Published in final edited form as:

Appetite. 2013 February ; 61(1): 19–29. doi:10.1016/j.appet.2012.09.021.

Moderate High Fat Diet Increases Sucrose Self-Administration In Young Rats

Dianne P. Figlewicz^{1,2}, Jennifer L. Jay², Molly A. Acheson, Irwin J. Magrisso⁴, Constance H. West¹, Aryana Zavosh², Stephen C. Benoit³, and Jon F. Davis³

¹VA Puget Sound Health Care System (151), Seattle WA 98108; University of Washington, Seattle WA 98195, U.S.A

²Dept of Psychiatry & Behavioral Sciences, University of Washington, Seattle WA 98195, U.S.A

³Dept of Psychiatry Metabolic Diseases Institute, University of Cincinnati, Cincinnati OH 45237, U.S.A

⁴Dept of Internal Medicine, Metabolic Diseases Institute, University of Cincinnati, Cincinnati OH 45237, U.S.A

Abstract

We have previously reported that a moderately high fat diet increases motivation for sucrose in adult rats. In this study, we tested the motivational, neurochemical, and metabolic effects of the high fat diet in male rats transitioning through puberty, during 5-8 weeks of age. We observed that the high fat diet increased motivated responding for sucrose, which was independent of either metabolic changes or changes in catecholamine neurotransmitter metabolites in the nucleus accumbens. However, AGRP mRNA levels in the hypothalamus were significantly elevated. We demonstrated that increased activation of AGRP neurons is associated with motivated behavior, and that exogenous (third cerebroventricular) AGRP administration resulted in significantly increased motivation for sucrose. These observations suggest that increased expression and activity of AGRP in the medial hypothalamus may underlie the increased responding for sucrose caused by the high fat diet intervention. Finally, we compared motivation for sucrose in pubertal vs. adult rats and observed increased motivation for sucrose in the pubertal rats, which is consistent with previous reports that young animals and humans have an increased preference for sweet taste, compared with adults. Together, our studies suggest that background diet plays a strong modulatory role in motivation for sweet taste in adolescent animals.

Keywords

Motivation; food reward; high fat diet; youth

Introduction

We have previously reported that a short exposure to a moderately high fat (31.8%) diet results in increased motivation for sucrose in adult rats (Figlewicz et al., 2006). The environmental vs. biological influences, or their synergy, on food preferences and motivation for energy dense foods have come to be appreciated over the past decade. This has heightened relevance in the young, as pediatric obesity has increased dramatically across

To whom correspondence should be addressed: Dianne Figlewicz Lattemann, Ph.D., Metabolism/Endocrinology (151), VA Puget Sound Health Care System, 1660 So. Columbian Way, Seattle WA 98108, Phone: 206-768-5240, FAX: 206-764-2164, latte@u.washington.edu.

the past decade (Ogden & Carroll, 2010). Increased preference for sweet taste has been documented in both young animals and the human pediatric population (Coldwell, Oswald, & Reed, 2009; Desor & Beauchamp, 1987; Desor, Greene, & Maller, 1975; Mennella, Pepino, & Reed, 2005; Meyers & Sclafani, 2006)), and is the presumptive basis for the food industry to design and market packaged foods and beverages with a high sugar content, for children. However, the impact of environmental influences, such as background diet, on motivation for sucrose in juvenile rats has not been evaluated systematically.

Current estimates suggest that 10-20% of children and adolescents in the U.S. are considered obese (Ogden & Carroll, 2010). On average the US population consumes 336 kcal of added sugar daily (National Cancer Institute Applied Research Program). When the population is separated into adults (19+ years old) and the pediatric population (2-18 years old), this number is slightly higher for children/adolescents and slightly lower for adults. For adolescents, the majority of added sugars come from soda, energy drinks, and sports drinks (National Cancer Institute Applied Research Program). An extensive systematic review and meta-analysis has shown that soft drink intake is associated with increased energy intake and body weight (Vartanian, Schwartz, & Brownell, 2007). The adolescent population (14--18 years old) consumes 444 kcal worth of added sugar daily, and children between the ages of 9 and 13 years of age consume 381 kcal added sugar daily (National Cancer Institute Applied Research Program). This additional consumption may be attributable in part to an elevated sweet preference in younger individuals versus adults (Coldwell, Oswald, & Reed, 2009; Desor & Beauchamp, 1987; Desor, Greene, & Maller, 1975; Mennella, Pepino, & Reed, 2005). Studies have demonstrated that children between the ages of 9 and 15 years of age prefer sugar solutions at higher concentrations than the preferred concentration of an adult sample (Desor, Greene, & Maller, 1975). Longitudinal studies have tested the sweet preference of these children a decade later in life, at which point their preference had decreased and was not significantly different than adult preference (Desor & Beauchamp, 1987). Studies have also demonstrated a preference for higher sucrose concentrations in children in comparison with their mothers (Mennella, Pepino, & Reed, 2005). This suggests that the heightened childhood sugar preference is not caused by genetics, but rather may reflect a developmental phenomenon. Studies have also demonstrated this heightened sucrose preference in rats (Meyers & Sclafani, 2006).

Many CNS systems and connectivities are plastic during adolescence in humans and rodents, including the mesocorticolimbic system and dopaminergic activity in the nucleus accumbens, a key site for the mediation of reward and motivation (Ikemoto & Panksepp, 1996; Kelley & Berridge, 2002) (see Andersen & Teicher [2008] for recent review). The functional significance of these anatomical and neurochemical changes is now being elucidated. Recent research from Bolaños and colleagues, and others, has been examining the post-treatment effects of the dopamine re-uptake transporter antagonist methylphenidate (Ritalin) in the post-weaning, juvenile rodent. There are reports of altered neurochemistry and behavior in adult life as a function of peri-adolescent treatment with methylphenidate (Bolaños et al., 2003; Bolaños, Glatt, & Jackson, 1998; Brandon, Marinelli, Baker, & White, 2001; Brandon, Marinelli, & White, 2003). While the findings are not entirely consistent, perhaps due to different animal models studied, collectively these studies emphasize that the adolescent period seems to be a developmental window for changing dopamine function. Food is a natural stimulus for the release of dopamine from ventral tegmental area (VTA) projections to the nucleus accumbens, and operant intake of sucrose by rats results in very acute release of dopamine (Roitman et al., 2004). We hypothesize that motivation for sucrose is associated with increases of nucleus accumbens dopamine, and modulation by environmental influences may be uniquely sensitive during this adolescent, peri-pubertal stage in the rat.

