Physiology & Behavior 141 (2015) 69–77

Contents lists available at ScienceDirect

Physiology & Behavior

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

Characterization of attenuated food motivation in high-fat diet-induced obesity: Critical roles for time on diet and reinforcer familiarity

Andrea L. Tracy *, Colin J.M. Wee, Grace E. Hazeltine, Rebecca A. Carter

Grinnell College, Department of Psychology, United States

HIGHLIGHTS

- Prior results on the role of obesigenic diets on food motivation have been inconsistent.
- Two factors appear critical: time consuming obesigenic diet and reinforcer familiarity.
- Increased time on obesigenic diet reduces food motivation, familiarity attenuates this.
- This helps reconcile prior results and contributes to an understanding of food motivation.
- Since prior food experience is critical, a varied diet would improve animal models of human obesity.

ARTICLE INFO

Article history:
Received 16 August 2014
Received in revised form 6 January 2015
Accepted 8 January 2015
Available online 9 January 2015

Keywords:
Obesity
Motivation
High-fat diet
Operant conditioning
Reward
Rat

ABSTRACT

Prior work using animal models to study the effects of obesogenic diets on food motivation have generated inconsistent results, with some reporting increases and others reporting decreases in responding on food-reinforced tasks. Here, we identified two specific variables that may account for these discrepant outcomes – the length of time on the obesigenic diet and the familiarity of the food reinforcer – and examined the independent roles of these factors. Time on diet was found to be inversely related to food motivation, as rats consuming a 40% high-fat diet (HFD) for only 3 weeks did not differ from chow-fed rats when responding for a sucrose reinforcer on a progressive ratio (PR) schedule, but responding was suppressed after 6 weeks of ad lib HFD consumption. Explicitly manipulating experience with the sucrose reinforcer by pre-exposing half the rats prior to 10 weeks of HFD consumption attenuated the motivational deficit seen in the absence of this familiarity, resulting in obese rats performing at the same level as lean rats. Finally, after 8 weeks on a HFD, rats did not express a conditioned place preference for sucrose, indicating a decrement in reward value independent of motivation. These findings are consistent with prior literature showing an increase in food motivation for rats with a shorter time consuming the obesigenic diet, and for those with more prior experience with the reinforcer. This account also helps reconcile these findings with increased food motivation in obese humans due to extensive experience with palatable food and suggests that researchers engaging in non-human animal studies of obesity would better model the conditions under which human obesity develops by using a varied, cafeteria-style diet to increase the breadth of food experiences.

1. Introduction

Motivation to obtain and consume palatable, energy dense foods is an important factor in the control of food intake and plays a key role in the development and maintenance of obesity. Obese individuals appear to be more highly motivated by palatable food than their lean counterparts [1,2]. This finding makes intuitive sense, but studies in human subjects are unable to clearly distinguish between motivational changes as a cause versus a consequence of obesity. Furthermore, there are a number of aspects that are difficult to probe in humans, such as the neural substrates of these changes and specific environmental factors that affect food-motivated behaviors.

However, the work to date on the effect of obesity on food-motivated behaviors in animal models has produced inconsistent outcomes. Studies in which high-fat diet (HFD) consumption occurs for a period of 12–15 weeks have shown reduced progressive ratio breakpoints for sucrose pellet reinforcers [3,4]. In contrast to these findings, la Fleur et al. [5] reported a significant increase PR responses for a sucrose reinforcer in rats on a high-fat, high-sugar choice diet, as did Figlewicz and colleagues [6,7] in both juvenile and adult rats on a commercial high-fat mixed diet.

* Corresponding author at: Grinnell College, Department of Psychology, 1116 8th Ave, Grinnell, IA 50112, United States.
E-mail address: tracyand@grinnell.edu (A.L. Tracy).

http://dx.doi.org/10.1016/j.physbeh.2015.01.008
0031-9384/

At first glance, it might appear that these discrepant outcomes represent a failure to replicate an effect across laboratories. We submit that this is not the only explanation; rather, differences in the methods and conditions used in these experiments may have contributed to the differences in the outcomes. Elucidating the role of key variables may yield insight into underlying mechanisms of food motivation as it relates to obesity.

We identified two primary factors that differed between the studies finding reductions in food-motivated behaviors and those finding increases in these same behaviors. First, these studies differed in the length of time that animals had subsisted on the high-fat diet prior to behavioral assays being carried out. Specifically, duration of high-fat diet consumption ranged from 3 weeks to 5 weeks in studies reporting increases in operant responding [5–7], while studies reporting decreases in responding were conducted after 12 weeks of diet exposure [3,4]. This suggests that the physiological and behavioral effects of these diets may shift as a result of persistent consumption and the corresponding gain in body weight and body fat. However plausible this seems, we can't conclusively attribute the differences in motivation across studies to the duration of diet consumption alone, as a number of other variables also differed between these experiments.

The second factor that we noted as a difference between these studies is the exposure to the taste of the reinforcer – sucrose – prior to any physiological changes induced by high-fat diet consumption or weight gain. This exposure is most apparent by contrasting the method of la Fleur et al. [5], in which rats tasted sucrose as a separate solution and consumed nearly 15% of their calories in this form, with that of Davis et al. [3], in which rats were given a commercial pre-mixed diet containing only 8% kcal from sucrose. This lead us to hypothesize that animals would show higher levels of motivation for a more familiar reinforcer.

The present studies aimed to provide an explanation for previously discrepant findings on the effect of high-fat diet consumption on food motivation by isolating and explicitly manipulating the duration of the ad lib diet consumption period and experience with the reinforcer, independent of diet composition and other factors. In Experiment 1, we tested PR responding for a sucrose reinforcer in the same group of rats following 3 and 6 weeks ad lib consumption of a HFD or chow, while Experiments 2 and 3 evaluated reinforcer familiarity by exposing half the rats to either the sucrose reinforcer or one of two specific reinforcer flavors prior to beginning a 10-week ad lib HFD consumption period. Finally, in order to assess the role of food reward in these processes, we tested the effect of diet-induced obesity on the development of conditioned place preference for a novel sucrose reinforcer (Experiment 4).

2. Methods

2.1. General methods

2.1.1. Subjects

Subjects for all experiments were male Long–Evans rats (Harlan Laboratories, Indianapolis, IN) approximately 60 days of age and weighing 225–250 g on arrival in the laboratory. All subjects were housed individually in Plexiglas "shoebox" style cages with wire lids. Room temperature was maintained at 20–23 °C with a 12 h:12 h light cycle. All handling and behavioral procedures occurred during the second half of the light period. Water was available ad libitum in the home cage. Food availability is described below. Animal care followed the Guide for Care and Use of Laboratory Animals and all procedures were approved by the Grinnell College Institutional Animal Care and Use Committee.

2.1.2. Diets

The standard chow diet contained 14% calories from fat with a caloric density of 3.0 kcal/g (Harlan Teklad Rodent Diet #8604, Indianapolis, IN). The high-fat diet (HFD) contained 40% calories from fat, almost entirely from a saturated fat source (butter), with a caloric density of 4.54 kcal/g (Research Diets, New Brunswick, NJ). Except where noted (Experiment 1), animals were maintained on these diets for 10 weeks prior to beginning food restriction and conditioning procedures. During the ad lib feeding periods, body weight and food intake was recorded weekly. All 45 mg pellets used in operant conditioning and conditioned place preference procedures were obtained from Test Diet (Richmond, IN).

