compared to extinction context. Before learning and after renewal testing, rats were tested for consumption of palatable food and chow in their home cage (1hr test/day per food). We found that adults and adolescents of both sexes were able to learn, extinguish, and renew conditioned responding regardless of hunger. Adolescents consistently had higher responding than adults across the learning and memory protocol. During consumption testing, females of both ages ate more palatable food than males, particularly when sated. Adolescent consumption was dependent on hunger state. Sated adolescents ate more palatable food than adults, and both sated adults and adolescents showed a preference for palatable food over chow. Food-deprived adolescents and adults ate similar amounts of chow, but only adults showed a preference for palatable food over chow. Palatable food consumption and conditioned responding during early acquisition training for that same food were positively correlated, indicating that hunger and satiety similarly impact palatable food-cue learning and consumption. Overall, these findings suggest that physiological hunger is not a prerequisite for successful associative learning and memory during adolescence or adulthood, and that female sensitivity to palatable food is present during adolescence.

TABLE OF CONTENTS

TABLE OF CONTENTS	iv
LIST OF TABLES	v
LIST OF FIGURES	vi
[1] Introduction	1
[2] Materials and methods	4
[2.1] Subjects	4
[2.1.1] Experiment 1	
[2.2] Apparatus	7
[2.3] Palatable food	7
[2.4] Behavioral training and testing procedures	
[2.4.1] Habituation	
[2.4.2] Food Consumption tests	9
[2.4.3] Cue-food learning, extinction, and renewal	9
[2.4.4] Quantification of conditioned behavior	10
[2.5] Statistical analysis	10
[3] Results	12
[3.1] Experiment 1	
[3.1] Experiment 1	12 12
[3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training	12 12 15
 [3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training [3.1.3] Renewal Tests 	12 12 15 18
 [3.1] Experiment 1	12 12 15 18 19
 [3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training [3.1.3] Renewal Tests [3.1.4] Consumption Tests [3.1.5] Correlations between learning and consumption 	12 12 15 18 19 21
 [3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training [3.1.3] Renewal Tests [3.1.4] Consumption Tests [3.1.5] Correlations between learning and consumption [3.2] Experiment 2 	12 12 15 18 19 21 23
 [3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training [3.1.3] Renewal Tests [3.1.4] Consumption Tests [3.1.5] Correlations between learning and consumption [3.2] Experiment 2 [3.2.1] Acquisition Training 	12 12 15 18 19 21 23 23
 [3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training [3.1.3] Renewal Tests [3.1.4] Consumption Tests [3.1.5] Correlations between learning and consumption [3.2] Experiment 2 [3.2.1] Acquisition Training [3.2.2] Extinction Training 	12 12 15 15 18 19 21 23 23 23 26
 [3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training [3.1.3] Renewal Tests [3.1.4] Consumption Tests [3.1.5] Correlations between learning and consumption [3.2] Experiment 2 [3.2.1] Acquisition Training [3.2.2] Extinction Training [3.2.3] Renewal Tests 	12 12 15 15 18 19 21 23 23 23 26
 [3.1] Experiment 1	12 12 15 15 18 19 21 23 23 23 26
 [3.1] Experiment 1	12 12 15 15 18 19 21 23 23 23 26 30 31 34
 [3.1] Experiment 1	12 12 15 18 19 21 23 23 23 23 30 31 34 37
 [3.1] Experiment 1	12 12 15 18 19 21 23 23 23 26
 [3.1] Experiment 1 [3.1.1] Acquisition Training [3.1.2] Extinction Training [3.1.3] Renewal Tests [3.1.4] Consumption Tests [3.1.5] Correlations between learning and consumption [3.2] Experiment 2 [3.2.1] Acquisition Training [3.2.2] Extinction Training [3.2.3] Renewal Tests [3.2.4] Consumption Tests [3.2.5] Correlations between learning and consumption 	12 12 15 18 19 21 23 23 23 26 30 31 34 37 40
 [3.1] Experiment 1	12 12 15 18 19 21 23 23 23 26 30 31 34 37 37 4 0 4 6

LIST OF TABLES

Table 1 Foodcup behavior during extinction training sessions 1-4	17
Table 2 Foodcup behavior during extinction training sessions 1-4	28

LIST OF FIGURES

Figure 1 Acquisition training sessions 1-8 (A1-A8)	13
Figure 2 Acquisition sessions 1 and 2, by trial	14
Figure 3 Extinction training sessions 1-4 (E1-E4)	16
Table 1 Foodcup behavior during extinction training sessions 1-4	17
Figure 4 Context-induced renewal of conditioned responding during tests	18
Figure 5 Food consumption tests	20
Figure 6 Correlations between consumption and learning	22
Figure 7 Acquisition training sessions 1 through 8 (A1-A8)	23
Figure 8 Acquisition training sessions 1 and 2, by trial	25
Figure 9 Extinction training sessions 1-4 (E1-E4)	27
Figure 10 Extinction training sessions 1-4 (E1-E4)	29
Figure 11 Context-induced renewal of conditioned responding after extinction	31
Figure 12 Food consumption tests	33
Figure 13 Correlations between consumption and learning	35

[1] Introduction

In animals, the motivation to consume food relies on the interplay between homeostatic and hedonic mechanisms. Homeostatic mechanisms influence food intake by integrating neural, hormonal, and gastric signals to meet an organism's continuous metabolic demands. Food intake is also impacted by factors independent of the internal need to regulate energy balance. Many foods with palatable properties can stimulate appetite and drive feeding, even in the absence of physiological hunger (Egecioglu et al., 2011; Yeomans et al., 2004).

In addition to internal hunger and pleasure signals, external stimuli can impact food seeking and consumption. These include sensory signals from the environment which can indicate the presence of food (food cues). Through associative learning, individuals form connections between these environmental cues and food rewards. Over time, these learned associations become robust modulators of motivation to seek and consume food and can activate neural reward systems without hunger, or the presence of food itself (Berridge et al., 2010; Kanoski & Boutelle, 2022; Weingarten, 1983). Especially when primed with food-cues, palatable foods are harder to resist, which is known to contribute to obesity and other maladaptive overeating behaviors (Belfort-DeAguiar & Seo, 2018; Cornell et al., 1989; Johnson, 2013; Petrovich, 2013; Yeomans et al., 2001).

Like other motivated behaviors, feeding can be dissected into two phases: appetitive and consummatory (Immelmann & Beer, 1989; Keen-Rhinehart et al., 2013; Craig, 1917). The appetitive phase, also known as the anticipatory phase, is characterized by its flexibility and occurs prior to attaining a goal. It encompasses food-seeking

behaviors such as approach, foraging, and searching, all aimed at reaching the desired outcome. On the other hand, the consummatory phase involves a series of actions that fulfill the end goal, such as food ingestion or drinking (Craig, 1917; Wallace, 1979).

One method to assess appetitive motivation for food involves measuring an animal's response to food-cues. Specifically, the acquisition of Pavlovian conditioned responding, its extinction, and the subsequent renewal of that behavior can serve as valuable behavioral indicators of motivation to learn and access memory about food-cues (Bouton, 2011). However, it is currently unclear how the motivation to seek food and learn about food-predicting cues may vary across the spectrums of hunger and palatability. It is assumed that food seeking and consumption of palatable foods would be impacted similarly, but this has not been examined systematically. Even less is known about how these behaviors may differ between sexes. Most prior studies on food motivation have been conducted using only male subjects (Barbano & Cador, 2005), but it is important to consider that males and females often respond to hunger and palatable food differently. For instance, binge eating and overeating behaviors are significantly more prevalent in females than males (Klump et al., 2013; Lovejoy & Sainsbury, 2009; Sample & Davidson, 2018; Sinclair et al., 2017). Importantly, females tend to be more sensitive to consuming palatable food than males (Babbs et al., 2011; Freeman et al., 2021), particularly when sated (Buczek et al., 2020). Furthermore, prior work found that food-deprived males and females learned and extinguished food-cue associations similarly, but the sexes differed in context-mediated renewal of extinguished conditioned behavior (Anderson & Petrovich, 2015, 2017). Similar sex differences during contextmediated renewal have also been found with alcohol rewards (Segal et al., 2022). To our

knowledge, behavioral patterns of males and females during cue-food learning and memory have not been investigated across different levels of hunger and satiety.

In addition to overlooking potential sex differences in food motivation, prior research in this field has also predominantly focused on adult subjects. However, it is well documented that sensitivity to food rewards differs between adolescence and adulthood (Spear, 2000). Compared to adults, adolescents are at a higher risk for developing eating disorders linked to both overeating and undereating (Klump, 2013; Klump et al., 2011). Adolescents exhibit heightened reward-seeking behavior, and their reward processing is more goal-directed, rather than stimulus-directed (Anderson & Spear, 2011; Doremus-Fitzwater et al., 2010; Rode et al., 2020). Previous work has shown that adolescent rats display increased consumption of palatable food, as well as enhanced motivation to work for that food compared to adults (Friemel et al., 2010; Marshall et al., 2017; Stolyarova & Izquierdo, 2015; Wilmouth & Spear, 2009). Nevertheless, direct comparisons between adolescents and adults of both sexes under sated and hungry conditions have yet to be explored.