Given the high preference for sweet taste in children and young rodents, we felt it important to also determine parameters of motivation for sucrose in adolescent rodents. In this series of studies, we evaluated the effect of a high fat diet intervention on motivation for sucrose in rats as they grew from post-weaning through puberty. We subsequently carried out metabolic and CNS evaluations to discern metabolic, endocrine, or neural changes associated with the diet intervention. Comparable to what we have reported in adult rats, a moderate high fat (31.8%) diet was effective in increasing sucrose self-administration. We also tested whether there was a post-diet treatment effect on sucrose motivation in the rats as young adults, comparable to the types of later-life effects reported for other behaviors. Our studies show that young rats exhibit increased motivation for sucrose when fed a moderately high fat diet which may be mediated by the orexigenic, hypothalamic peptide AGRP; that there appears to be no carryover effect of the early diet intervention, into post-pubertal adulthood; and that the behavior is manifest although the rats are metabolically normal, and pre-obese. Finally, peripubertal rats exhibit increased motivation for sucrose relative to young adult rats.

Materials and Methods

Subjects

Subjects were male Albino rats from Simonsen (Gilroy, CA). Rats were maintained on chow (Laboratory Rodent Diet 5001, LabDiet) or moderate high fat diet (31.8%; Research Diets Inc) *ad libitum*. The diets are matched for overall carbohydrate content (58% kcal vs. 51% kcal for low fat vs. high fat, respectively). The low fat chow has 6.23 gm% free sugars and the high fat diet has 29 gm% sucrose. They were maintained on a 12:12 h light-dark cycle with lights on at 6 AM. Unless otherwise indicated, rats were brought in at 3 weeks of age, immediately post-weaning, and were housed for acclimation until 5 weeks of age. At this age, diet and/or behavioral training and testing were begun. Specific protocols are described in detail below, and summarized in Table 1. Because male rats go through puberty at the 6th-7th week of age, the timing of studies was designed to study rats as they traverse this developmental stage. All procedures performed on the rats followed the NIH guidelines for animal care, and were approved by the Animal Care and Use Sub-Committee of the Research and Development Committee at the VA Puget Sound Health Care System.

Sucrose self-administration

General Protocol. Procedures were based upon our published methodology (Figlewicz et al., 2008; Figlewicz et al., 2006). All training and testing procedures were carried out between 0700 and 1200 hr. The experiment included 2-3 phases: autoshaping and fixed ratio (FR) training; surgery and recovery in specified cohorts (see Table 1); and progressive ratios (PR) training using the PR algorithm of Richardson and Roberts (Richardson & Roberts, 1996). The PR algorithm requires 1, 2, 4, 6, 9, 12, 16, 20, 28, 36, 48, 63, 83, 110, 145, 191, 251, 331, 437, 575, 759, 999, 999(etc) lever presses for succeeding reward deliveries within a session, and is a rigorous test for motivation and reward (27). Rats were trained to self-administer 5% sucrose (0.5 ml reward) delivered into a liquid drop receptacle. The operant boxes, controlled by a Med Associates (Georgia, VT) system, had two levers, but only one lever (an active, retractable lever) activated the infusion pump. Presses on the other lever (an inactive, stationary lever) were also recorded. The sucrose solution was delivered into a liquid drop receptacle for oral consumption (Med Associates). Initial training was conducted during one-h sessions for 10 days under a continuous reinforcement schedule (FR1: each lever press was reinforced), with a maximum possible of 50 sucrose rewards delivered per session. Each session began with the insertion of the active lever and the illumination of a white houselight that remained on for the entire session. A 5-s tone (2900 Hz, 20 dB above background)+light (7.5 W white light above the active lever) discrete compound cue

accompanied each reward delivery, followed by a 20-sec time out after each sucrose delivery. PR training was carried out for a maximum possible 3 h/day for ten days. Daily sessions ended after 30 min of no active lever press responding, at which point the house light was turned off and the active lever retracted.

Effect of AGRP on sucrose self-administration

As our results showed an increase of AGRP mRNA expression in pubertal rats fed the high fat diet, we wanted to confirm that AGRP could increase sucrose self-administration. 5-wk old chow-fed rats were taken through FR training, then received cannulas into the third cerebral ventricle (ICV). Following a week of recovery, confirmation of placement with an angiotensin II drinking response test (see Figlewicz et al., [2006]), and one session of FR re-training, rats were started on the PR self-administration paradigm. After PR Day 1, rats were assigned to one of two groups such that mean PR Day 1 performance did not differ between the two groups (artificial CSF vehicle, aCSF; or AGRP, 2 μ l of 0.01 nmol). They received injections of aCSF (n=8) or AGRP (n=7) on PR days 2, 5, and 8. Total daily food intake was quantitated during the PR training time.

Effect of age on sucrose self-administration

We compared self-administration behavior between the pubertal rats and young adults, fed chow or the 31.8% fat diet. Rats had two weeks of acclimation to the VAPSHCS vivarium (3–5 wk or 8–10 wk). They then received the diet during the entire test/training period (4 wk). Thus, as in the initial experiment, pubertal rats were studied at 5-8 wk of age. Young adults were studied at 10-13 wk of age.

Body composition determination

Body composition was measured using quantitative magnetic resonance spectroscopy (QMR [Nixon et al., 2010]) to determine the body water content of individual rats, from which relative body fat is calculated. The animals were placed in cylindrical holders un-anesthetized, and then the holders are inserted into the QMR machine for a 2 minute scan, which performs triplicate measurements. Data are saved to an integrated computer (EchoMRI, Echo Medical Systems, Houston, TX) for immediate calculation of whole-body water, fat, and lean mass.