2.1.3. Food restriction

For operant conditioning and conditioned place preference (CPP) procedures, all animals were reduced to 85% of their ad lib body weights prior to beginning training sessions and maintained at this weight throughout the experiment (except where noted). To achieve this weight, animals were given a small daily ration of their assigned food (i.e., animals consuming chow continued to receive chow and animals consuming HFD continued to receive HFD). Body weight was monitored and food rations were adjusted accordingly. Weight was reduced gradually over approximately 7 days and maintained throughout the experiment. Daily rations were given approximately 1 h before the onset of dark during the weight reduction phase and 30 min–1 h following the end of the behavioral session during the conditioning/testing phase.

2.1.4. Operant conditioning procedure

All operant conditioning was carried out in four identical chambers (Lafayette Instrument, Lafayette, IN). Each chamber had internal dimensions of approximately 12″ × 10″ × 8″ with two stainless steel end walls, Plexiglas sidewalls and top, and a stainless steel rod floor. On one end wall were two stainless steel levers, present during all sessions, and a single recessed food magazine with a Plexiglas entry flap positioned in the center between the two levers. Three infrared detectors were placed along the side walls of the chamber to detect activity. Each chamber was housed within a larger sound-attenuating chamber with a house light illuminated throughout each session and a ventilation fan, which served to provide a consistent auditory environment and minimize interference from outside noise. ABET II software (Lafayette Instrument, Lafayette, IN) was used to control, monitor and record from all chambers.

In all operant conditioning sessions, one lever was designated the active lever and remained active during all sessions, the second lever was designated inactive and presses on this lever never yielded any outcome. The reinforcer was one 45 mg pellet (pellet type specified for each experiment). All training sessions were 1 h in duration and one training session was conducted per day. The training schedule was as follows: two shaping sessions, two sessions of fixed ratio 1 (FR1), two sessions of FR3, two sessions of FR5. During shaping sessions, reinforcers were delivered on an FR1 schedule with additional reinforcer delivered at the end of every 5 min interval in which no reinforcers were earned via lever pressing, in order to familiarize the animals with the availability and delivery location of reinforcer pellets. During progressive ratio (PR) test sessions, the number of lever presses required to earn each reinforcer was determined according to the following schedule, which raised the response requirement by increasing increment for each subsequent reinforcer [8]: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 693, 737, 901. PR sessions were untimed and ended for each animal when 20 min elapsed without a reinforcer being earned. The final ratio completed prior to this was defined as the animal's "breakpoint". Responses on both levers, magazine entries, and general activity were recorded for all training and testing sessions.

2.1.5. Conditioned place preference procedure

The conditioned place preference (CPP) procedure was conducted in a three compartment chamber (Med Associates, St. Albans, VT), comprising two 25 × 21 × 21 cm side chambers, one with black walls and a floor with a floor of smooth stainless steel parallel rods and one with white wall and a steel mesh grid floor, and a smaller center chamber (12 × 21 × 21 cm) with gray walls and a solid plastic floor. Motorized

doors could be raised or lowered between the center chamber and each side chamber. Doors were controlled and activity (monitored by infrared beams in each chamber) was recorded by computer software (Med Associates, St. Albans, VT). Pre-test and post-tests consisted of a 10 minute session in which all doors were open and animals were permitted to move freely between the three chambers. Time spent in each chamber was recorded during these sessions. For training sessions, each animal was assigned either the black or the white chamber as the "paired" chamber and the opposite side as the "unpaired" chamber. Training consisted of 6 consecutive days in which the animals were placed in the paired chamber with their designated reinforcer for 15 min or placed in the unpaired chamber with no reinforcer. These sessions were 15 min long and occurred on alternating days for a total of 3 paired sessions and 3 unpaired sessions.

2.1.6. Statistics

All statistical analyses were conducted using STATISTICA 12 (StatSoft, Inc.).

2.2. Experiment 1

2.2.1. Dietary exposure

After one week habituation to the lab, animals were weight-matched; half the animals were continued on standard lab chow ($n = 7$) and half were switched to HFD ($n = 8$). Animals were allowed to consume this food ad lib in their home cages for three weeks, before beginning the 7-day food restriction procedure.

2.2.2. Operant conditioning

Reinforcers in this experiment were 45 mg plain sucrose pellets. After reaching the target body weight, animals were trained on the series of operant conditioning schedules described above, then given a single PR test session. Following that session, animals were returned to ad lib feeding on their assigned diet for 3 additional weeks. Food restriction was repeated and animals were given a single sessions of FR3 and FR5 schedules, followed by a final PR test session.

2.3. Experiment 2

2.3.1. Reinforcer pre-exposure

After one week of habituation to the lab, animals were weight-matched and assigned to one of four conditions: Chow-Pre-Exposure ($n = 8$), Chow-No Exposure ($n = 8$), HFD-Pre-Exposure ($n = 8$), and HFD-No Exposure ($n = 8$). Each day for 5 days, all animals received 50 45 mg pellets in a small dish in their home cage (chow and water were available ad lib during this period). For animals in the two Pre-Exposure conditions, the pellets were plain sucrose pellets, while the two No Exposure groups received unsweetened grain-based pellets. The exposure period began approximately 1 h before lights out (increasing the likelihood that animals would be hungry and consume the pellets without forced food restriction) and lasted 15 min and all animals consumed all of the pellets they were given during this phase.

2.3.2. Dietary exposure

One day following the final pre-exposure session, animals in the two HFD conditions were switched to ad lib feeding on the HFD, while the two Chow groups were maintained on the standard lab chow. Ad lib feeding on these diets was continued for 10 weeks, at which time the food restriction procedure was implemented.

2.3.3. Operant conditioning

Reinforcers for all animals in this experiment were 45 mg plain sucrose pellets. After reaching the target body weight, animals were trained on the series of operant conditioning schedules described above, then given a single PR test session.

2.4. Experiment 3

2.4.1. Pilot flavor testing

Prior to beginning this experiment, a separate group of rats was tested to ensure that the two pellet flavors were discriminable. Eight rats were given exposure on two separate days to 2.5 g of each flavor (peanut butter and fruit punch). For one group ($n = 4$), peanut butter exposure was followed by a 20 ml/kg injection of 0.15 M LiCl and fruit punch was followed by an injection of 20 ml/kg physiological saline. For the second group ($n = 4$), the flavor-drug contingencies were reversed. All rats were then given a single two-cup choice test and intake of the two pellet flavors was measured. Rats consumed 0.86 ± 0.18 g of the saline-paired flavor and 0.02 ± 0.01 g of the LiCl-paired flavor [$t(7) = 4.21$, $p < 0.01$]. We concluded that rats readily and reliably discriminated these flavors. There also appeared to be no preference for one flavor over another, as intake of the two flavors did not differ during initial exposure (FP $= 1.89 \pm 0.29$ g, PB $= 2.10 \pm 0.18$ g), nor for the "safe" flavor during testing (FP $= 0.78 \pm 0.18$ g, PB $= 0.93 \pm 0.37$ g).

2.4.2. Reinforcer pre-exposure

After one week of habituation to the lab, animals were weight-matched and assigned to one of four conditions: Chow-Pre-Exposure ($n = 8$), Chow-No Exposure ($n = 8$), HFD-Pre-Exposure ($n = 8$), and HFD-No Exposure ($n = 8$). Each day for 5 days, all animals received fifty (50) 45 mg pellets in a small dish in their home cage (chow and water were available ad lib during this period). Half the animals in each of the 4 conditions received peanut butter flavored sucrose pellets, while the other half received fruit punch flavored sucrose pellets. The exposure period again began approximately 1 h before lights out and lasted 15 min and all animals consumed all of the pellets they were given during this phase.