The primary goal of this project was to compare food reward learning and consumption of male and female adult and adolescent rats in sated versus hungry (fooddeprived) states to determine if hunger state impacts these behaviors similarly, and whether there are sex and/or age differences. To address this, we conducted two experiments. In the first experiment, we compared adult male and female rats, and in the second experiment, we compared adolescent and adult rats. The behavioral protocol included a food cue learning and memory phase, before and after which food consumption was tested. During the learning and memory protocol, subjects underwent

cue-food acquisition training using a Pavlovian conditioning protocol, extinction training, and testing to assess the renewal of conditioned behavior. We used context-induced renewal, which involves training subjects in different contexts during the acquisition and extinction and then returning them to the acquisition context—where they initially learned cue-food associations—which induces robust renewal of responding to the food cue (Bouton & Ricker, 1994). We assessed consumption during feeding tests and conditioned responding during acquisition, extinction, and renewal, and analyzed correlations between these two behaviors.

[2] Materials and methods

[2.1] Subjects

[2.1.1] Experiment 1

Experimentally naïve 32 adult (72-76 days) Sprague Dawley rats (16 male, 16 female) were used. The subjects were bred in-house from our transgenic *Fos-lacZ* colony and confirmed with genotyping as wild type. Rats from the same litter were balanced across conditions to the extent possible. The rats weighed 283-383g (males) and 190-235g (females) at the beginning of the behavioral protocol. At the start of the experiment, rats were moved to a new housing room that was different from their breeding room and were allowed 24h to acclimate prior to handling and habituation procedures. For the duration of the experiment, males and females were individually housed in the same colony room on two separate shelves and maintained on a 12h light/dark cycle (lights on 06:00). Behavioral testing was conducted during the light phase between 10:00 and 14:00.

On the fourth day of handling, male and female rats were assigned to either food restricted (deprived) or *ad libitum* (sated) eating schedules (n = 8 per group; 4 groups) and remained in these conditions throughout the experiment. Rats in the sated condition had *ad libitum* access to standard laboratory chow (Purina Lab Diet Prolab RMH 3000; 3.47 kcal/g: 26% protein, 15% fat, 59% carbohydrates (89% starch)). Rats in the deprived condition were fed restricted amounts of chow to maintain 85% *ad libitum* body weight. They were fed a baseline of 14g (males) or 12g (females) chow that was adjusted based on daily weight (+1/2g for each gram underweight and -1g for each gram overweight). All rats were weighed and fed daily at the end of behavioral training and testing. All rats had *ad libitum* access to water. All housing and testing procedures were compliant 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

Experimentally naïve 32 adolescent (21-25 days) and 32 adult (72-76 days) Sprague Dawley rats (32 male, 32 female) were purchased from Envigo and used as subjects. At the beginning of the behavioral protocol, adolescents were 32-34 days old and weighed 48-57g (males) and 50-55g (females). Adults were 81-83 days old and weighed 211-250g (males) and 158-195g (females). All subjects were individually housed in the same room and given 48 hours to adjust to colony life prior to handling. Adolescent and adult subjects were housed on two separate shelves and were grouped by sex. For the duration of the experiment, subjects were maintained on a 12h light/dark cycle (lights on 06:00). Behavioral testing was conducted during the light phase between 10:00 and 14:00.

The food restriction protocol was modified for adolescent rats to ensure proper growth trajectory while maintaining adequate food deprivation necessary for associative learning. On the third day of handling, 24h ad libitum consumption of chow was measured for all adolescent subjects. The next day, yoked pairs were created between adolescent rats of the same weight and sex, and to the extent possible, by similar amounts of chow consumed in the previous 24h to ensure a similarly paired growth trajectory. Per yoked pair, one adolescent rat was fed *ad libitum* (sated), and one adolescent rat was assigned to a restricted diet (deprived), where the weight of the sated rat was used to calculate 85% of the deprived rat's growing weight. Adolescent rats in the deprivation group were given 7g of chow on the first day of food restriction, from which they initially gained 1-2g overnight (Anderson, Bush, and Spear 2013). For every feeding thereafter, rats were fed a 10g baseline that was adjusted based on daily weight (+1/2 g for each)gram underweight and -1g for each gram overweight). The baseline amount fed was increased by 2g each week for males, and by 1g every week for females. Once per week, daily consumption of chow was measured for the sated rats and 85% of their 24h chow consumption was used as a benchmark for maintaining the deprived rats on their 85% food restriction protocol.

On the fourth day of handling, adolescent and adult male and female rats were assigned to either food restricted (deprived) or *ad libitum* (sated) eating schedules (n = 8per group; 8 groups) and remained in these conditions throughout the experiment. All rats had *ad libitum* access to water. All rats were weighed daily at the end of the experimental procedures. All housing and testing procedures were compliant 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

Pavlovian conditioning (acquisition training), extinction training, and renewal testing took place in a set of 8 identical behavioral chambers (30 x 28 x 30cm; Coulbourn Instruments, Allentown, PA) located in a separate space from the colony room. Chambers were made of transparent Plexiglas in the front and back, aluminum top and sides, with grid floors and a recessed food dispenser (3.2 x 4.2cm) on one wall. Each chamber was enclosed in a plastic isolation cubicle (79 x 53 x 53cm; Coulbourn Instruments, Allentown, PA) with monolithic rigid foam walls to reduce noise and light. The back wall of each isolation cubicle contained a mounted video camera to record behavior and a ventilation fan that provided background noise (55 dB). All sessions were recorded for behavioral analysis. Rats underwent training and testing in two distinctly different chambers (context 1 and context 2), each of which had unique visual, tactile, and olfactory properties. Context 1 contained an opaque Plexiglas insert over the grid floor and had isolation cubicle doors closed throughout the duration of the session. Context 2 contained an opaque Plexiglas insert placed upright along the left side wall, and had isolation cubicle doors open, and a 1% acetic acid olfactory cue. Both contexts contained an illuminated house light. The two contexts were counterbalanced in regard to behavioral training and testing assignments (see below).

[2.3] Palatable food

The palatable food used was Test Diet (TD) pellets (5TUL 45mg Scott Pharma: cat#1811155; 3.44 kcal/g; 20% protein, 13% fat, 67% carbohydrates (100% sucrose).

Previous work from our group shows that rats strongly prefer these pellets compared to palatable foods with different sugar-to-fat ratios (Reppucci, 2010).

[2.4] Behavioral training and testing procedures

All training and testing began after deprived rats reached 85% *ad libitum* body weight. Subjects underwent a learning and memory protocol that included acquisition training, extinction training, and renewal testing. Acquisition and extinction training occurred in different contexts. Rats learned cue-food associations across 8 Pavlovian conditioning sessions, followed by cue-only presentations for 4 extinction sessions in a different context. They were then tested for renewal of conditioned responding to the food cue in the acquisition compared to extinction context, counterbalanced. Prior to and following the learning and memory protocol, rats were tested for consumption of TD and chow. Each test was conducted over two days with one-hour access to TD or chow in the home cage, with food type order counterbalanced.

[2.4.1] Habituation

After the 24h acclimation period, all subjects were handled daily for 7 days and habituated to the testing food and feeding dishes. First, rats were habituated to TD pellets with 1g given in their home cage over 30 minutes. On a separate occasion, rats were habituated to empty ceramic food dishes with one 30-minute exposure. The next day, rats were habituated to eating TD from the food dishes with 1g of TD in the dishes for 30 minutes. The following day rats were habituated to eating chow from the food dishes in a similar manner.

[2.4.2] Food Consumption tests

All rats were tested for their consumption of TD and chow in two counterbalanced sessions on two separate days. Consumption tests occurred in home cages and were 60min long. Prior to each test all food was removed from the cages and rats were given either 20g of TD pellets or 20g of standard chow in food dishes placed on the cage floor. Half of the rats in each condition received TD and the other half received chow on the first test day, with food type counterbalanced on the second test day. After 60min elapsed, all remaining uneaten food was weighed to determine the amounts consumed. Cages were thoroughly checked by hand for any buried pellets to obtain accurate consumption calculations. Food intake during consumption tests counted toward daily food allotment for deprived rats. In the first cohort of Experiment 1, two adult male rats in the *deprived* condition consumed all 20g of TD during the consumption test, and the amount of TD and chow given to subsequent cohorts for consumption testing was raised to 25g.