Intravenous glucose tolerance testing (IVGTT)

Conscious IVGTTs were carried out in rats with chronically implanted IV cannulas, that were fasted overnight prior to study, utilizing methodology based upon Frangioudakis, Gyte, Loxham, & Poucher (2008). Bilateral intravenous cannulas were implanted two weeks prior to study, as per our established methodology (Figlewicz et al., 2009). Baseline samples were drawn at t-10 min (0.5 ml for determination of insulin and glucose, at all time points) and t0 min. Rats received an infusion of 1 gm glucose/2ml/kg over 15-20 seconds followed by 0.5 ml flush of saline. Blood samples were taken at 5, 15, 30, 60, 90, and 120 min. Owing to plugging of a catheter during the procedure (hence, inability to obtain blood samples), final 'n's for the baseline/IVGTT data presented are 7-8 for chow-fed rats and 8 for rats fed 31.8% fat diet (Table 3). Plasma insulin was determined using Linco rat insulin RIA kits (#RI-13K and SRI-13K, Linco) and plasma glucose was determined on a YSI Glucose Analyzer). Area under the curve (AUC) for the response from baseline was calculated at 5 min and 120 min. The HOMA index was calculated as $\text{fasting (glucose [mM]} \times \text{insulin [U/L]} \text{) / 22.5}$ and was calculated using terminal fasting samples measured for insulin and glucose.

Fasting metabolic parameters

Rats from Experiment 1 were fasted overnight prior to euthanasia, a few days after completion of the IVGTT. Rats were deeply anesthetized with isoflurane inhalation and exsanguinated. Brains were rapidly removed and frozen in liquid nitrogen for measurement of hypothalamic peptide mRNA and nucleus accumbens catecholamines. Terminal plasma or serum were used for measurement of fasting insulin, glucose, leptin, and triglycerides. For triglycerides, Point Scientific Triglyceride GPO Kit #T7531-400 (Fisher #23-666-418) and standards KIT #7531-STD (Fisher #23-666-422) were utilized, and 3 μ l of serum was assayed in duplicate. Plasma leptin was measured with Millipore Linco RIA Kit# RL 83K.

Catecholamine HPLC Methods [Davis et al., 2008]

Rats were euthanized with isoflurane anesthesia, and brains were rapidly removed, frozen, and stored at -80°C . Bilateral micro-punches of the nucleus accumbens (NAcc) were isolated from each animal. Although substantial care was taken to minimize contamination by neighboring brain regions, due to the nature and size of each micro-punch our method did not allow us to distinguish subregions (i.e. NAcc core vs. shell) within the NAcc. For high-performance liquid chromatography (HPLC) analysis, an antioxidant solution (0.4 N perchlorate, 1.343 mM ethylenediaminetetraacetic acid (EDTA) and 0.526 mM sodium metabisulfite) was added to the samples followed by homogenization using an ultrasonic tissue homogenizer (Biologics; Gainesville, VA). A small portion of the tissue homogenate was dissolved in 2% sodium dodecyl sulfate (SDS) (w/v) for protein determination (Pierce BCA Protein Reagent Kit; Rockford, IL). The remaining suspension was spun at 14,000 g for 20 min in a refrigerated centrifuge. The supernatant was reserved for HPLC.

Samples were separated on a Microsorb MV C-18 column (5 μ m, 4.6 $\times$ 250 mm, Varian; Walnut Creek, CA) and simultaneously examined for DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), both of which are markers of dopamine degradation, 5-HT and 5-HIAA. Compounds were detected using a 12-channel coulometric array detector (CoulArray 5200, ESA; Chelmsford, MA) attached to a Waters 2695 Solvent Delivery System (Waters; Milford, MA) under the following conditions: flow rate of 1 ml/min; detection potentials of 50, 175, 350, 400 and 525 mV, and; scrubbing potential of 650 mV. The mobile phase consisted of a 10% methanol solution in distilled H_2O containing 21 g/l (0.1 M) citric acid, 10.65 g/l (0.075 M) Na_2HPO_4 , 176 mg/l (0.8 M) heptanesulfonic acid and 36 mg/l (0.097 mM) EDTA at a pH of 4.1. Unknown samples were quantified against a 6-point standard curve with a minimum R^2 of 0.97. Quality control samples were interspersed with each run to ensure HPLC calibration.

Orexigenic peptides mRNA qPCR

We measured expression of hypothalamic peptides that stimulate feeding and have been implicated in motivation and reward behaviors (Figlewicz & Sipols, 2010): neuropeptide Y (NPY [Hahn, Breininger, Baskin, & Schwartz, 1998; Jewett et al., 1995; Kelley, Nannini, Bratt, & Hodge, 2001]); agouti-related peptide (AGRP [Aponte, Atasoy, & Sternson, 2011; Barnes, Argyropoulos, & Bray, 2010; Broberger et al., 1998; Hagan et al., 2000; Hahn, Breininger, Baskin, & Schwartz, 1998; Kaushik et al., 2011; Krashes et al., 2011; Rossi et al., 1998; Tracy et al., 2008]); and orexin (Cason et al., 2010; Choi, Davis, Fitzgerald, & Benoit, 2010). Rats were euthanized with isoflurane anesthesia, and brains were rapidly removed, frozen and stored at -80°C until processing. The medial and lateral hypothalamus were microdissected as one block using an AHP-1200CPV freezing plane (Thermoelectric Cooling America, Chicago, IL) which maintained a constant temperature of 12°C throughout the dissection process. Total RNA from microdissected tissue was isolated by Trizol reagent (Invitrogen, Carlsbad, CA) and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. The total RNA was treated to remove any potential