2.4.3. Dietary exposure

One day following the final pre-exposure session, animals in the two HFD conditions were switched to ad lib feeding on the HFD, while the two Chow groups were maintained on the standard lab chow. Ad lib feeding on these diets was continued for 10 weeks, at which time the food restriction procedure was implemented.

2.4.4. Operant conditioning

Reinforcers for all animals in this experiment were 45 mg flavored sucrose pellets. For animals in the two Pre-Exposure conditions, the flavor of the pellets during operant conditioning matched the flavor that they were given during the pre-exposure phase (that is, animals that had previously consumed peanut butter pellets received peanut butter pellets and animals that had consumed fruit punch flavored pellets received fruit punch flavored pellets). For animals in the two No Exposure conditions, the flavor of pellets received during operant conditioning was the opposite of what they had been given during pre-exposure (that is, animals that had previously consumed peanut butter pellets were given fruit punch pellets as a reinforcer and vice versa). In this way, all animals in the Pre-Exposure groups had previously consumed the reinforcer, while the reinforcer received by the animals in the No Exposure groups was entirely novel, but the specific flavors were counterbalanced across groups, ensuring that any pre-existing preference for a particular flavor could not account for any differences in outcome. After reaching the target body weight, animals were trained on the series of operant conditioning schedules described above, then given a single PR test session.

2.5. Experiment 4

2.5.1. Dietary exposure

After one week of habituation to the lab, animals were weight-matched and assigned to receive either chow ($n = 8$) or HFD ($n = 8$). Animals were allowed to consume their assigned diet ad lib in their

home cages for 8 weeks, before beginning the 7-day food restriction procedure.

2.5.2. Conditioned place preference

After reaching their target body weights, a pre-test was conducted for each animal, in which all doors were open and the animal could move freely through the three chambers. The pre-test determined which of the two side chambers (white or black) was preferred by each animal. The non-preferred side was then assigned as the paired chamber for each individual. Training sessions were then conducted across 6 consecutive days (1 session per day), alternating paired and unpaired sides of the chamber. Each day, the animal was placed in the designated side and the door closed. On paired trials, 5 g of plain 45 mg sucrose pellets were placed in a small stainless steel cup affixed to the floor in one corner of the chamber. On unpaired trials, an empty cup was placed in the chamber. Following the training trials, a post-test was conducted in which the animals were again allowed to move freely through the three chambers for 10 min.

3. Results

3.1. Experiment 1

3.1.1. Body weight

As expected, rats consuming HFD were significantly heavier than rats consuming chow after 6 weeks (HFD: 455.4 ± 10.7 g, Chow: 422.3 ± 11.9 g, see Fig. 1a), but there were no differences between the groups at Week 0 (prior to the initiation of HFD), at Week 3 (HFD: 386.8 ± 7.6 g, Chow: 371.7 ± 8.1 g), or following the first period of food restriction and operant training, prior to the second 3 weeks of ad lib food availability (HFD: 328.0 ± 6.1 g, Chow: 316.8.3 ± 6.1 g). A repeated-measures (Weeks 0, 3, 6) ANOVA yielded a main effect of Duration [$F(2,26) = 460.65$, $p < 0.01$] and a Duration × Diet interaction [$F(2,26) = 7.74$, $p < 0.01$]. One-way ANOVAs at each time point confirmed a significant difference between diet conditions only at Week 6.

3.1.2. Progressive ratio breakpoint

As shown in Fig. 1b, breakpoints in the PR test did not differ between the two diet conditions at 3 weeks, but breakpoint was significantly lower for animals after 6 weeks of HFD consumption than for rats eating only standard chow. Progressive ratio breakpoints were analyzed using a 2 × 2 ANOVA though with only two time points (Week 3 vs Week 6). Again a significant Diet × Duration interaction was found [$F(1,13) = 7.02$, $p < 0.05$] and post-hoc analyses (Tukey's HSD) indicated that this was due to a significant reduction in breakpoint for HFD-fed compared to chow-fed rats at Week 6, but no difference between the groups at Week 3. No differences in inactive lever presses (HFD: 17.1 ± 4.0, Chow: 23.3 ± 4.4) or total activity counts (HFD: 327.4 ± 17.0, Chow: 353.6 ± 25.9) were observed between groups, supporting the notion that this reduction in breakpoint at Week 6 does not reflect a general reduction in activity in obese animals.

3.2. Experiment 2

Two animals were removed from the data set (one from the Chow-No Exposure condition and one from the HFD-No Exposure condition) due to failure to respond during the PR test.

3.2.1. Body weight

Body weights were analyzed by repeated measures ANOVA with Weeks (1–10) as a within-subjects factor and Pre-Exposure (Exposure vs No Exposure) and Diet (HFD vs Chow) as between-subjects factors. This analysis yielded main effects of Week [$F(9,252) = 643.04$, $p < 0.01$] and Diet [$F(1,28) = 6.69$, $p < 0.05$] and a Week × Diet interaction [$F(9,252) = 3.65$, $p < 0.01$], indicating that all rats gained weight over the dietary exposure period, but that HFD-fed rats did so to a greater extent and at a greater rate than chow-fed rats and this was not affected by reinforcer pre-exposure. This was confirmed by post-hoc Tukey's HSD analysis, indicating that body weights between the two diet conditions did not differ at Week 1, but that HFD-fed rats were significantly heavier starting in Week 7 and continuing to Week 10 when experimental food deprivation began (Week 10 mean body weights: Chow = 507.8 ± 9.8 g, HFD = 538.1 ± 9.8 g).

3.2.2. Progressive ratio breakpoint

A 2 × 2 ANOVA (Pre-Exposure × Diet) yielded no significant main effects or interactions. Based on a priori hypotheses, comparisons were made between the two diet conditions for animals that had been given pre-exposure or no pre-exposure to the sucrose reinforcer. As shown in Fig. 2, consistent with the findings of Experiment 1, HFD-fed animals that were not exposed to reinforcer prior to operant training showed significantly reduced breakpoints relative to chow-fed animals [$t(12) = 3.26$, $p < 0.01$]. However, pre-exposure to the reinforcer mitigated this effect resulting in no difference in breakpoint between the two diet conditions [$t(14) = 0.89$, $p > 0.05$].