[2.4.3] Cue-food learning, extinction, and renewal

The learning and memory protocol included 8 sessions of cue-food Pavlovian conditioning (acquisition), 4 sessions of extinction training, and 2 sessions of testing to assess renewal of the conditioned behavior. The protocol followed an "ABA" design in which acquisition training occurred in a distinct context (context A), extinction training occurred in a different context (context B), and renewal of conditioned behavior was induced by a return to the acquisition context (context A) (Bouton, 1993). Contexts 1 and 2 described in "Apparatus" were counterbalanced as contexts A and B. All training and testing sessions were conducted on separate days. Prior to both acquisition and extinction

training blocks, rats were habituated to their assigned contexts in a session lasting 38min. During each habituation session, rats were placed in their assigned context with the house light on, but with no TD pellet delivery or tone presentations. Following habituation to context A, acquisition training took place across eight sessions, each lasting 34min. During each session, rats were presented with a tone (CS) followed by the delivery of 2 TD pellets (US) into the recessed food dispenser for 8 CS-US trials per session on a random interval schedule. Following habituation session to context B, extinction training consisted of four 34min sessions in which rats were presented with 8 trials of CS alone. After extinction, renewal testing consisted of 8 CS presentations alone in both contexts A and B across two counterbalanced sessions (one context/session/day), each lasting 34min.

[2.4.4] Quantification of conditioned behavior

To assess and quantify learning, trained observers who were unaware of the experimental conditions analyzed the subjects' conditioned responses from videos of the learning and memory sessions. Conditioned response (CR) was measured by the expression of 'food cup behavior'. Food cup behavior was operationally defined by rats either poking their nose into the recessed food cup or by anticipatorily facing the food cup while standing directly in front of it. Behavior was scored over 20s per each trial: 10s before each CS presentation (preCS) and 10s during each CS presentation. Every 1.25 seconds a behavior was recorded in a binary manner (food cup or non-food cup) for a total of 8 observations per each 10s epoch, and the percentage of time that each rat spent at the food cup during each preCS and each CS was calculated using these observations. **[2.5] Statistical analysis**

SPSS software (v.28) was used for all statistical analyses and significance was set at p ≤ 0.05 . Food cup behavior and consumption data were analyzed using two-way mixed repeated measures of analysis of variance (ANOVAs) with sex and hunger state as between-subjects factors. PreCS and CS period responding and session were used as repeated measures when analyzing acquisition, extinction, and renewal testing data. Elevation of responding was examined separately in order to evaluate learning independent of baseline differences, and was calculated by subtracting baseline (PreCS) responding from CS responding. Consumption data (denoted as grams eaten per 100 grams of bodyweight) was analyzed separately using the same between-subjects factors, but with food type (TD, chow) as repeated measures. For pre-planned analyses, simple effects pairwise comparisons (Bonferroni-adjusted) were analyzed when appropriate. Correlations between the amounts of food consumed during the first consumption test and conditioned responding during acquisition training were calculated using Pearson correlation coefficients. In certain cases, such as follow-up consumption test analyses and follow-up correlations, data were split by hunger state and/or sex.

Acquisition training sessions 1 and 2 were analyzed separately using multivariate ANOVAs, with the sessions split into two 4-trial blocks (first half and second half of each session). During these early acquisition training sessions, we also calculated the rate of change (ROC) in responding from the first half of the first training session (H1) to the second half of the second training session (H4) using a simplified slope formula (ROC = $\frac{\Delta y}{\Delta x} = \frac{H4-H1}{4-1}$). Greenhouse-Geisser or Huynh-Feldt corrections were used when sphericity was violated.

[3] Results

Experiments 1 and 2 compared how adult and adolescent male and female rats learn about and consume palatable food under sated and hungry conditions. Experiment 1 compared adult male and female rats; experiment 2 compared adolescent and adult subjects of the same sex. Subjects underwent a three-phase learning and memory protocol that consisted of Pavlovian conditioning, extinction, and context-mediated renewal of responding. Prior to and following the learning and memory protocol, rats were tested for their consumption of palatable food (TD) and chow.

[3.1] Experiment 1

[3.1.1] Acquisition Training

Across the eight acquisition sessions, all rats increased foodcup behavior (conditioned response; CR) during CS periods but not during preCS periods, indicating successful learning. During the first three sessions, deprived rats of both sexes had higher CRs during CS periods than sated rats (Figure 1).

A mixed repeated measures (MRM) ANOVA of CRs with sex and hunger state as between subjects measures and training session and period (preCS and CS) as repeated measures found main effects of session (F(7, 196)=23.457, p<.001), period (F(1, 196)=557.797, p<.001) and hunger state (F(1, 28)=10.843, p=.003), but not sex (F(1,28)=2.344, p=.137). There were interactions between session and hunger state (F(7, 196)=7.334, p<.001), period by hunger state (F(1, 196)=5.028, p=.033), session by period (F(7, 196)=66.441, p<.001), and session by period by hunger state (F(7,196)=3.772, p=.004). Follow-up pairwise comparisons indicated that all rats had higher CRs during CS compared to preCS periods from sessions 2-8 (p<.05). During the first session,



Figure 1 Acquisition training sessions 1-8 (A1-A8). Acquisition training sessions 1-8 (A1-A8). Graph shows conditioned responding, quantified as average percent of time (mean ± SEM) spent exhibiting foodcup behavior during preCS (dotted lines) and CS (solid lines) periods throughout each training session. Caret symbols (^) indicate a difference between deprived and sated groups during preCS periods; ampersands (&) indicate a difference between preCS and CS responding; and asterisks (*) indicate a difference between deprived and sated groups during CS presentations (p<.05).

deprived rats had higher CS than preCS responding, whereas sated rats did not (p=.028, p=.390). During acquisition sessions 1 and 2, deprived rats responded more than sated rats during the CS and preCS (session 1: p=.005, p=.002, session 2: p<.001, p=.019), and this difference was most apparent during training session 2 (Figure 1). During session 3, deprived rats of both sexes had higher CRs than sated rats during the CS periods (p=.003), but not during preCS periods (p=.057). For the remaining acquisition sessions, all groups exhibited similar high responding during CS periods (p>.05; except session 5

(effect of hunger state, p=.017)), and similar low responding during preCS periods (p>.05).

To assess learning during the first two sessions, we compared CRs during CS periods between the first half and second half of each session. For both sessions, CRs during the second half were higher than during the first half (*session 1* F(1, 28)=11.274, p=.002; *session 2* F(1, 28)=22.692, p<.001). Deprived rats had higher CRs than sated rats (*session 1* F(1, 28)=9.264, p=.005; *session 2* F(1, 28)=38.483, p<.001), but there were no sex differences (*session 1* F(1, 28)=.933, p=.342; *session 2* F(1, 28)=2.889, p=.100). As



Figure 2 Acquisition sessions 1 and 2, by trial. Conditioned responding during each of the eight CS presentations (trials) during acquisition sessions 1 (left) and 2 (right), quantified as average percent of time (mean \pm SEM) spent exhibiting foodcup behavior during CS presentation. Bar graph insert shows average rate of change in conditioned responding from the first half of acquisition session 1 to the second half of acquisition session 2. Asterisks indicate a significant difference between deprived and sated groups: * p<.05, *** p<.001.

an additional assessment of learning, we quantified the rate of change (ROC) in conditioned responding during CS presentations across the first two sessions. Because the biggest differences in responding between sated and hungry groups were during session 2, we calculated ROC in responding from the first half of the first training session (H1) and the second half of the second training session (H4) (see Methods). The rate of change was higher in deprived subjects (F(1, 28)=6.682, p=.015), but did not differ between sexes (F(1, 28)=2.376, p=.134) (Figure 2).

Because of prior evidence that males and females respond differently to palatable food under sated, but not hungry conditions (Buczek et al., 2020), we conducted additional analyses for sessions 1 and 2 by hunger state. During these sessions, sated females had higher CRs than sated males (F(1, 14)=6.442, p=.024), while deprived males and females did not differ in their CRs during CS periods (F(1, 14)=.433, p=.521).

[3.1.2] Extinction Training

Across extinction training, all rats decreased their CRs during CS periods, as expected. Deprived subjects had higher CRs than sated subjects during CS presentations during early, but not late extinction training. All groups decreased their CRs to the CS at the same rate; however, only deprived rats reached preCS responding levels by the end of training (Figure 3).