genomic DNA contamination using RNase free DNase (Promega, Madison, WI), and was quantitated using a NanoVue spectrophotometer (GE Healthcare, Cambridge, UK). RNA quality was confirmed by standard agarose gel electrophoresis. Complementary DNA (cDNA) was then retrotranscribed (RT) from 1-2 µg of total RNA by a mixture of random hexamers and oligo DT priming using the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, CA). Non-retrotranscribed (no RT) reactions were also prepared from each sample to control for potential genomic DNA contamination. The cDNA and no-RT controls were diluted, and 5-10 ng of template cDNA from each sample was used to measure mRNA expression of selected genes by real time quantitative PCR utilizing the MyIQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Triplicate measurements for each sample were run on standard iCycler 96 well plates, along with no template controls (NTC) to detect potential cross contamination, in 20 µl reaction volumes consisting of 10 µl 2× iQ Sybr Green Supermix (Bio-Rad, Hercules, CA), 2 µl of 0.2-0.5 µM each primer, 3 µl DEPC water, and 5 µl of template. All qPCR reactions included a melt curve analysis to ensure specificity of signal. Relative expression for each gene of interest was calculated by extrapolation to a standard curve individually run on each plate and derived from serial dilutions of a pooled sample of reference cDNA, and normalized to relative expression of reference genes (acidic ribosomal phosphoprotein 36B4 for gene expression in hypothalamic tissue, and mitochondrial ribosomal protein L32 for expression in the nucleus accumbens). The following primer sequences (IDT, San Diego, CA) were used to amplify rat prepro-orexin, NPY, and AGRP: Prepro-orexin, Forward: 5'-TTCCTTCTACAAAGGTTCCCT-3', 5'-GCAACAGTTCGTAGAGACGGCAG-3'; NPY: Forward, 5'-TACTCCGCTCTGCGACACTACATC-3'; Reverse: 5'-CACATGGAAGGGTCTTCAAGCC-3'; AGRP, Forward: 5'-GCAGAAGGCAGAAGCTTTGGC-3'; Reverse: 5'-CCCAAGCAGGACTCGTGACAG-3'.

cFos Immunocytochemistry (ICC) and Quantitation

Fluorescence ICC was used to identify Fos-positive and AGRP-positive neuronal cell bodies in the medial hypothalamus, according to our established methodology (Figlewicz et al., 2011). On the final day (PR Day 10), rats were placed in their self-administration chambers as usual, for 90 min. Immediately following that last 90 min session, rats were deeply anesthetized with isoflurane inhalation and perfused with 0.9% NaCl followed with cold 4% paraformaldehyde solution. The timing for anesthetic and euthanasia was based upon the known timecourse of peak expression of cFos protein at 90--120 min-post event. Thus cFos expression would reflect the activation of the CNS at the onset of the behavioral task, rather than being the result of the animals experiencing the task. Brains were removed and post-fixed in paraformaldehyde several days, then subsequently placed in 20% sucrose-PBS, then 30% sucrose-PBS solution. Brains were sectioned on a cryostat (Leica CM 3050S cryostat) for immunohistochemistry. We used our established methodology to quantitate immunoreactive cFos protein in brain sections (Figlewicz et al., 2011). Slide-mounted 12 µm whole-brain coronal sections were washed three times in phosphate buffered saline (PBS, OXOID, Hampshire, England). Sections were washed for 20 min with 100% ethanol/DI water (50%, v/v) followed by a PBS wash, then blocked for 1 hour at room temperature in PBS containing 5% normal goat or donkey serum. Sections were then washed multiple times in PBS and incubated overnight at 4°C in primary antibody solutions made up in PBS. Sections were washed three times in PBS and then incubated in the dark at room temperature in secondary antibody solution made up in PBS for 1 hour. Sections were subsequently washed again in PBS, and mounted and cover-slipped in Vectashield hard set mounting medium (Vector; Burlingame, CA) mounting medium. Digital images of sections were acquired using a Nikon Eclipse E-800 fluorescence microscope connected to a Qimaging Retiga digital capture camera using NIS Elements (Nikon) software.

Based upon PCR studies demonstrating increased AGRP mRNA levels, we focused upon medial hypothalamic regions, particularly the ventromedial nucleus and the arcuate nucleus (ARC)). Atlas-matched 12 μm sections were evaluated for cFos expression and quantitation in matched sections and regions, based upon the atlas of Paxinos and Watson [2005]. For quantitation (at 40 $\times$ magnification), atlas-matched regions were selected. NIS Elements software (Nikon) was utilized to capture an image of the desired area. An area was delineated for counting and threshold for positive cell counts was established. The identical area and background (threshold) were utilized for sections from the respective experimental groups, and software counting of positive cells (quantitation) was carried out in the same session for all experimental groups, to prevent between-session changes in background setting. For statistical analysis, counts were taken from an individual rat only if corresponding or complete sections through each area were available; data for a specific area were not taken from a rat if there was incomplete bilateral representation for that area.

In addition to cFos quantitation, quantitative double-label immunohistochemistry for cFos and AGRP was conducted. Because we did not wish to disturb the animals' behavioral performance, they were not pre-treated with colchicine to optimize visualization of AGRP. Therefore visualization of AGRP-positive neurons might be underestimated. The dual staining procedure for AGRP was comparable to the assay of cFos-immunoreactivity on its own, except that sections were blocked for one hour at room temperature in PBS-5% donkey serum. Then, a mixture of fos-Ab and AGRP primary antibodies was used for overnight incubation at 4°C; likewise both secondary antibodies were in the same solution and incubated for one hour in the dark at room temperature. Initial optimization assays were carried out to determine an appropriate dilution of the primary antibodies. Primary antibodies used were rabbit anti-cFos (1:500) (sc-52) and goat anti-AGRP (1:100) (18634) (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Secondary antibodies used were Cy3-conjugated donkey anti-rabbit (Jackson ImmunoResearch; West Grove, PA), and Alexa fluor 488 donkey anti-goat IgG (Molecular Probes, Eugene, OR); all secondary antibodies were diluted at 1:500.