3.3. Experiment 3

3.3.1. Body weight

Body weights were analyzed by repeated measures ANOVA with Weeks (1–10) as a within-subjects factor and Pre-Exposure (Exposure vs No Exposure) and Diet (HFD vs Chow) as between-subjects factors. This analysis yielded main effects of Week [$F(9,252) = 634.26$, $p < 0.01$] and Diet [$F(1,28) = 14.06$, $p < 0.01$] and a Week × Diet interaction [$F(9,252) = 21.61$, $p < 0.01$], indicating that all rats gained weight over the dietary exposure period, but that HFD-fed rats did so to a

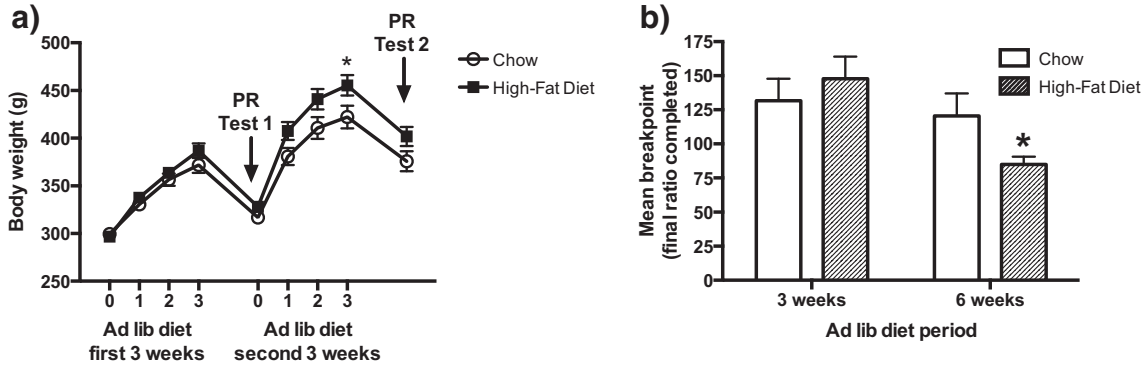

Fig. 1. (a) Body weights of rats maintained on either chow (n = 7) or HFD (n = 7). Tests of progressive ratio (PR) responding were conducted while food restricted to ~85% of ad lib body weight at the end of each 3-week ad lib period. (b) Sucrose-reinforced progressive ratio breakpoints (mean ± SEM) for rats maintained on chow (n = 7) or HFD (n = 8) for either 3 weeks or 6 weeks prior to PR testing. *p ≥ 0.05.

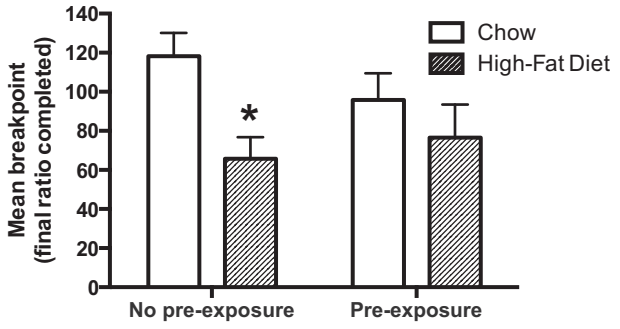

Fig. 2. Progressive ratio breakpoints (mean ± SEM) for rats maintained on chow or HFD for 10 weeks prior to operant conditioning. Half the rats in each diet condition (Pre-exposure; n = 8 per diet condition) were pre-exposed to the sucrose pellets used as the operant reinforcer prior to beginning the 10 weeks of ad lib diet intake, while the other half (No pre-exposure; n = 7 per diet condition) received exposure to plain grain pellets. *p ≥ 0.05.

greater extent and at a greater rate than chow-fed rats and this was not affected by reinforcer pre-exposure. This was confirmed by post-hoc Tukey's HSD analysis, indicating that body weights between the two diet conditions did not differ at Week 1, but that HFD-fed rats were significantly heavier starting in Week 6 and continuing to Week 10 when experimental food deprivation began (Week 10 mean body weights: Chow = 495.4 ± 11.8 g, HFD = 562.1 ± 11.8 g).

3.3.2. Progressive ratio breakpoint

As seen in Fig. 3, breakpoints were reduced in both HFD- and chow-fed rats when responding for a flavored sucrose pellet that they had been exposed to prior to operant training and testing. However, this effect did not appear to be influenced by diet condition. Indeed, a 2 × 2 × 2 ANOVA (Pre-Exposure × Diet × Pellet Flavor) confirmed this, yielding only a main effect of Pre-exposure [$F(1,24) = 4.43$, $p < 0.05$]. The lack of either a main effect of Diet or a Pre-exposure × Diet interaction indicates that, although there was no learning about the specific flavor of the pellets, the exposure to any sucrose pellets did mediate the typical reduction in responding observed in the HFD condition. Pellet Flavor did not interact with either of the critical variables, nor was there a significant main effect of Pellet Flavor alone, indicating that there was not an overall or systematic difference in preference for peanut butter or fruit punch flavored pellets.

3.4. Experiment 4

3.4.1. Body weight

Body weights were analyzed by repeated measures ANOVA with Weeks (0–8) as a within-subjects factor and Diet (HFD vs Chow) as a between-subjects factor. This analysis yielded main effects of Week [$F(8,112) = 31.62$, $p < 0.01$] and Diet [$F(1,14) = 7.28$, $p < 0.05$], indicating that all rats gained weight over the dietary exposure period and that HFD-fed rats were significantly heavier than chow-fed rats. Although there was not a significant Week × Diet interaction [$F(8,112) = 1.98$, $p = 0.055$], independent t-tests confirmed that body weights were not significantly different at Week 0 [$t(14) = 0.24$, $p = 0.8$, Chow = 323.0 ± 4.2 g, HFD = 321.6 ± 4.1 g], but HFD rats were significantly heavier at Week 8 [$t(14) = -4.32$, $p < 0.01$; Chow = 456.8 ± 10.1 g, HFD = 548.1 ± 18.6 g].

3.4.2. Conditioned place preference

Percent time spent in the sucrose-paired chamber was calculated for the pre- and post-test for each animal. Means for these values were compared using paired samples t-tests. A significant increase in percent time spent in the sucrose-paired chamber was observed following six training sessions for chow-fed rats [$t(7) = -4.31$, $p > 0.01$], but not for high-fat diet-fed rats [$t(7) = -0.86$, $p = 0.42$] (Fig. 4).

4. Discussion

Previous attempts to assess the effects of obesigenic diets on food-motivated behaviors in rat models have yielded inconsistent results, with some studies reporting an increase in motivation [5–7], while others have found a reduction in these behaviors [3,4]. While this research has consistently used the same basic measure to assess motivation (operant responding on a progressive ratio schedule), there have been a number of methodological variations across studies. The current investigation focused on two of these differences: duration of HFD consumption and degree of exposure to the reinforcer prior to chronic HFD consumption. Our results clearly indicate that both of these factors play a role in the effect of HFD-induced obesity on food-reinforced behaviors, providing not only a potential explanation of the discrepant results from a methodological perspective, but also conceptual insight into the motivational changes that develop as a result of chronic consumption of a palatable, energy-dense diet.

In the present study, after 3 weeks of diet exposure, there was no difference in PR performance between the animals consuming HFD and those consuming low-fat chow. After an additional 3 weeks of ad lib feeding, however, PR responding was significantly reduced in the HFD condition. Although responding at 3 weeks was not different between the two diet conditions, this result fits the general pattern observed in the literature, as shorter dietary exposure in prior work was associated with increases in responding for a sucrose reinforcer [5–7], while relatively lengthy exposures were associated with reductions in responding [3,4,9]. Here, however, we were able to isolate the effect of duration from variations in dietary composition and other differences

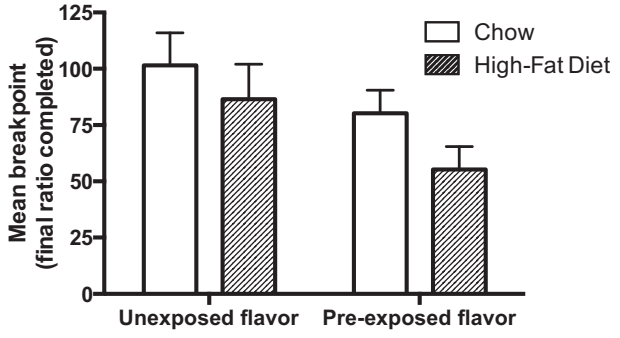

Fig. 3. Progressive ratio breakpoints (mean ± SEM) for rats maintained on chow or HFD for 10 weeks prior to operant conditioning. Half the rats in each diet condition (pre-exposed flavor; n = 8 per diet condition) were pre-exposed to the same flavor of sucrose pellets used as the operant reinforcer prior to beginning the 10 weeks of ad lib diet intake, while the other half (unexposed flavor; n = 8 per diet condition) received exposure to sucrose pellets of a different flavor.