A MRM ANOVA of CRs found main effects of session (F(3, 84)=5.5776, p=.004) and period (F(1, 28)=62.836, p<.001), but not hunger state (F(1, 28)=.529, p=.473) or sex (F(1, 28)=2.259, p=.144). There were session by period (F(3, 84)=33.134, p<.001) and session by period by hunger state interactions (F(3, 84)=8.158, p<.001). Follow-up pairwise comparisons revealed that during CS presentations, deprived rats of



Figure 3 Extinction training sessions 1-4 (E1-E4). Graphs show conditioned responding during extinction training, quantified as average percent of time (mean \pm SEM) spent exhibiting foodcup behavior during preCS and CS periods in **deprived (A)** and **sated (B) subjects** throughout each extinction session. Males and females are collapsed due to lack of statistically significant sex differences; see Table 1 for data values for each sex. Asterisks indicate a significant difference in responding between preCS and CS periods (* p<.05).

both sexes had higher responding than sated rats during the first extinction session (p=.014), but not during the remaining three sessions (p=.172, p=.194, p=.840). Furthermore, although both deprived and sated rats exhibited higher CRs during CS compared to preCS periods during the first two extinction sessions (p<.001), deprived rats' responding during CS periods decreased by the third session and into the fourth session to preCS responding levels (p=.249, p=.263), whereas sated rats' CRs during CS periods remained higher than their preCS responding across all four extinction sessions (p<.001, p=.024, p=.016) (Figure 3). To confirm extinction learning, we compared CS period responding during the first and final extinction session, and found that all groups decreased responding to the CS from the first to the last extinction session (F(1, 28)=17.496, p<.001).

Similar to the acquisition training, the most apparent change in responding occurred between the first and second extinction sessions (Figure 3). Thus, to further evaluate group differences during extinction, we compared responding during the first half of the first session (H1) and the second half of the second session (H4). CRs decreased from H1 to H4 (F(1, 28)=36.457, p<.001). Deprived rats responded higher than sated rats, (F(1, 28)=6.341, p=.018), but there were no sex differences (F(1, 28)=1.029, p=.319). We also calculated the ROC in conditioned responding between H1 and H4 (see Methods). There was no effect of hunger (F(1, 28)=.810, p=.376) or sex (F(1, 28)=.625, p=.436) on ROC.

Extinction Sessions						
			1	2	3	4
Deprived	PreCS	Male	1.4 ± .8	2.7 ± 1.2	0.8 ± 0.8	4.3 ± 2.0
		Female	4.7 ± 1.5	5.3 ± 1.7	10.0 ± 4.2	14.1 ± 5.8
	CS	Male	29.5 ± 4.7	15.6 ± 4.9	6.4 ± 2.4	7.0 ± 3.8
		Female	25.3 ± 2.7	12.7 ± 3.3	9.8 ± 3.1	15.2 ± 3.8
Sated	PreCS	Male	5.3 ± 1.9	0.8 ± 0.5	6.8 ± 3.2	4.1 ± 3.0
		Female	6.1 ± 2.7	3.1 ± 2.1	7.0 ± 3.3	7.8 ± 4.0
	CS	Male	17.0 ± 0.8	5.9 ± 1.3	11.3 ± 3.6	9.4 ± 3.1
		Female	19.1 ± 4.5	12.3 ± 4.1	13.7 ± 4.0	11.3 ± 4.6

Table 1 Foodcup behavior during extinction training sessions 1-4. The table shows conditioned responding during extinction training for deprived and sated groups of each sex, quantified as average percent of time (mean ± SEM) spent exhibiting foodcup behavior during preCS and CS periods.

[3.1.3] Renewal Tests

During renewal testing, all groups had higher CRs during CS presentations in the acquisition context compared to the extinction context, indicating successful context-induced renewal effect across all conditions (Figure 4).

A MRM ANOVA of CRs found main effects of period (F(1, 28)=101.033, p<.001), context (F(1, 28)=18.353, p<.001), and sex (F(1, 28)=77.546, p=.022) but not hunger (F(1, 28)=1.642, p=.211). There were interactions of period by hunger (F(1, 28)=4.856, p=.036), period by context (F(1, 28)=26.819, p<.001), and period by hunger by sex (F(1, 28)=5.204, p=.030). The renewal effect was confirmed through follow-up pairwise comparisons, which showed that during CS periods, all groups had higher CRs in the acquisition context compared to the extinction context (p<.001). As expected, responding during preCS remained lower than during CS periods in both contexts (acquisition: p<.001, extinction: p=.005), and did not differ between contexts (p=.323).



Figure 4 Context-induced renewal of conditioned responding during tests. Graphs show conditioned responding, quantified as the average percent of time (mean \pm SEM) spent exhibiting foodcup behavior for deprived (empty bars) and sated (filled bars) in the acquisition context (left) and extinction context (right) during **preCS (A)** and **CS (B)** presentations. Asterisks indicate a difference between groups (* p<.05), contexts (*** p<.001).

Additional comparisons indicated that during CS periods, deprived males had lower CRs than sated males (p=.025) and deprived females (p=.006). Females responded similarly during CS periods regardless of hunger state (p=.807).

[3.1.4] Consumption Tests

All rats underwent food consumption tests with TD and chow, prior to and following the learning and memory protocol. During the first consumption test, which occurred before learning and memory protocol, all groups ate more TD than chow, and deprived rats consumed more food than sated rats. There were also sex differences. Females ate more overall, and sated females ate more TD than sated males, while deprived males and females ate similar amounts of TD. Consumption patterns were similar across both tests (Figure 5).

A MRM ANOVA of grams of food consumed found main effects of food type (F(1, 28)=242.254, p<.001), hunger (F(1, 28)=154.859, p<.001), and sex (F(1, 28)=10.628, p=.003), as well as a food type by sex interaction (F(1, 28)=5.705, p=.024). Follow-up pairwise comparisons revealed that while females ate overall more food than males, the difference between sexes was greater for TD consumption (p=.003) than chow consumption (p=.015).

During the second consumption test, which occurred after all learning and memory testing was completed, patterns were similar to those in the first consumption test. Deprived rats ate more of each food than sated rats (F(1, 28)=247.743, p<.001), and both groups ate more TD than chow (F(1, 28)=178.613, p<.001). Females ate more than males (F(1, 28)=10.994, p=.003). There was an interaction between food type and hunger (F(1, 28)=7.417, p=.011).



Figure 5 Food consumption tests. Graphs show the amounts of TD (filled bars) and chow (empty bars) that deprived (left bars) and sated (right bars) rats consumed, shown as grams eaten per 100 grams of bodyweight (BW), during the **first (A)**, and **second (B)** consumption tests. Asterisks indicate a difference between sexes (** p<.01), hunger states (*** p<.001), food types (*** p<.001), tests (*** <.001).

To gain a better understanding of this interaction, additional analyses were split by hunger state. During each test, sated rats ate more TD than chow (*Test 1* F(1, 28)=239.365, p<.001; *Test 2* F(1, 28)=211.299, p<.001), and females ate more than males (*Test 1* F(1, 14)=13.978, p=.002; *Test 2* (F(1, 14)=9.376, p=.008). There was a food type by sex interaction (*Test 1* F(1, 14)=6.940, p=.020; *Test 2* F(1, 14)=8.452, p=.011), which indicated that sated females ate more TD than sated males during each test (p=.003, p=.006), and that they ate more chow than sated males during the first test, but not during the second test (p=.019, p=.193). The same analysis for deprived rats found that they also ate more TD than chow during each test (*Test 1* F(1, 28)=71.772, p<.001; *Test 2* F(1, 28)=74.716, p<.001), and that females ate more than males during the second, but not the first test (*Test* 1 (F(1, 14)=3.827, p=.071; *Test 2* (F(1, 14)=5.618, p=.033). There were no food type by sex interactions during either test (*Test 1* F(1, 14)=1.276, p=.278; *Test 2* F(1, 14)=.038, p=.849).

Next, we compared consumption patterns across both tests. All groups ate more during the second consumption test (F(1, 28)=17.653, p<.001), and ate more TD than chow (F(1, 28)=295.229, p<.001). Deprived rats ate more than sated (F(1, 28)=229.182, p<.001), and females ate more than males (F(1, 28)=12.478, p=.001). There were interactions of test session by hunger (F(1, 28)=20.065), p<.001), test session by food type (F(1, 28)=7.244, p=.012), food type by sex (F(1, 28)=4.247, p=.049), and test session by food type by hunger (F(1, 28)=13.763, p<.001). Post-hoc analyses indicated that deprived rats ate more TD, but not chow, during the second compared to the first consumption test (p<.001, p=.087), whereas sated rats ate similar amounts of both foods between the two tests (p=.600, p=.635).

[3.1.5] Correlations between learning and consumption

To examine the relationship between consumption and learning, Pearson correlation coefficients were calculated for the first consumption test (subjects tested prior to learning) and conditioned responding during preCS and CS periods for each acquisition training session. The amounts of TD and chow consumed were each positively correlated with conditioned responding to the CS during the first three acquisition training sessions.

During the first acquisition session, there was a moderately positive correlation between CRs during the CS presentations, and the amounts of TD (r=.529, p=.002) and chow (r=.507, p=.003) consumed. The strongest correlation was during the second acquisition training session (TD: r=.667, p<.001, chow: r=.719, p<.001) (Figure 6).