Statistical analyses

Group data are presented as means $\pm$ standard error of the mean (SEM) in the text, Tables, and Figures. Significance is defined as $p < 0.05$. Statistical comparisons are made between experimental groups, as presented under "Results" using unpaired Student's 't' test (e.g., diet, age, or treatment comparison). 'Normalization' of data is defined as it is used.

Results

Effect of moderate high fat diet on peri-pubertal motivation for sucrose

Rats fed the 31.8% fat diet during wks 5-8, while in self-administration sessions, had a significantly elevated motivation for sucrose, compared with chow-fed rats. As shown in Figure 1a, there was no difference in performance during the initial FR training (averaged FRDays 1-10 active lever presses, 38 ± 5 vs. 39 ± 2 for chow vs. 31.8% fat diet, respectively). However, when the rats were switched to the more stringent PR task, there was a significant increase of number of active lever presses, and number of sucrose rewards taken, but not in overall session length (Figure 1b). There was no effect of the chronic diet treatment on the number of inactive lever presses. When rats were fed the high fat diet during wks 5-8 but subsequently returned to a chow diet taken through FR and PR training during weeks 9-12, there was a trend but no significant difference in active lever presses. Thus, there appears to be no behavioral carry-over effect of a moderately high fat diet consumed during the peri-pubertal timeframe. PR parameter data for these cohorts are summarized in Table 2. In order

to begin to elucidate contributing mechanism(s) to the diet-induced increase in sucrose motivation, we carried out a number of metabolic and CNS measurements.

Effect of moderate high fat diet on metabolic parameters

Immediately after the conclusion of behavioral testing, body fat composition was determined on rats that had the diet intervention and behavioral paradigm during wks 5-8. Rats then received chronic intravenous cannulas for (conscious) IV glucose tolerance tests (IVGTTs). Subsequently, terminal fasting plasma and serum were obtained for additional metabolic measures. As shown in Table 3, there were no differences in body composition, body weight, fasting insulin or glucose measures, insulin sensitivity (HOMA calculation) or responses to the IVGTT, between the chow-fed and high fat diet-fed rats. Terminal fasting leptin and triglyceride measurements did not differ between the two groups. Thus, although the diet treatment had a significant effect on motivation for sucrose, it reflects a behavioral response in high fat-fed rats that are pre-obese.

Effect of moderate high fat diet on CNS homeostatic and reward neurochemistry

In addition to terminal metabolic measurements, brains from the cohort that had both diet intervention and behavioral training during weeks 5-8 were measured for nucleus accumbens amine profiles (n=4 per diet group) or mRNA levels of hypothalamic orexigenic peptides. As shown in Table 4, there was no significant effect of the high fat diet on dopamine, norepinephrine, or serotonin metabolites in the nucleus accumbens, a central site of reward and motivational activity (Ikemoto & Panksepp, 1996; Kelley & Berridge, 2002) in which each of these neurotransmitter systems plays a key regulatory role. Within hypothalamic extracts, mRNA levels of the orexigenic peptides, NPY, AGRP, and orexin were measured. A strong but non-significant trend for increased AGRP in the fat-fed rats was observed in this cohort (n=8 for either diet); we therefore repeated the diet/behavioral training paradigm in an additional cohort and measured NPY, AGRP, and orexin mRNA in the hypothalamus. In the combined cohorts, we observed a significant ($p<0.05$) increase in AGRP mRNA in rats fed the high fat diet vs. chow controls (Figure 2), but no significant change in NPY or orexin expression. To evaluate possible connections between AGRP expression and self-administration behavior, we measured cFos and AGRP immunopositive neurons in the mediobasal hypothalamus. Groups of rats were fed the chow or 31.8% fat diet; some were taken through the self-administration protocol (weeks 5-8) and others were handled as behavioral controls. Figure 3a shows an example of co-localization of cFos and AGRP in an arcuate nucleus neuron. As summarized in Table 5, activation of AGRP neurons (co-expression of cFos-ICC and AGRP-ICC within the same cells) was associated with self-administration activity. This is demonstrated in Figure 3b, where the number of activated (cFos-positive) neurons is shown as the neuronal cell count, or as percent of total AGRP-positive neurons: there is significant activation of AGRP neurons in the rats self-administering sucrose, vs. the handling controls, in the combined diet groups. A within-diet treatment comparison for the number of activated AGRP neurons in the self-administration group vs. handling controls displayed a trend that did not reach statistical significance (chow, $p=.078$; 31.8% fat diet, $p=.073$). Importantly, not only do these data link AGRP neuronal activation with self-administration behavior, but because of the timing for the cFos measurement (90 minutes after the rats were placed in their self-administration chambers), cFos expression reflects activity of AGRP neurons in anticipation of, or at the onset of, self-administration activity. There was a non-significant trend for increased total AGRP-positive neurons in the self-administration group (vs. handling controls, $p=0.16$). In those rats, where lever-pressing was matched between the diet groups, the number of AGRP-positive neurons was also matched. There was no effect of the diet treatment alone on number of AGRP-positive neurons in the behavioral control rats.

Effect of AGRP administration on sucrose motivation

Our interpretation of this finding is that AGRP expression in the pubertal rats is a key mechanism underlying the enhanced sucrose self-administration of the high fat diet-fed rats. To confirm for the efficacy of AGRP to increase motivation for sucrose, AGRP was administered via the third ventricle to chow-fed peri-pubertal rats during the PR portion of the behavioral paradigm. This dose regimen of AGRP was sub-threshold for stimulation of chow intake across the two weeks of PR paradigm, but resulted in significantly increased sucrose self-administration, as shown in Figure 4. (Note that each sucrose reward has a caloric content of 0.1 kcal, therefore the sucrose self-administration activity contributes negligible calories to total daily intake.) Table 6 shows self-administration parameter data across the 9-day PR paradigm, with AGRP or aCSF injected ICV on Days 2, 5, and 8. In AGRP-treated rats, the number of active lever presses was significantly increased overall across PR Days 2-10 ($p=0.03$), and on non-injection days ($p=0.048$) with a trend towards an increase on the (averaged) injection days. Additionally, Stop Time (which reflects the total time spent engaged in the self-administration task) was significantly increased on non-injection days ($p=0.02$) with trends towards an increase overall, and on injection days. The number of sucrose rewards was increased overall across PR Days 2-10 ($p=0.03$). There was no effect of AGRP treatment on inactive lever pressing, compared with aCSF-treated controls, or between injection- and non-injection days. The results support an interpretation of a sustained effect of AGRP to increase sucrose self-administration: rats pressed more on the rewarding lever, received more sucrose rewards, and spent more time engaged with the task.