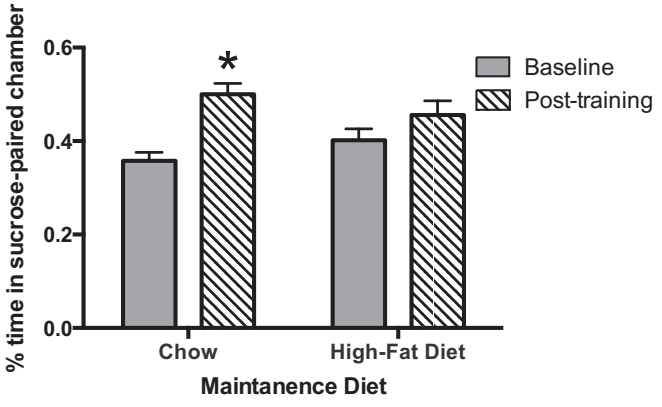

Fig. 4. Time spent in sucrose-paired chamber pre- and post-conditioning by rats maintained on either chow (n = 8) or HFD (n = 8) for 10 weeks prior to beginning conditioning. *p ≥ 0.05.

across laboratories and, by testing the same group of animals at different time points, to demonstrate a specific effect of diet duration on motivation for sucrose pellets, independent of other environmental or experimental factors.

The fact that we observed significant increases in body weight only at the second time point tested, coincident with the reductions in responding for sucrose, suggests that weight gain is a critical factor contributing to motivational deficits. This is consistent with other studies in which animals that were significantly heavier showed reductions in motivated behaviors, while those that were not showed increased responses [3–7]. This interpretation is also supported by a study in which rats were fed a lower (10%) fat diet, but still gained significantly more weight than a control group fed a 13% fat diet, but produced fewer PR responses and by reports of depressed motivation in rats genetically prone to obesity that are heavier even when fed the same low-fat diet as their obesity-resistant counterparts [3,9,10]. On the other hand, Davis et al. [3] included a pair-fed group that also suppressed PR responding while consuming the HFD in the absence of weight gain, indicating that weight gain cannot exclusively account for this effect. However, the nature of the present study in which rats on the same diet were tested at multiple time points and the collective weight of existing evidence lead us to conclude that significant and chronic overweight is a substantial contributor to deficits in food-motivated behavior.

We have also shown here that, independent of weight gain or duration of HFD consumption, experience with the reinforcer can modulate motivated behaviors. Specifically, exposure to sucrose prior to the onset of diet-induced obesity attenuated the decreased PR responding observed, as compared to rats under the same dietary conditions and weight status who had no sucrose exposure prior to operant training. Again, this supports the pattern that we observed in the literature in which animals exposed to higher sucrose content as a part of their maintenance diet showed increases in sucrose motivation, while those with little or no sucrose exposure showed decreases [3–7]. However, in these previous studies, the degree of familiarity with sucrose was confounded with duration of HFD consumption, as well as other possible cross-laboratory procedural or environmental variations. By explicitly manipulating only exposure to the sucrose reinforcer prior to introducing the HFD, while holding all other variables constant, we have demonstrated that even a brief introduction to sucrose is sufficient to attenuate the reduction in motivation that would otherwise occur after 10 weeks on the HFD, producing responding at the same level as chow-fed rats.

In Experiment 3, we asked about the specificity of this attenuation by exposing animals to sucrose pellets with a particular flavor, then assessing their responding for either that same flavor or for a novel pellet flavor, and found no evidence of specific learning about the flavor. Rather, we observed a general effect of sucrose pre-exposure to attenuate motivational deficits, replicating the effect from Experiment 2. Regardless of whether rats were tested with the pre-exposed pellet flavor or a novel flavor, PR breakpoints were equivalent for chow-fed and HFD-fed rats in this experiment; there was no motivational deficit. This result indicates that the learning about the pellets that occurred during the pre-exposure phase was generalized to the sweet taste of sucrose, not specific to particular flavors. As described above (Section 2.4.1), pilot testing conducted prior to this experiment indicated that the two flavors were discriminable by rats, so this result cannot be due to the inability of the animals to tell one flavor from the other, nor was it due to preference for one flavor over another. In fact, we observed that, regardless of diet, all rats responded significantly less during the PR test if they were responding for their pre-exposed pellet flavor compared to rats responding for an unexposed flavor, indicating that some learning about the flavor did occur. This decrement in responding may be due to latent inhibition — that is, the formation of a weaker association between the flavor and the conditioning context as a result of prior experience receiving the flavor in the absence of the training context, which would require discrimination between the two flavors. At the same time, the value assigned to the reinforcer during the pre-exposure period accrued to the sweet taste of the sucrose rather than the specific flavor (see below for further discussion).

There are a number of possible explanations for the observed differences in lever pressing that do not rely on motivation or reward processing. First, the rats in the HFD condition may have failed to successfully acquire the operant task. While diet-induced obese rats do exhibit learning impairments across a variety of tasks [11–13], we do not believe that the reduction in bar pressing we observed can be attributed to a failure to form the association between the behavioral response and the outcome. Animals in both diet conditions demonstrated equivalent learning after the initial training at 3 weeks ad lib HFD exposure in Experiment 1, but still showed differences in later PR responding. As well, rats in Experiments 2 and 3 increased their pressing across sessions and with increasing fixed ratios during the FR training sessions, which were initiated after 10 weeks of HFD consumption. Presses on the inactive lever were significantly lower than active lever presses for all dietary and exposure conditions in all experiments, demonstrating that animals were not simply responding indiscriminately, but had appropriately associated the outcome with the correct lever-pressing response. Secondly, the lower levels of pressing by HFD-consuming rats also cannot be explained by reductions in overall activity, as total activity counts did not differ significantly between the groups, nor did the number of lever presses on the inactive lever. Finally, the lack of motivational deficit in Experiments 2 and 3 after sucrose exposure argues against the possibility that the reduction in goal-directed behavior is due to a negative contrast effect in which sucrose compares unfavorably to the palatable HFD, as the sucrose pre-exposure should not affect the comparison between the two foods.