Albeit weaker, this positive correlation continued into the third session of training (TD: r=.414, p=.018, chow: r=.535, p=.002). For acquisition sessions 4 through 8, all groups were responding similarly during CS periods, and therefore there were no correlations between responding and food consumption for those sessions. The only exception to this was during acquisition session 5, where there was a weakly positive correlation between CRs during the CS and TD (r: .375, p=.034) and chow (r=.469, p=.007) consumption.

Conditioned responding during preCS periods was also correlated with food consumption during early acquisition training. There was a positive correlation between the amounts of TD and chow consumed and CRs during the first and second sessions (TD: r=.531, p=.002, chow: r=.523, p=.002; TD: r=.373, p=.035, chow: r=.374, p=.026), but not during the remaining sessions (p>.05).



Figure 6 Correlations between consumption and learning. Graphs show Pearson correlations between consumption (grams eaten per 100 grams of bodyweight (BW)) of **TD (A)** and **chow (B)** and conditioned responses (percent of time spent exhibiting foodcup behavior) during CS presentations during acquisition training session 2.

[3.2] Experiment 2

[3.2.1] Acquisition Training

All groups exhibited successful learning during acquisition training, as evidenced by an increase in their CRs during CS periods, and a decrease and overall low CRs during preCS periods. Adolescents had overall higher responding than adults throughout training. During early training, deprived groups initially surpassed sated groups in their conditioned responding during CS presentations; however, sated subjects reached comparable CR levels by the fourth training session (Figure 7).





A MRM ANOVA of CRs found main effects of session (F(7, 392)=36.928,

p<.001), period (F(1, 56)=774.665, p<.001), age (F(1, 56)=4.030, p=.050), and hunger (F(1, 56)=11.102, p=.002), but not sex (F(1, 56)=1.682, p=.200). There were interactions between session and hunger (F(7, 392)=2.736, p=.009), session and period (F(7, 392)=113.984, p<.001), session, period, and hunger (F(7, 392)=2.495, p=.016), and session, period, hunger, and sex (F(7, 392)=2.044, p=.049). Follow-up pairwise comparisons found that after the first session, responding during CS periods was higher than during preCS periods for all groups (sessions 2-8: p<.001). There were also hunger state differences in both sexes during CS and preCS periods in early acquisition training. Deprived rats of both sexes had higher CRs during CS presentations than their sated counterparts during the second (males: p<.001, females: p=.002) and third (males: p=.029, females: p<.001) sessions. By acquisition session 4 and for the remaining sessions, there were no longer any hunger state differences (p>.05). During first and second session preCS periods, deprived males had higher CRs than sated males (p=.002, p=.043), while this effect was not observed in females (p=.218, p=.432).

To evaluate age differences in responding during CS presentations, we conducted additional analyses for deprived and sated subjects separately. Deprived rats increased their CRs across training (F(7, 196)=57.398, p<.001), regardless of age (F(1, 28)=.021, p=.886) or sex (F(1, 28)=.811, p=.376). Sated rats also increased their CRs across training (F(7, 196)=69.290, p<.001), and adolescents had higher responding than adults (F(1, 28)=6.230, p=.019). There were no sex differences (F(1, 28)=1.159, p=.291).

Since the greatest increase in conditioned responding occurred during the first two sessions, we compared responding during the first and second half of each of these

sessions (Figure 8). For session 1, CRs during the second half were higher than during the first half (F(1, 56)=19.438, p<.001), and deprived rats responded more than sated rats (F(1, 56)=4.705, p=.034), regardless of age (F(1, 56)=1.848, p=.180) or sex (F(1, 56)=.059, p=.808). The same analysis for session 2 also indicated higher CRs during the second half compared to the first half (F(1, 56)=44.411, p<.001), with deprived rats responding more than sated (F(1, 56)=26.150, p<.001). There were no main effects of age (F(1, 56)=1.525, p=.222) or sex (F(1, 56)=.039, p=.844), but there was an interaction between session half, age, and sex (F(1, 56)=4.419, p=.040). Follow-up pairwise



Figure 8 Acquisition training sessions 1 and 2, by trial. Deprived rats are shown in teal, sated rats are shown in black; adults shown in solid lines, adolescents in dashed lines. Graphs show conditioned responding during each of the eight CS presentations (trials) in **males (A)** and **females (B)** during acquisition **session 1 (left side of each graph)** and **session 2 (right side of each graph)**, quantified as average percent of time (mean \pm SEM) spent exhibiting foodcup behavior during cue presentation. Bar graph inserts show average rate of change in conditioned responding from the first half of acquisition session 1 to the second half of acquisition session 2. Within the bar graph inserts, teal bars denote deprived rats, black bars denote sated rats; empty bars denote adolescents, solid bars denote adults. Asterisks in the main graphs indicate a significant difference between hunger states (* p<.001), sessions (*** p<.001). Asterisks in the inserts indicate a crossover effect for males and females (p<.05, see text for details). Pairwise comparisons were not significant for the crossover interaction shown in the bar graph inserts.

comparisons found differences during the first and second halves of the second session. During the first half of the second session, there were no age or sex differences (p>.05). During the second half of the second session, adolescent females had higher CRs than adult females (p=.022), whereas adolescent and adult males did not differ (p=.471). Adolescent females also had higher CRs than adolescent males (p=.047). Adult males and females did not differ during the second half (p=.298).

Then, we assessed the ROC during CS periods from the first half of the first session to the second half of the second session (see Methods). A univariate ANOVA found a close to significance main effect of hunger (F(1, 56)=3.944, p=.052), but no main effects of age (F(1, 56)=.009, p=.923) or sex (F(1, 56)=.237, p=.628). There was a crossover interaction between age and sex (F(1, 56)=4.259, p=.044) such that adolescent females had a higher ROC than adult females, whereas adolescent males had a lower ROC than adult males (Figure 8; bar graph inserts). However, follow-up pairwise comparisons were not significant.

[3.2.2] Extinction Training

During extinction training, all rats decreased CRs during CS periods, indicating successful extinction learning. Adolescent rats responded higher than adults, and deprived rats higher than sated rats (Figure 9).

To assess memory of the CS, we examined responding during the first trial of extinction training. There were no main effects of age, hunger, or sex (p>.05). Next, a MRM ANOVA of CRs across extinction found main effects of session (F(3, 168)=15.651, p<.001), period (F(1, 56)=126.342, p<.001), age (F(1, 56)=20.131, p<.001), and hunger (F(1, 56)=10.390, p=.002), but not sex (F(1, 56)=45.810, p=.771).

There were interactions of age by period (F(1, 56)=12.951, p<.001) and session by period (F(3, 168)=24.452, p<.001). To address these interaction effects, we analyzed CS and preCS periods separately.



Figure 9 Extinction training sessions 1-4 (E1-E4). Graphs show conditioned responding during each extinction session, quantified as average percent of time (mean \pm SEM) spent exhibiting foodcup behavior during **preCS (A)** and **CS (B)**. See Table 2 for data values for each sex. Asterisks indicate a significant difference between hunger states (** p<.01), ages (*** p<.001).

Because there were no sex differences, subsequent analyses were conducted with the sexes collapsed (Figure 10). Responding during CS periods decreased across extinction sessions (F(3, 180)=28.830, p<.001). Adolescents had higher responding than adults (F(1, 60)=23.608, p<.001), and deprived rats had higher responding than sated rats (F(1, 60)=7.423, p=.008). Responding during preCS periods remained low and did not change across extinction sessions (F(3, 180)=1.005, p=.392). Adolescents had higher responding than adults (F(1, 60)=9.400, p=.003), and deprived rats had higher responding than sated rats (F(1, 60)=11.258, p=.001). To further assess extinction training, we compared responding during preCS and CS periods for each group separately. Deprived adults had higher responding during CS than preCS periods during the first two, but not during the last two sessions ((F(3, 45), 6.151, p=.001); p<.001, p=.006, p=.092, p=.133). Sated adults showed a similar pattern ((F(3, 45), 3.268, p=.030); p=.007, p=.048, p=.157, p=.224). Sated adolescents decreased CRs during CS periods to preCS levels by the fourth session ((F(3, 45), 10.307, p<.001); p<.001, p=.001, p=.121), whereas deprived adolescents maintained higher responding during CS compared to preCS periods throughout all four sessions ((F(3, 45), 8.294, p<.001); p<.001, p=.001, p=.006, p=.047) (Figure 10).