Effect of life stage on preference and motivation for sucrose

In the final experiment, we evaluated whether motivation for sucrose differs between pubertal and adult rats. Initially, 5- and 10-wk old rats were given a sucrose preference test with choices of solutions ranging from 0 to 20% sucrose, prior to beginning self-administration testing and training. As shown in Figure 5a, and consistent with findings reported in the literature, the pre-pubertal rats appeared to prefer a sweeter solution than the young adult rats: most pre-pubertal rats had a peak intake of 20% sucrose solution, whereas the adult rats showed a peak intake of 15% sucrose. Subsequently, both age groups were split between rat chow and high fat diet during the self-administration training and testing. There was a small but statistically significant increase in the number of active lever presses by the peri-pubertal vs. adult rats (45 ± 3 vs. 37 ± 2 , $p=0.05$) averaged across the FR sessions, with no difference in number of sucrose rewards or number of presses on the inactive lever. As shown in Figure 5b, there was a highly significant overall effect of age, across the PR sessions, with significantly increased active lever pressing for the pubertal ($n=15$) vs. young adult ($n=14$) rats (2-way ANOVA, PRDay $\times$ age; effect of age, $p=0.017$, no independent effect of PRDay, no significant interaction). There was a trend for a greater effect of age in the high fat diet-fed condition but this did not achieve statistical significance ($p=.13$). Table 7 lists PR behavioral parameters: in addition to increased active lever presses, peri-pubertal rats received significantly more sucrose rewards, and showed a trend towards increased Stop Time. Additionally, the peri-pubertal rats had a small but significant increase in presses on the inactive (i.e., non-rewarding) lever, although for both peri-pubertal and adult rats, the number of inactive lever presses was approximately 10% of the number of active lever presses. These results suggest that peri-pubertal rats prefer and will more avidly seek out sweet-tasting foods, and the effect may be amplified with a background of high fat diet.

Discussion

The main finding of this study is that a moderately high fat diet consumed during the peri-pubertal period (just before, during, and just after the age of transition into puberty)

significantly increased motivation for sucrose solutions. This finding is consistent with our previous, similar, observation in adult rats (Figlewicz et al., 2006). In these animals, and in additional age- and treatment-matched cohorts, we determined through extensive metabolic characterization that the rats were non-obese or pre-obese and were not peripherally insulin resistant. We cannot rule out the possibility that the rats had CNS-localized resistance to the actions of insulin or leptin, however: both of these hormones contribute to CNS site-specific modulation of food reward (Davis, Choi, & Benoit, 2010; Davis et al., 2011a; Figlewicz & Sipols, 2010).

In a subset of rats, we measured amine neurotransmitters and related metabolites in the nucleus accumbens, which receives a heavy investment of dopaminergic projections from the midbrain, and is considered a key and central CNS site for the mediation of reward and motivated behavior (Ikemoto & Panksepp, 1996; Kelley & Berridge, 2002). We observed no change in absolute levels or ratios of any of these transmitter metabolites which suggests that altered catecholaminergic or serotonergic activity within the nucleus accumbens is not a primary or major CNS mechanism underlying the increased sucrose motivation. This is consistent with the recent report from Davis et al. [2011 b], who demonstrated in adult rats that ICV AGRP increases dopamine turnover in the medial prefrontal cortex but not the nucleus accumbens. Further, we observed no ‘behavioral carryover’ effect of the diet when tested on rats immediately post-puberty, as young adults. This is in contrast to the findings from Bolaños and others, on both behavioral and catecholaminergic parameters, in adult rodents treated with methylphenidate (Bolaños et al., 2003; Bolaños, Glatt, & Jackson, 1998; Brandon, Marinelli, Baker, & White, 2001; Brandon, Marinelli, & White, 2003). This is likely due to the direct targeting of dopaminergic neurons by methylphenidate, and may also be a function of timing of the diet intervention and time of testing of animals. Finally, we may not have observed carryover effects, because in this study, a primary locus of diet effect appears to be the medial hypothalamus.

In this study, three lines of evidence support a key role for the medial hypothalamic neuropeptide AGRP in the increased self-administration of sucrose in the high fat diet-fed rats. First, we observed an increase of AGRP expression (mRNA) in extracts of whole hypothalamus in rats fed the 31.8% fat diet relative to chow controls. However, orexin mRNA and NPY mRNA levels were not changed. Thus, the effect of the high fat diet/behavioral paradigm appear to be specific to AGRP, and not generalized to orexigenic neuropeptides. This emphasizes a role for AGRP in motivation for, or seeking of, food, and is consistent with a number of recent reports in the literature (discussed below). Our recent work has demonstrated a key role of medial hypothalamic activation in association with PR performance in our motivation paradigm, with increased cFos expression in several medial hypothalamic nuclei (Figlewicz et al., 2011). We have also identified the ARC as a key region for the effect of (exogenous) insulin to decrease sucrose self-administration (Figlewicz et al., 2008). The ARC contains AGRP/NPY neurons (Broberger et al., 1998; Hahn, Breininger, Baskin, & Schwartz, 1998) which act within the medial hypothalamus to stimulate feeding by multiple mechanisms. In this study, immunocytochemical quantitation of activated AGRP neurons demonstrated an increase of cFos/AGRP neurons in rats that were trained to self-administer sucrose, compared to non-trained behavioral controls. This is a second approach leading to the interpretation that AGRP neuronal activation contributes to (the onset of) sucrose self-administration. Both earlier and more recent studies associated AGRP expression and action with a preferential intake of fat, either as a diet (Hagan et al., 2000) or in the context of a motivational paradigm (Tracy et al., 2008); and in adult rats ICV AGRP preferentially conditions a place preference to fat (Davis et al., 2011b). Recent studies utilizing targeted molecular techniques that permit specific activation of AGRP neurons in mice (Aponte, Atasoy, & Sternson, 2011; Krashes et al., 2011) have confirmed that AGRP robustly stimulates feeding, increases food-seeking, and decreases energy