In order to more directly assess the reward value of sucrose pellets independent of the requirement to acquire operant conditioning or engage in effortful behavior, we employed a conditioned place preference (CPP) procedure in which no response was required for rats to receive the food. In this procedure, a stimulus is considered to be "rewarding" if the animal displays a preference for the location in which the stimulus was received. This process is distinct from (though not necessarily orthogonal to) the one underlying operant reinforcement [14]. Prior work has reported failure to acquire CPP for amphetamine in obese rats and for nicotine in obese mice [3,15], but the present study is the first to test CPP for a food stimulus. We found that the rats on the HFD did not develop a CPP for sucrose pellets under conditions that produced a place preference in lean rats, indicating that this palatable food has a decreased reward value following chronic HFD consumption, supporting the interpretation that a reward deficit underlies the reduction in motivation seen in the operant task. An alternative interpretation of this finding is that the obese rats are impaired at forming the association between the context and the sucrose pellets. However, the acquisition of simple Pavlovian discrimination has previously been shown to be unimpaired in an obese rat model [12], suggesting that this is unlikely to account for our results. Furthermore, a recent study demonstrated that obese women are impaired at forming an association between a visual cue and a food reinforcer, but not a monetary reinforcer, supporting our conclusion that this is not a general associative learning deficit, but a function of the alteration of the reward value of select stimuli [16].

One of the most reliable physiological consequences of chronic HFD consumption and the attendant weight gain is an increase in circulating levels of insulin and leptin [17], which function as adiposity signals that provide feedback to the CNS on the repository of energy in body fat stores [18]. Central administration of exogenous leptin and insulin reduces lever pressing for sucrose in lean rats and impairs expression of a previously learned food-conditioned place preference, and leptin prevents learning of this preference under food-deprived conditions [6,19,20]. It seems plausible, then, that elevated endogenous leptin and insulin in obese rats are contributing factors to the reduced PR responding and failure to show a CPP in the present experiments. Leptin levels are directly correlated with BMI in rats, which is consistent with the finding

that the longer the rats are subject to the HFD and the corresponding weight gain, the more likely deficits are to appear in food-motivated behaviors. This is also largely consistent with other studies — for example, leptin levels were significantly elevated after 13 weeks of HFD in mice with PR deficits, but plasma leptin did not differ following 5 weeks of HFD feeding and elevated PR responding [4,7]. Though there are exceptions, as leptin levels were significantly increased in rats showing increased PR responding after only 2 weeks of a high-fat, high-sugar choice diet [5]. That measurement was conducted after the completion of training and testing, however, and may not reflect leptin levels during the behavioral assay. Given that in a number of cases, plasma leptin and/or insulin levels were not collected, and the inconsistent timing relative to behavioral assays when they were measured, a study that systematically evaluates the relationship between plasma leptin and insulin levels and food-reinforced PR responding is needed to better assess the role of these endogenous hormones. Interestingly, exogenous administration of insulin and leptin did not have suppressive effects on operant performance in HFD-fed rats after 5 weeks of diet consumption [6]. However, in this study, the HFD-fed rats were already producing elevated PR responding, suggesting another process may be sensitizing behavior and overriding any effects of leptin or insulin to suppress motivation, again necessitating further study of the role of these hormones in these behaviors under different dietary conditions.

The present results experimentally confirm that the longer consumption of the HFD persists (along with the consequent weight gain), the greater the decrement in food-motivated behaviors. Midbrain dopamine pathways are critically involved in feeding behavior, and, more importantly, in motivational processes [21–23], with impaired DA function decreasing motivated behaviors and elevated DA activity having the opposite effect [24,25]. There is ample evidence that obese humans and non-human animals exhibit both structural and functional changes in this brain circuitry. High-fat-diet-induced obese rats have reduced numbers of D2 receptors in the nucleus accumbens (NAcc), decreased DA turnover, and lower levels of DA release in response to palatable food intake [3,26–28]. Similarly, obese humans have reduced striatal D2 receptor availability and decrease activation in these regions in response to tasting palatable foods [29–31]. These studies represent chronic periods of overweight, with a minimum period of 28 days consuming the HFD for animals and humans meeting BMI standards for obesity. In contrast, feeding mice a 40% high-fat diet for only 20 days significantly increased NAcc D2 receptors [32]. Though no studies have looked specifically at changes in DA function over time, these findings, along with D2 receptor number in rats and D2 binding availability in humans being negatively correlated with body weight/BMI [26,29], strongly suggest that DA activity shifts during the process of chronic intake of palatable food, and decrements are progressive with increases in weight gain and persistent intake of palatable, energy dense foods. The rats consuming HFD in the present study were significantly heavier at the 6-week time point, but not the 3-week time point, compared to chow-fed rats, and in previous studies of food motivation, significantly higher body weights were observed in rats that showed decreased PR breakpoint, while in studies where breakpoint was increased, body weights were not significantly different between diet conditions. Furthermore, it is likely that the action of leptin and insulin on food-motivated behavior occurs via mesolimbic DA circuitry [33]. Leptin receptor knockdown in the mid-brain increases sucrose-reinforced operant behavior [34] and insulin in the VTA suppresses activity in DA neurons [35], providing a potential mechanism for down-regulation of this system under conditions of elevated insulin levels. Collectively, these data support the notion that DA dysfunction, as a consequence of diet-induced weight gain and persistent consumption of palatable, energy-dense foods, may be one basis for the observed findings and worthy of future exploration. Over time, this impairment leads to motivational deficits, but between initial exposure to obesigenic foods and the occurrence of significant weight gain, there may be a phase of elevated DA activity that promotes motivation to obtain and consume these foods.

This DA-based incentive salience hypothesis may also provide a mechanism for the pre-exposure effect that we observed. One proposed mechanism for dopaminergic function in driving motivated behavior is that it acts to generate incentive salience or "wanting" for a particular reinforcer [36]. Within this framework, incentive salience is determined as a function of two primary components: the stimulus representation in memory, which has an assigned value based on prior experience, and the current physiological state of the animal relevant to the reinforcer (e.g., hunger level, in the case of food). DA functions to integrate this information and produce a signal of incentive salience that drives performance of goal-directed behaviors [23]. In the case of the present studies, consumption of palatable sugar pellets has a high reward value for lean rats regardless of when they are consumed, while for obese rats, the assigned value is low due largely to adiposity signals providing information about a replete physiological state to mesolimbic circuitry [34,35,37]. However, if rats were previously exposed to the sucrose pellets their memory of the high value from that experience will contribute to determining incentive salience and maintain a higher level of responding.

One challenge in interpreting the current findings is that we did not observe an increase in PR responding under any of our conditions, failing to replicate the effects seen by others when using shorter duration of dietary exposure and different diet composition [5–7]. Our short-duration test (3 weeks) was selected to be a time point intermediate to those used in prior studies (2–5 weeks), while our longer duration time point was just slightly longer than the longest duration shown to increase responding. It is possible that a shorter time on our diet would have yielded increased responding, but what is almost certain is that these effects are a result of an interaction between multiple variables, including, but likely not limited to, those under investigation here. In fact, another study reporting a decrement in PR responses in diet-induced obese rats used a diet with a relatively high (34.5%) sucrose content, but also an ad lib consumption period of 13 weeks, suggesting that the reinforcer pre-exposure effect may be overridden by an extended period of obesity and HFD intake. In addition, the pre-exposure to sucrose that was given in Experiments 2 and 3 was very limited compared to the sucrose exposure received by animals in previous studies prior to showing increased PR breakpoints [5,7]. The fact that, as noted above, even this brief exposure was sufficient to attenuate the deficit in PR responding is consistent with the notion that more extensive exposure could reverse the deficit and lead to increases in responding.