Extinction Sessions						
			1	2	3	4
Densitived	Adult PreCS	Male	14.1 ± 3.9	16.2 ± 4.8	8.2 ± 3.6	3.9 ± 2.0
		Female	11.7 ± 6.6	7.4 ± 3.2	4.9 ± 2.3	5.3 ± 2.3
	Adolescent PreCS	Male	18.4 ± 5.8	16.4 ± 4.5	11.1 ± 4.5	17.6 ± 5.7
		Female	14.1 ± 3.8	13.9 ± 4.5	17.4 ± 7.8	17.8 ± 4.8
Deprived	Adult CS	Male	37.7 ± 6.0	25.2 ± 5.5	11.7 ± 3.9	12.3 ± 8.0
		Female	30.9 ± 8.0	17.2 ± 5.3	9.0 ± 4.4	5.3 ± 2.3
	Adolescent CS	Male	42.8 ± 3.9	36.1 ± 5.7	22.1 ± 6.7	25.7 ± 5.4
		Female	40.2 ± 6.1	26.6 ± 6.1	29.5 ± 8.1	22.1 ± 5.2
	Adult PreCS	Male	4.9 ± 1.5	4.3 ± 2.6	1.0 ± 1.0	4.9 ± 4.7
		Female	6.8 ± 6.4	2.7 ± 1.2	6.3 ± 3.4	4.7 ± 2.9
Sated	Adolescent PreCS	Male	11.3 ± 3.8	8.4 ± 2.1	6.8 ± 3.0	7.2 ± 1.9
		Female	3.9 ± 3.1	10.4 ± 5.0	8.2 ± 3.1	9.0 ± 2.0
	Adult CS	Male	15.2 ± 5.0	6.1 ± 2.1	3.3 ± 1.6	6.4 ± 2.8
		Female	23.0 ± 7.7	6.4 ± 1.7	13.9 ± 6.3	9.0 ± 4.3
	Adolescent CS	Male	42.8 ± 7.0	21.3 ± 2.8	21.1 ± 3.8	9.6 ± 4.0
		Female	33.6 ± 7.3	22.7 ± 5.0	23.2 ± 5.5	20.5 ± 7.2

Table 2 Foodcup behavior during extinction training sessions 1-4. The table shows conditioned responding during extinction training for deprived and sated groups of each age and sex, quantified as average percent of time (mean ± SEM) spent exhibiting foodcup behavior during preCS and CS periods.



Figure 10 Extinction training sessions 1-4 (E1-E4), by group. Graphs show conditioned responding during each extinction session, quantified as average percent of time (mean \pm SEM) spent exhibiting foodcup behavior during preCS and CS periods in **deprived adults (A)**, sated adults (B), deprived adolescents (C), and sated adolescents (D). Asterisks indicate a significant difference in responding between preCS and CS periods (* p<.05).

In addition, we compared responding during CS periods between the first and last extinction session and found main effects of session (F(1, 60)=52.517, p<.001), hunger (F(1, 60)=5.076, p=.028), and age (F(1, 60)=14.929, p<.001), but the interaction between session, hunger, and age did not reach significance (F(1, 56)=3.356, p=.072). We also

calculated the rate of change in responding between these sessions, and found that the groups did not differ (p>.05).

[3.2.3] Renewal Tests

During renewal testing, all groups had higher responding during CS presentations in the acquisition context compared to the extinction context, confirming the contextinduced renewal effect. Adolescents exhibited higher CRs than adults overall (Figure 11).

A MRM ANOVA of CRs found main effects of context (F(1, 56)=10.452, p=.002), period (F(1, 56)=124.016, p<.001), and age (F(1, 56)=13.808, p<.001), but not hunger (F(1, 56)=.245, p=.622). The effect of sex did not reach significance (F(1, 56)=3.723, p=.059). There were interactions of period by age (F(1, 56)=5.941, p=.018) and period by context (F(1, 56)=22.286, p<.001).

Based on these interactions, we analyzed responding during preCS and CS periods separately. During CS periods, CRs in the acquisition context were higher than in the extinction context (F(1, 56)=19.303, p<.001) and adolescents responded higher than adults (F(1, 56)=13.236, p<.001). There were no main effects of sex (F(1, 56)=2.904, p=.094) or hunger (F(1, 56)=.003, p=.954). During preCS periods, adolescents had higher CRs than adults (F, 1, 56)=9.273, p=.004) but there were no main effects of context (F(1, 56)=.023, p=.880) or sex (F(1, 56)=3.575)=.064).

Because adolescents differed from adults during both preCS and CS periods, we analyzed elevation of responding (see Methods) across both contexts during renewal. There were main effects of context (P(1, 56)=21.995, p<.001) and age (F(1, 56)=6.406), p=.014), but not hunger (P(1, 56)=.817, p=.370) or sex (F(1, 56)=.727, p=.398).



Figure 11 Context-induced renewal of conditioned responding after extinction. Graphs show conditioned responding, quantified as the average percent of time (mean \pm SEM) spent exhibiting foodcup behavior during preCS (left side; **A and C**) and CS (right side; **B and D**) prtiofd for deprived (top row; **A and B**) and sated (bottom row; **C and D**) groups in the acquisition and extinction contexts. Asterisks indicate a main effect of age (* p<.05), context (*** p<.001).

[3.2.4] Consumption Tests

Subjects underwent a consumption test prior to and following the learning and memory protocol. We found age and sex differences in consumption. During the first consumption test, all adults, as well as sated adolescents, ate more TD than chow, whereas deprived adolescents ate similar amounts of both foods. Additionally, sated adolescents ate more TD than sated adults, while chow consumption was similar across these groups. Females ate more TD than males, whereas both sexes ate similar amounts of chow (Figure 12).

A MRM ANOVA of grams of food consumed for the first consumption test found main effects of food type (F(1, 56)=70.728, p<.001), hunger (F(1, 56)=323.472, p<.001), and sex (F(1, 56)=4.013, p=.050), but not age (F(1, 56)=.958, p=.332). There were interactions between food type and hunger (F(1, 56)=11.410, p=.001), food type and sex (F(1, 56)=5.572, p=.022), and food type, hunger, and age (F(1, 56)=4.470, p=.039). Follow-up pairwise comparisons indicated that females ate more TD than males (p=.007), while both sexes ate similar amounts of chow (p=.909). Sated adolescents ate more TD than adults (p=.032), and they ate similar amounts of chow (p=.307), whereas deprived adolescents and adults ate similar amounts of TD (p=.090) and chow (p=.491). Both sated adolescents and adults ate more TD than chow (p<.001). Deprived adults ate more TD than chow (p<.001), whereas deprived adolescents ate comparable amounts of both foods (p=.238) (Figure 12).

During the second consumption test, all groups ate more TD than chow (F(1, 56)=181.443, p<.001), deprived ate more than sated (F(1, 56)=245.005, p<.001), and females ate more than males (F(1, 56)=6.767, p=.012) (Figure 12). There was an interaction between age and hunger (F(1, 56)=22.059), p<.001). Deprived adults at more than deprived adolescents (p<.001), while sated adults and adolescents ate similar amounts (p<.418).



Figure 12 Food consumption tests. Adolescents are shown in teal; adults are shown in black. Graphs show consumption of deprived and sated (left and right half of graphs) rats, shown as grams eaten as a percentage of bodyweight (mean \pm SEM) for chow and TD (empty and filled bars, respectively) during the first **(A and B)**, and second **(C and D)** consumption tests in males (left column, A and C) and females (right column, B and D). Asterisks indicate a significant difference between food types, ages (* p<.05, *** p<.001).

Next, we compared consumption patterns from the first to the second test. We analyzed deprived and sated groups separately to discern hunger state-specific changes in consumption across tests. We found that deprived adults increased their consumption from the first to the second test, whereas deprived adolescents did not. Neither sated adults nor adolescents increased their consumption from the first to second test.

In deprived rats, a MRM ANOVA found main effects of food type (F(1, 28)=44.467, p<.001), test session (F(1, 28)=8.782, p=.006), age (F(1, 28)=11.380, p=.002), and sex (F(1, 28)=5.210, p=.030). There were interactions between test session and age (F(1, 28)=18.314, p<.001), food type and test session (F(1, 28)=22.636, p<.001), and food type, test session, and sex (F(1, 28)=9.066, p=.005), while the interaction between food and age did not reach significance (F(1, 28)=2.932, p=.098). Follow-up pairwise comparisons for the interaction between test session and age found that deprived adults ate more during the second test than during the first (p<.001), whereas deprived adolescents did not (p=.360). Follow-up comparisons for the interaction between food type, test session, and sex found that both sexes increased their TD consumption from the first to the second test (males: p<.001, females: p=.039), whereas male chow consumption decreased, and female chow consumption did not change (p=.030, p=.270).

The same analysis in sated rats found main effects of food type (F(1, 28)=230.068, p<.001) and age (F(1, 28)=9.154, p=.005). Main effects of test session and sex did not reach significance (*test session:* (F(1, 28)=3.611, p=.068); *sex:* (F(1, 28)=3.098, p=.089)), and neither did an interaction between food type, test session, and sex (F(1, 28)=3.457, p=.074).