expenditure. It is interesting to note that in the experimental groups fed the high fat diet, total caloric intake was significantly lower compared with the control chow-fed rats (Table 8), which would be consistent with an endogenous AGRP effect to decrease energy expenditure. These effects are consistent with the earlier findings of Hagan et al. [2000], that exogenous AGRP effects on some aspects of energy balance can be quite prolonged. Thus, as a third approach, our results showing increased sucrose self-administration by (chow-fed) pubertal rats given ICV AGRP likewise suggest an action that is sustained. The specific increase of AGRP mRNA expression in rats fed the high fat diet for four weeks is consistent with the recent research from Kaushik and colleagues [2011] which connects exogenous fatty acids, intracellularly generated fatty acids, and increased AGRP expression in hypothalamic neurons. Thus, addition of oleic or palmitic acid to cultured hypothalamic cells resulted in increased AGRP expression. While the diet we utilized had increased stearic, palmitic, and oleic acid, it is not possible to know whether these fatty acids are increased in the *in vivo* hypothalamic milieu, whether their localized concentrations would correspond to the dietary fatty acid profile, and whether one or more of these would specifically lead to increased AGRP expression. Nonetheless, it is tempting to speculate that dietary subcomponents may contribute to increased motivation for sweets through a primary action at the medial hypothalamus.

Our study demonstrates that young rats have increased motivation for sucrose compared with adult rats. This was apparent across the entire time of PR self-administration, and there was a trend for the high fat diet to enhance the age effect. It is possible that this did not reach statistical significance because of the relatively small group sizes; thus, the data suggest that in pubertal animals (and perhaps humans) moderately elevated fat in the diet may contribute to enhanced seeking behaviors to obtain sweetened beverages or foods. From a societal perspective, it emphasizes the need to pay attention to the fat component of “tweens” ‘or teens’ diets, not only because of direct, negative metabolic consequences of excess dietary fat, but also because it can contribute to behaviors that result in enhanced intake of sugars. As recently reviewed by Stanhope [2012], co-ingestion of sugars with fat can have substantial negative metabolic consequences. High fat/sugar combinations in humans also are a relatively less satiating diet (Drewnowski, 1998). With the increase in incidence of diabetes (Cizza, Brown, & Rother, 2012) and fatty liver (Kohli et al., 2010) occurring in the pediatric population, the importance of a healthy and balanced diet in youth is clear. We observed a significant increase in presses on the inactive lever in the pubertal rats (vs. adult rats), although the number of lever presses was still very low. It is possible but seems unlikely that the enhanced active lever pressing could be accounted for as a ‘non-specific’ effect of overall activity, as most activity was goal-directed towards the active lever. Although the actual number of inactive lever presses was increased, the proportion relative to active lever presses was comparable between peri-pubertal and adult rats, and the increased lever presses may reflect the longer active time in the self-administration chambers. In a different paradigm (some food restriction, use of food pellets rather than a sweet reward, and an FR1 schedule) Sturman, Mandell, & Moghaddam [2010] have recently reported altered instrumental performance in adolescent vs. adult rats. They observed no difference in nosepokes that delivered food pellets, between juvenile and adult rats. They did, however, observe increased perseverative behavior during extinction, in the juvenile rats. Taken together, the two studies emphasize an influence of age and developmental stage on motivation for food, consistent with the rapid growth of pubertal rats. In this study we evaluated male, but not female, rats. Currently there are limited studies directly comparing male and female rats in food motivation paradigm, and systematic evaluation during the pubertal period is warranted. It should be noted that in the study of (human) adolescents, Coldwell and colleagues (2009) observed an association between a marker of growth, and not gonadal steroids *per se*. Nonetheless, gender effects in this age group deserve further investigation.

In conclusion, our studies demonstrate increased motivation for sucrose in pubertal rats compared with adults, and this is enhanced by access to a moderately high fat diet. The effect of high fat diet upon sucrose motivation may be mediated by increased AGRP activity in the medial hypothalamus. This is further evidence of the strong intrinsic CNS functional connectivity of circuitry that regulates energy homeostasis with circuitry that regulates reward and motivation. The enhancement of motivation for sucrose by a moderately high fat diet precedes metabolic derangements and overt obesity and suggests that behavior may initially drive metabolic changes, rather than vice versa. Ingestion of high fat, and fructose-containing sweet foods, would jointly contribute to a metabolic profile that is high risk for both type2 diabetes and cardiovascular disease. These findings emphasize the importance of focusing upon eating patterns and diet during puberty, as being affected not only by socio-environmental influences, but also by CNS neurochemical and behavioral adjustments as an animal or human transitions through a period of multiple maturational changes for the acquisition of reproductive competency.

Acknowledgments

This research was supported by NIH grant DK40963. Dianne Figlewicz Lattemann is a Senior Research Career Scientist, Biomedical Laboratory Research Program, Department of Veterans Affairs Puget Sound Health Care System, Seattle, Washington. Stephen Benoit was supported by NIH DK066223 and Ethicon Endosurgery Inc. The authors thank Dr. Tami Wolden-Hanson for support with body composition measurements; Dr. William Banks and Lucy Dillman for support with the triglyceride measurements; and Amalie Alver, and Samantha Thomas-Nadler for assistance with the behavioral studies.