It is also worth noting that, in order to produce robust responding across diet conditions, we food restricted our animals during PR testing, which was not the case in the studies conducted by La Fleur et al. [5] or Figlewicz et al. [7], who found increases in PR responding after HFD consumption. While it is plausible that obese rats may respond differently to food restriction or be less sensitive to changes in states of hunger and satiety than their lean counterparts, we do not believe that this can account for the differences between the present study (and that of Davis et al., in which rats were also food restricted and PR responding was reduced in animal on the HFD). In the study by Finger et al. [4], mice on a HFD produced fewer responses during PR testing under non-food-restricted conditions, indicating that the satiety state of the animals does not reliably predict the direction of the difference between HFD- and chow-fed rats. Further, when looking at the number of PR bar presses across studies, the rats in our experiments or those of Davis et al. [3] press at levels that are intermediate to those of La Fleur et al. and Figlewicz et al. (Chow: ~325–525 (present, [3]), vs ~100 [7] vs ~500 [5]; HFD: ~150–850 (present, [3]), vs ~175 [7] vs ~500 [5]). It is evident by this range that there are other factors playing a role in overall levels of responding, but this leads us to conclude that the inconsistent response to food restriction across studies makes this an unlikely explanation for the systematic differences that we observed.

Unlike the rats in the present studies, obese humans are consistently found to have higher food motivation, as tested by PR responding, attentional bias toward food cues, and neural activation in response to food cues and food anticipation [1,2,30,38]. This is in spite of demonstrated decrements in DA receptor number and function and high leptin levels; in many cases, these are subjects with very high BMI values (≥40) indicative of a relatively lengthy duration of overweight and overeating, which we would expected to reduce food-motivated responding [29,31,39]. Applying the results of Experiments 2 and 3 to this situation can help explain the apparent differences between humans and the rodent model. Due to the prevalence of high-fat, high-sugar, high-energy foods in our current obesigenic environment, people are exposed to a wide variety of foods prior to the onset of weight gain. This "pre-exposure" establishes a high value for these foods based on the memory of the initial encounters, which is retrieved on subsequent encounters in an obese state, leading to "cravings" for the food and increased motivation to obtain and consume the food. The experience accrued by humans is much greater than the relatively brief pre-exposure given to our rats and, thus, is likely to have a much greater impact on motivation. And, as the results of Experiment 3 indicate, if the incentive salience accrues to general tastes, such as sweet or salty, rather than specific flavors, this would increase motivation for a very wide variety of readily available foods (e.g., "sweets" rather than just chocolate chip cookies). Further, if there is an initial period of reinforcer sensitization prior to the onset of obesity, the animals in the present studies did not experience the sweet sucrose pellets during that time, limiting a further opportunity to establish a high value, while humans presumably continue to consume these palatable foods during this period raising the value that will be recalled later.

5. Conclusion

The current investigation into the effects of high-fat-diet-induced obesity on food-motivated behaviors makes a significant step toward reconciling prior, apparently discrepant, findings in the literature. By explicitly manipulating the length of time consuming the HFD and prior exposure to the operant reinforcer, we were able to establish that these variables are, independently, critical determinants of food motivation when modeling the effects of obesity.

In particular, this work is of interest with respect to the relevance of animal models of obesity in explaining human food motivation. The present obesigenic environment provides a great deal of experience with palatable, high-energy foods, which functions similarly to the pre-exposure in the present studies, establishing a high incentive salience for many palatable foods that leads to persistent drive to seek out and consume these foods throughout the development and maintenance of obesity. This elevated motivation due to extensive prior food experience may contribute to the difficulty in achieving successful weight loss.

Based on the differences between our findings here and those typically observed in obese humans, we offer a suggestion for future research: in order to best extrapolate results from animal subjects to human obesity, using an obesigenic diet that provides variety in taste and nutrient sources, such as a cafeteria diet, will serve to most accurately model the experience and conditions that contribute to human weight gain in the present, western, obesigenic environment and lead to a clearer understanding of the behavioral and physiological consequences of more typical diet-induced obesity.

Acknowledgments

This work was supported by the Grinnell College Committee on Support of Faculty Scholarship and Harris Faculty Fellowship (ALT).