[3.2.5] Correlations between learning and consumption

Similar to the first experiment, we evaluated the relationship between consumption and learning. To do so, we calculated Pearson correlation coefficients between food consumption during the first consumption test and conditioned responding during preCS and CS periods for each acquisition training session across all groups. Both TD and chow consumption were correlated with CRs during the CS periods during early acquisition training. We observed the strongest correlations during sessions 2 and 3.



Figure 13 Correlations between consumption and learning. Graphs show Pearson correlations between conditioned responding (average percent of time (mean ± SEM) spent exhibiting foodcup behavior during CS periods) during acquisition training session 2 and TD consumption during the first consumption test (grams eaten per 100 grams of bodyweight (BW)) for adult males (A), adolescent males (B), adult females (C), and adolescent females (D). Empty circles denote deprived subjects; filled circles denote sated subjects.

During the first acquisition training session there was a weak positive relationship between CRs during CS presentations and the amounts of both TD and chow consumed that did not reach significance (TD: r=.236, p=.061; chow: r=.216, p=.086). The correlations were the strongest during the second (TD: r=.554, p<.001; chow: r=.502, p<001) and third (TD: r=.515, p<.001; chow: r=.434, p<.001) sessions. For training sessions 4 through 8, the relationship remained moderately positive for TD, but not chow (session 4-8: .007>p>.030, .331>r>.271).

Responding during preCS was also correlated with food consumption, during early training. The amounts of both TD and chow that rats consumed were moderately correlated with their preCS responding during session 1 (TD: r=.338, p=.006; chow: r=.356, p=.004). Although weaker, the positive correlations were sustained for the next two training sessions for TD but not for chow (session 2: TD: r=.300, p=.016; chow: r=.231, p=.067; session 3: TD: r=.293, p=.019; chow: r=.216, p=.087). For the remaining sessions the correlations were insignificant (p>.05), except sessions 7 and 8 (session 7: TD (p<.001), chow (p=.046); session 8: TD (p=.017), chow (p=.065)).

Because the strongest correlation occurred during session 2, we next examined group differences in correlations between responding (CRs during CS presentations) during acquisition session 2 and the amounts consumed during the first consumption test. The amounts of TD consumed were moderately positive in adult males (r=.619, p=.011), adult females (r=.571, p=.021), and adolescent females (r=.606, p=.013), but not in adolescent males (r=.217, p=.419) (Figure 13). The same analysis for chow consumption found moderately positive correlations in adult males (r=.724, p=.002) and

adolescent females (r=.527, p=.036), but not in adolescent males (r=.453, p=.078) or adult females (r=.308, p=.247) (data not shown).

[4] Discussion

The present study investigated the effects of physiological hunger and satiety on food motivation in adult and adolescent rats of both sexes. Here we assessed the motivation to learn cues for palatable food through a Pavlovian learning paradigm, and the motivation to consume the same palatable food. To our knowledge, this is the first study to comprehensively examine the impact of age, sex, and hunger state on appetitive and consummatory motivation for palatable food. We evaluated these measures independently, as well as in conjunction, to examine their relationship to each other.

Our main finding was that neither adult nor adolescent rats require hunger in order to successfully learn, extinguish, and renew conditioned responding during rewardassociative learning. In addition, adolescents are more responsive to cues predicting palatable food, as well as at baseline, than adults. We also observed that females have a stronger drive to consume palatable food than males, and that this sex difference is present in adolescence.

[4.1] Experiment 1

The first experiment compared how food-deprived and sated adult male and female rats consume and learn cues for palatable food. The subjects underwent an appetitive learning and memory protocol, before and after which their *ad libitum* palatable food consumption was measured. Throughout the appetitive protocol, both sexes were able to learn, extinguish, and renew food-cue associations regardless of their hunger state.

We observed that food-deprived rats initially learned faster than sated rats, but they reached a plateau in responding by the third acquisition training session. Sated rats reached the level of responding of the food-deprived rats by the fourth session, and they continued to respond similarly for the remaining sessions at a level previously observed using a similar protocol (Anderson & Petrovich, 2015). During early learning sessions, there were no sex differences in food-deprived rats. However, sated females exhibited higher responding than sated males. Although all groups converged in responding later in training, this early sex-specific difference supports previous findings that sated females are more sensitive to palatable food than sated males, which will be discussed below.

During extinction training, all groups decreased their conditioned responding, and did so at the same rate. Food-deprived rats initially exhibited higher levels of conditioned responding than sated rats. Despite this, food-deprived rats reduced their conditioned responding to baseline levels by the end of training. In contrast, sated rats did not reach baseline responding levels by the same point in extinction. This indicates that fooddeprived rats exhibit enhanced learning and extinction of food-cue associations compared to sated rats. However, successful associative learning and extinction can occur in rats without physiological hunger.

Following acquisition and extinction training, which occurred in two distinctly different contexts, subjects were tested for renewal of conditioned responding. All groups successfully renewed food seeking when placed back into the context where they initially learned the food-cue associations, thus confirming the renewal effect regardless of sex or hunger state. This is intriguing considering that some prior studies did not find a consistent renewal effect in female subjects (L. C. Anderson & Petrovich, 2015, 2017;

Lafferty, 2022; Segal et al., 2022), whereas other work has demonstrated renewal in females using instrumental/operant conditioning procedures (Bouton et al., 2011; Bouton & Schepers, 2014; Schepers & Bouton, 2017). However, there are some procedural differences to note between the current study and previous studies that found inconsistent renewal in females in Pavlovian settings. Most notably, subjects in the current study were habituated to the experimental contexts prior to training, and the acquisition and extinction training protocol was longer than in previous studies. Our findings suggest that the additional training and experience with contexts in the current protocol was critical for successful renewal in both sexes.

Prior to and following the appetitive learning and memory protocol, subjects were tested for their consumption of the same palatable food used during the Pavlovian protocol. We found that females ate more than males overall. Further, sated females ate more palatable food than sated males, whereas food-deprived males and females ate similar amounts. These patterns were similar during the tests that occurred before and after appetitive training, and they are in line with previous work that showed that females are particularly sensitive to consuming palatable food, even when sated (Buczek et al., 2020; Klump et al., 2013; Sinclair et al., 2017). Next, we used correlation analyses to directly compare and examine the relationship between the consumption of palatable food and learning about a cue that predicts that same palatable food. There was a positive correlation between the amounts of the food consumed and the level of responding to the food cue during early learning, which suggests that similar motivation underlies both behaviors. The subjects that consumed more palatable food also exhibited more cue-specific responding and faster learning during early training sessions. These subjects

were more often food-deprived than sated, and more often female than male. Consumption of neutral-tasting chow also positively correlated with conditioned responding during early learning. Food-deprived rats tended to have higher chow consumption and conditioned responding than sated rats, while males and females behaved similarly. This suggests that states of physiological hunger and satiety similarly impact palatable food-cue learning and consumption, and that there is increased general responsivity among females to palatable food compared to males.

Beyond consummatory behavior, there is a growing body of evidence that males and females differ in their responding to palatable food cues. For example, females have been found to exhibit elevated approach behavior and responding for palatable food than males in Pavlovian and operant paradigms, particularly during early training sessions (Grimm et al., 2022; Lefner et al., 2022; Pitchers et al., 2015; Reichelt et al., 2016; Tapia et al., 2019). Females also have higher preference for chambers paired with palatable food in conditioned place preference tests (Sinclair et al., 2017). Our findings that sated females responded higher than males during initial learning and consumed more of the palatable food than males add to previous research by providing additional behavioral evidence that palatable food is a more salient reward to females than males.

[4.2] Experiment 2

The second experiment examined appetitive learning and consumption in fooddeprived and sated adolescents and adults of both sexes. Overall, we found that both food-deprived and sated adolescents successfully learned, extinguished, and renewed conditioned responding to Pavlovian food cues in the current protocol. Adolescents consistently had higher responding compared to the adult subjects across acquisition,

extinction, and renewal testing. This is in agreement with prior evidence that adolescents are more responsive to stimuli that predict reward compared to adults (DeAngeli et al., 2017; Galvan, 2013, Sturman & Moghaddam, 2011).

Similar to Experiment 1, there were group differences during early acquisition training, and all groups reached similar, high levels of responding by the end of training. Food-deprived rats initially exhibited faster learning than sated rats. The most robust learning occurred between the first and the second training sessions, and adolescent females acquired food-cue associations the fastest during this block, in terms of both their conditioned responses and the rate of change. Across acquisition training, food-deprived adults and adolescents had similar levels of responding, whereas sated adolescents responded more than sated adults.