References

[1] B.E. Saelens, L.H. Epstein, Reinforcing value of food in obese and non-obese women, Appetite 27 (1996) 41–50. http://dx.doi.org/10.1006/appe.1996.0032.
[2] L.H. Epstein, J.L. Temple, B.J. Neaderhiser, R.J. Salis, R.W. Erbe, J.J. Leddy, Food reinforcement, the dopamine D2 receptor genotype, and energy intake in obese and nonobese humans, Behav. Neurosci. 121 (2007) 877–886. http://dx.doi.org/10.1037/0735-7044.121.5.877.
[3] J.F. Davis, A.L. Tracy, J.D. Schurdak, M.H. Tschöp, J.W. Lipton, D.J. Clegg, et al., Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat, Behav. Neurosci. 122 (2008) 1257–1263. http://dx.doi.org/10.1037/a0013111.
[4] B.C. Finger, T.G. Dinan, J.F. Cryan, Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task, Psychopharmacology (Berlin) (2012) 173–181. http://dx.doi.org/10.1007/s00213-011-2468-0.
[5] S.E. la Fleur, L.J. Vanderschuren, M.C. Luijendijk, B.M. Kloeze, B. Tiesjema, R.A. Adan, A reciprocal interaction between food-motivated behavior and diet-induced obesity, Int. J. Obes. 31 (2007) 1286–1294. http://dx.doi.org/10.1038/sj.ijo.0803570.
[6] D.P. Figlewicz, J.L. Bennett, A.M. Naleid, C. Davis, J.W. Grimm, Intraventricular insulin and leptin decrease sucrose self-administration in rats, Physiol. Behav. 89 (2006) 611–616. http://dx.doi.org/10.1016/j.physbeh.2006.07.023.
[7] D.P. Figlewicz, J.L. Jay, M.A. Acheson, I.J. Magrisso, C.H. West, A. Zavosh, et al., Moderate high fat diet increases sucrose self-administration in young rats, Appetite 61 (2013) 19–29. http://dx.doi.org/10.1016/j.appet.2012.09.021.
[8] N.R. Richardson, D.C. Roberts, Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy, J. Neurosci. Methods 66 (1996) 1–11 (http://www.ncbi.nlm.nih.gov/pubmed/8794935, accessed July 28, 2014).
[9] A.P. Blaisdell, Y.L.M. Lau, E. Telminova, H.C. Lim, B. Fan, C.D. Fast, et al., Food quality and motivation: a refined low-fat diet induces obesity and impairs performance on a progressive ratio schedule of instrumental lever pressing in rats, Physiol. Behav. 128 (2014) 220–225. http://dx.doi.org/10.1016/j.physbeh.2014.02.025.
[10] E.B. Rasmussen, W. Reilly, C. Hillman, Demand for sucrose in the genetically obese Zucker (fa/fa) rat, Behav. Process. 85 (2010) 191–197. http://dx.doi.org/10.1016/j.beproc.2010.07.008.
[11] R. Molteni, R.J. Barnard, Z. Ying, C.K. Roberts, F. Gómez-Pinilla, A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning, Neuroscience 112 (2002) 803–814 (http://www.ncbi.nlm.nih.gov/pubmed/12088740).
[12] S.E. Kanoski, R.L. Meisel, A.J. Mullins, T.L. Davidson, The effects of energy-rich diets on discrimination reversal learning and on BDNF in the hippocampus and prefrontal cortex of the rat, Behav. Brain Res. 182 (2007) 57–66. http://dx.doi.org/10.1016/j.bbr.2007.05.004.
[13] G. Winocur, C.E. Greenwood, Studies of the effects of high fat diets on cognitive function in a rat model, Neurobiol. Aging 26 (Suppl. 1) (2005) 46–49. http://dx.doi.org/10.1016/j.neurobiolaging.2005.09.003.
[14] M.T. Bardo, R.A. Bevins, Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology (Berlin) 153 (2000) 31–43 (http://www.ncbi.nlm.nih.gov/pubmed/11255927, accessed July 21, 2014).
[15] J.A. Blendy, A. Strasser, C.L. Walters, K.A. Perkins, F. Patterson, R. Berkowitz, et al., Reduced nicotine reward in obesity: cross-comparison in human and mouse, Psychopharmacology (Berlin) 180 (2005) 306–315. http://dx.doi.org/10.1007/s00213-005-2167-9.
[16] Z. Zhang, K.F. Manson, D. Schiller, I. Levy, Impaired associative learning with food rewards in obese women, Curr. Biol. (2014). http://dx.doi.org/10.1016/j.cub.2014.05.075.
[17] S.C. Woods, R.J. Seeley, P.A. Rushing, D.D. Alessio, P. Tso, A controlled high-Fat diet induces an obese syndrome in rats 1, J. Nutr. (2003) 1081–1087.
[18] S.C. Benoit, D.J. Clegg, R.J. Seeley, S.C. Woods, Insulin and leptin as adiposity signals, Recent Prog. Horm. Res. 59 (2004) 267–285 (http://www.ncbi.nlm.nih.gov/pubmed/14749506, accessed August 13, 2014).
[19] D.P. Figlewicz, M.S. Higgins, S.B. Ng-Evans, P.J. Havel, Leptin reverses sucrose-conditioned place preference in food-restricted rats, Physiol. Behav. 73 (2001) 229–234 (http://www.ncbi.nlm.nih.gov/pubmed/11399316).
[20] D.P. Figlewicz, J. Bennett, S.B. Evans, K. Kaiyala, A.J. Sipols, S.C. Benoit, Intraventricular insulin and leptin reverse place preference conditioned with high-fat diet in rats, Behav. Neurosci. 118 (2004) 479–487. http://dx.doi.org/10.1037/0735-7044.118.3.479.
[21] J.D. Salamone, M. Correa, The mysterious motivational functions of mesolimbic dopamine, Neuron 76 (2012) 470–485. http://dx.doi.org/10.1016/j.neuron.2012.10.021.
[22] R.D. Palmiter, Is dopamine a physiologically relevant mediator of feeding behavior? Trends Neurosci. 30 (2007) 375–381. http://dx.doi.org/10.1016/j.tins.2007.06.004.
[23] K.C. Berridge, The debate over dopamine's role in reward: the case for incentive salience, Psychopharmacology (Berlin) 191 (2007) 391–431. http://dx.doi.org/10.1007/s00213-006-0578-x.
[24] K.S. Smith, K.C. Berridge, J.W. Aldridge, Disentangling pleasure from incentive salience and learning signals in brain reward circuitry, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) E255–E264. http://dx.doi.org/10.1073/pnas.1101920108.
[25] B. Cagniard, P.D. Balsam, D. Brunner, X. Zhuang, Mice with chronically elevated dopamine exhibit enhanced motivation, but not learning, for a food reward, Neuropsychopharmacology 31 (2006) 1362–1370. http://dx.doi.org/10.1038/sj.npp.1300966.
[26] P.M. Johnson, P.J. Kenny, Addiction-like reward dysfunction and compulsive eating in obese rats: role for dopamine D2 receptors, 13 (2010) 635–641. http://dx.doi.org/10.1038/nn.2519 (Addiction-like).
[27] B.M. Geiger, M. Haburcak, N.M. Avena, M.C. Moyer, B.G. Hoebel, E.N. Pothos, Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity, Neuroscience 159 (2009) 1193–1199. http://dx.doi.org/10.1016/j.neuroscience.2009.02.007.

[28] E. van de Giessen, S.E. la Fleur, L. Eggels, K. de Bruin, W. van den Brink, J. Booij, High fat/carbohydrate ratio but not total energy intake induces lower striatal dopamine D2/3 receptor availability in diet-induced obesity, Int. J. Obes. (Lond.) 37 (2013) 754–757. http://dx.doi.org/10.1038/ijo.2012.128.
[29] G.J. Wang, N.D. Volkow, J. Logan, N.R. Pappas, C.T. Wong, W. Zhu, et al., Brain dopamine and obesity, Lancet 357 (2001) 354–357 (http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3098897&tool=pmcentrez&rendertype=abstract).
[30] E. Stice, S. Spoor, C. Bohon, M.G. Veldhuizen, D.M. Small, Relation of reward from food intake and anticipated food intake to obesity: a functional magnetic resonance imaging study, 117 (2008) 924–935. http://dx.doi.org/10.1037/a0013600.
[31] E. van de Giessen, F. Celik, D.H. Schweitzer, W. van den Brink, J. Booij, Dopamine D2/3 receptor availability and amphetamine-induced dopamine release in obesity, J. Psychopharmacol. (2014). http://dx.doi.org/10.1177/0269881114531664.
[32] T. South, X.-F. Huang, High-fat diet exposure increases dopamine D2 receptor and decreases dopamine transporter receptor binding density in the nucleus accumbens and caudate putamen of mice, Neurochem. Res. 33 (2008) 598–605. http://dx.doi.org/10.1007/s11064-007-9483-x.
[33] S. Fulton, P. Pissios, R.P. Manchon, L. Stiles, L. Frank, E.N. Pothos, et al., Leptin regulation of the mesoaccumbens dopamine pathway, Neuron 51 (2006) 811–822. http://dx.doi.org/10.1016/j.neuron.2006.09.006.
[34] J.F. Davis, D.L. Choi, J.D. Schurdak, M.F. Fitzgerald, D.J. Clegg, J.W. Lipton, et al., Leptin regulates energy balance and motivation through action at distinct neural circuits, Biol. Psychiatry 69 (2011) 668–674. http://dx.doi.org/10.1016/j.biopsych.2010.08.028.
[35] G. Labouèbe, S. Liu, C. Dias, H. Zou, J.C.Y. Wong, S. Karunakaran, et al., Insulin induces long-term depression of ventral tegmental area dopamine neurons via endocannabinoids, Nat. Neurosci. 16 (2013) 300–308. http://dx.doi.org/10.1038/nn.3321.
[36] K.C. Berridge, Food reward: brain substrates of wanting and liking, Neurosci. Biobehav. Rev. 20 (1996) 1–25 (http://www.ncbi.nlm.nih.gov/pubmed/8622814).
[37] S. Fulton, B. Woodside, P. Shizgal, Modulation of brain reward circuitry by leptin, Science 287 (2000) 125–128 (http://www.ncbi.nlm.nih.gov/pubmed/10615045).
[38] S. Yokum, J. Ng, E. Stice, Attentional bias to food images associated with elevated weight and future weight gain: an fMRI study, Obesity (Silver Spring) 19 (2011) 1775–1783. http://dx.doi.org/10.1038/oby.2011.168.
[39] M. Maffei, J. Halaas, E. Ravussin, R.E. Pratley, G.H. Lee, Y. Zhang, et al., Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects, Nat. Med. 1 (1995) 1155–1161. http://dx.doi.org/10.1038/nm1195-1155.