A prior study compared the influence of age and sex on a similar Pavlovian auditory conditioning paradigm using food-deprived adolescent and adult rats, and also found that both adult and adolescent females responded more than males (Hammerslag & Gulley, 2014). During early conditioning, they found that adult females acquired approach behavior faster than adult males. The same sex difference was seen in adolescents; however, it occurred in later sessions. There are some procedural differences between that and the current study. The former used a sucrose solution, while we used sucrose pellets. It has been theorized that rats may be differently motivated by liquid versus solid sucrose, particularly in learning and memory paradigms (Mook et al., 1983). Our subjects were maintained at 85% of their free-feeding bodyweight throughout the experiment, and they were housed individually. In contrast, their subjects were kept at 90-95% bodyweight and were double or tripled housed with same-sex littermates. These

differences in weight maintenance and housing conditions could affect reward-seeking behavior. Nonetheless, both studies point to the idea that females exhibit enhanced reward-seeking behavior compared to males.

During extinction training, all groups successfully decreased responding, and extinguished at the same rate. Adolescents exhibited higher overall and cue-specific responding compared to adults. Similarly, food-deprived rats had higher overall and cuespecific responding compared to sated rats. These differences were not due to memory. Prior to extinction training, all groups responded similarly during late acquisition and during the first extinction trial. This trial captures previously acquired food-cue memory, and a lack of group differences during this trial indicates similar cue-specific memory recall across groups, regardless of the introduction of a new context.

Adults reached baseline responding levels sooner than adolescents during extinction. These findings suggest that adolescent extinction differs from that of adults, even though they can successfully extinguish learned cues. Specifically, both fooddeprived and sated adults reduced conditioned responding to baseline levels by the third extinction session. In contrast, sated adolescents did not reach baseline responding levels until the final session, and food-deprived adolescents did not reach baseline responding levels at all. Likewise, prior studies have found that adolescents successfully extinguish food-cue associations in both Pavlovian and operant paradigms, but they exhibit higher levels of responding compared to adults (Amancio-Belmont et al., 2017; Andrzejewski et al., 2011; Meyer & Bucci, 2016; Sturman et al., 2010). However, these prior studies used food-deprived male subjects. Here we extend these findings to female rats, as there were no sex differences in either adults or adolescents during extinction training.

All groups showed robust renewal of responding when placed back into the context where they initially learned food-cue associations. Our findings demonstrate that adolescents renew responding to food cues in our paradigm, regardless of sex or hunger state. This is the first study to compare food-deprived and sated adolescent ABA renewal in an appetitive Pavlovian learning paradigm. In addition, we found that adolescents had higher overall responding, as well as higher cue-specific responding than adults across both contexts. This suggests that adolescents appear to exhibit greater sensitivity to cues previously associated with food rewards than adults, during both states of hunger and satiety. A prior study similarly found that food-deprived adolescent rats exhibited greater renewal of extinguished instrumental responding than adult rats (Eddy & Green, 2017), though here they did not observe age differences across acquisition or extinction.

Overall, we observed greater age-dependent behavioral differences during extinction training and renewal testing than during acquisition training. During extinction and renewal, adolescents exhibited higher cue-specific and baseline responding levels than adults. It is important to point out that extinction and renewal involved a different context from the one where acquisition learning took place, and it is possible that the context switch is the reason that adolescents and adults differed. Heightened adolescent responding may indicate that they did not encode new associations between context, cue, and food as efficiently as adults.

Subjects in Experiment 2 underwent consumption testing before and after learning and memory protocol using the same palatable food from training (TD), as well as their neutral-tasting chow. One caveat is that the adolescents were close to adulthood by the time of the second test (postnatal day (PND) 50-51). Their age during the first

consumption test is a better representative of adolescent behavior (PND 32-33). Therefore, our focus is on findings from the first consumption test.

We found differences in palatable food consumption between adolescents and adults that were dependent on hunger state. Food-deprived adolescents and adults ate similar amounts of TD, but adults ate more TD than chow, while adolescents ate similar amounts of both foods. In contrast, sated adolescents ate more TD than chow, and ate more TD than sated adults. These patterns suggest that sated adolescents are more sensitive to palatable food than sated adults. In contrast, when in a food-deprived state, adults exhibit a clear preference for palatable food, whereas adolescents demonstrate a more balanced approach for both foods equally, potentially driven by an adaptive response to meet their immediate physiological needs.

When examining the relationship between palatable food consumption and cuespecific learning, we observed positive linear correlations between consumption and early learning in adult males, adult females, and adolescent females, but not in adolescent males. However, this result should be interpreted with caution due to small sample size.

The finding that sated adolescents ate more TD than adults supports prior evidence that adolescents are more sensitive to the hedonic properties of palatable food than adults (Amancio-Belmont et al., 2017; Counotte et al., 2014; Doremus-Fitzwater et al., 2010; Friemel et al., 2010; Vaidya et al., 2004). A prior study compared positive taste responses to sucrose between non-food-deprived adolescents and adults, and found that they are greater in adolescents (Wilmouth & Spear, 2009).

However, we did not observe this in food-deprived adolescents. Their similar consumption of palatable and neutral tasting food suggests that the high hedonic value of

a palatable food does not supersede ongoing metabolic signals that are present in a state of hunger. In contrast, food-deprived adults ate more palatable than neutral tasting food. Their clear preference for palatable food may reflect that their consummatory decisionmaking is influenced by both the hedonic and homeostatic value of a food. Overall, there appears to be a difference in the extent to which food-deprived adolescents and adults prioritize homeostatic versus hedonic drives. To our knowledge, this is the first study to directly compare food-deprived and non-food deprived adolescents.

In addition to age and hunger state differences, we also found sex differences in consumption, where adolescent and adult females ate more palatable food than males. These sex differences, coupled with the finding that adolescent females also learned slightly faster than other groups, suggest that female sensitivity to palatable food begins from a young age. It is also interesting to note that we observed this difference in both sated and food-deprived conditions, particularly in adolescents. These results are different from the findings of a prior study which measured longitudinal adolescent and adult rat sucrose consumption (Marshall et al., 2017). They found that male, rather than female, adolescent rats were more sensitive to the palatable properties of liquid sucrose reward. However, they tracked pubertal onset in adolescent rats, and reported a mean onset of PND 41 for males and PND 34 for females. In our study, it is unlikely that either sex started or reached puberty, as our male and female adolescent subjects were PND 32-33. However, they were PND 50-51 during the second consumption test and had most likely reached puberty then, yet we did not observe male sensitivity to palatable food.

As mentioned above, the current study did not track pubertal maturation or monitor the reproductive cycle of female subjects. The puberty transition marks

significant changes in hormone levels that drive the final maturation of secondary sex characteristics and reproductive systems (Sisk & Foster, 2004). However, there is considerable variability in the timing of pubertal maturation between sexes. The pubertal window is approximately PND 30-42 in females, and PND 42-55 in males (Ojeda et al., 1980). The first consumption test in the current study occurred during PND 32-33 in both sexes. At this age, we infer that adolescent males in our study had not started the puberty transition. Similarly, most of our adolescent females were peripubertal and had most likely not yet started puberty. Therefore, it is unlikely that circulating androgen and estrogen hormones would have played a substantial role in shaping adolescent behavior during the first consumption test, as puberty-related changes had not yet taken place.

Fluctuations in estrogen and progesterone levels across the cycle may modulate learning, memory, and food intake (Almey et al., 2014; Dalla & Shors, 2009; Eckel, 2011; Somogyi et al., 2011). However, there are concerns regarding tracking the female cycle and establishing "typical" characteristics. The reproductive cycle shows natural variability in onset of sexual maturation, ovulation, and total cycle length, and such variation is often regarded as irregular (Long & Evans, 1922; Marcondes et al., 2002; Westwood, 2008). Furthermore, collecting samples via vaginal smear cytology long-term can cause stress-induced changes in behavior and memory (Becegato et al., 2021; Handy et al., 2016; Varol et al., 2022). As such, the influence of steroid hormones on our observed findings remains unclear and could be a topic of exploration in the future.

[4.3] Conclusions

This study provided novel insights into the contributions of hunger, age, and sex in food-motivated learning and behavior. By directly comparing learning and consumption

behavior under different hunger states, we addressed a crucial gap in the existing literature, which has predominantly tested food-deprived adult subjects. Non-food deprived rats were able to learn, extinguish, and renew conditioned responding as well as food-deprived rats in a Pavlovian associative learning paradigm. These results highlight the robustness of associative learning in states of *both* hunger and satiety, and suggest that physiological hunger is not a prerequisite for successful learning during adolescence or adulthood. The present findings also indicate that adolescents are more sensitive to palatable food than adults. Lastly, our comparisons of male and female behavior indicate that females have heightened sensitivity to palatable food, and that this sex difference is present during adolescence. Future research should aim to further explore the underlying mechanisms and developmental processes that contribute to these behavioral characteristics in adolescence, with a focus on the role of reward processing systems and potential manipulations to further elucidate the drivers of food motivation.

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