References

- Andersen SL, Teicher MH. Stress, sensitive periods and maturational events in adolescent depression. *Trends in Neuroscience*. 2008; 31:183–191.
- Aponte Y, Atasoy D, Sternson SM. AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nature Neuroscience*. 2011; 14:351–355.
- Barnes MJ, Argyropoulos G, Bray GA. Preference for a high fat diet, but not hyperphagia following activation of mu opioid receptors is blocked in AgRP knockout mice. *Brain Research*. 2010; 1317:100–107. [PubMed: 20051234]
- Bolaños CA, Barrot M, Berton O, Wallace-Black D, Nestler EJ. Methylphenidate treatment during pre- and periadolescence alters behavioral responses to emotional stimuli at adulthood. *Biological Psychiatry*. 2003; 54:1317–1329. [PubMed: 14675795]
- Bolaños CA, Glatt SJ, Jackson D. Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis. *Brain Research Developmental Brain Research*. 1998; 111:25–33. [PubMed: 9804875]
- Brandon CL, Marinelli M, Baker LK, White FJ. Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. *Neuropsychopharmacology*. 2001; 25:651–61. [PubMed: 11682248]
- Brandon CL, Marinelli M, White FJ. Adolescent exposure to methylphenidate alters the activity of rat midbrain dopamine neurons. *Biological Psychiatry*. 2003; 54:1338–1344. [PubMed: 14675797]
- Broberger C, Johansen J, Johansson C, Schalling M, Hokfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proceedings of the National Academy of Science*. 1998; 95:15043–15048.
- Cason AM, Smith RJ, Tahsili-Fahadan P, Moorman DE, Sartor GC, Aston-Jones G. Role of orexin/hypocretin in reward-seeking and addiction: implications for obesity. *Physiology & Behavior*. 2010; 100:419–428. [PubMed: 20338186]
- Choi DL, Davis JF, Fitzgerald ME, Benoit SC. The role of orexin-A in food motivation, reward-based feeding behavior and food-induced neuronal activation in rats. *Neuroscience*. 2010; 167:11–20. [PubMed: 20149847]
- Cizza G, Brown RJ, Rother KI. Rising incidence and challenges of childhood diabetes. A mini review. *Journal of Endocrinological Investigation*. 2012 epub May 8, 2012.

- Coldwell SE, Oswald TK, Reed DR. A marker of growth differs between adolescents with high vs. low sugar preference. *Physiology & Behavior*. 2009; 96:574–580. [PubMed: 19150454]
- Davis JF, Choi DL, Benoit SC. Insulin, leptin and reward. *Trends in Endocrinology and Metabolism*. 2010; 21:68–74. [PubMed: 19818643]
- Davis JF, Choi DL, Schurdak JD, Fitzgerald MF, Clegg DJ, Lipton JW, Figlewicz DP, Benoit SC. Leptin regulates energy balance and motivation through action at distinct neural circuits. *Biological Psychiatry*. 2011a; 69:668–674. [PubMed: 21035790]
- Davis JF, Choi DL, Schurdak JD, Krause EG, Fitzgerald MF, Lipton JW, Sakai RR, Benoit SC. Central melanocortins modulate mesocorticolimbic activity and food seeking behavior in the rat. *Physiology & Behavior*. 2011b; 102:491–495. [PubMed: 21172367]
- Davis JF, Tracy AL, Schurdak JD, Tschop MH, Clegg DJ, Benoit SC, Lipton JW. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. *Behavioral Neuroscience*. 2008; 122:1257–1263. [PubMed: 19045945]
- Desor JA, Beauchamp GK. Longitudinal changes in sweet preferences in humans. *Physiology & Behavior*. 1987; 39:639–641. [PubMed: 3588712]
- Desor JA, Greene LS, Maller O. Preferences for sweet and salty in 9- to 15-year-old and adult humans. *Science*. 1975; 190:686–687. [PubMed: 1188365]
- Drewnowski A. Energy density, palatability, and satiety: implications for weight control. *Nutrition Reviews*. 1998; 56:347–353. [PubMed: 9884582]
- Figlewicz DP, Bennett JL, Aliakbari S, Zavosh A, Sipols AJ. Insulin acts at different CNS sites to decrease acute sucrose feeding and sucrose self-administration in rats. *American Journal of Physiology*. 2008; 295:R388–R394. [PubMed: 18525010]
- Figlewicz DP, Bennett JL, Naleid AM, Davis C, Grimm JW. Intraventricular insulin and leptin decrease sucrose self-administration in rats. *Physiology & Behavior*. 2006; 89:611–616. [PubMed: 17045623]
- Figlewicz DP, Bennett-Jay JL, Kittleson S, Sipols AJ, Zavosh A. Sucrose self-administration and CNS activation in the rat. *Am J Physiol*. 2011; 300:R876–R884.
- Figlewicz DP, Ioannou G, Bennett Jay J, Kittleson S, Savard C, Roth CL. Effect of moderate intake of sweeteners on metabolic health in the rat. *Physiology & Behavior*. 2009; 98:618–624. [PubMed: 19815021]
- Figlewicz DP, Sipols AJ. Energy regulatory signals and food reward. *Pharmacology, Biochemistry, and Behavior*. 2010; 97:15–24.
- Frangioudakis G, Gyte AC, Loxham SJ, Poucher SM. The intravenous glucose tolerance test in cannulated Wistar rats: A robust method for the in vivo assessment of glucose-stimulated insulin secretion. *Journal of Pharmacological and Toxicological Methods*. 2008; 57:106–113. [PubMed: 18280184]
- Hagan MM, Rushing PA, Pritchard LM, Schwartz MW, Strack AM, Van Der Ploeg LHT, Woods SC, Seeley RJ. Long-term orexigenic effects of AgRP-(83-132) involve mechanisms other than melanocortin receptor blockade. *American Journal of Physiology*. 2000; 279:R47–R52. [PubMed: 10896863]
- Hahn TM, Breininger JF, Baskin DG, Schwartz MW. Coexpression of *Agrp* and *NPY* in fasting-activated hypothalamic neurons. *Nature Neuroscience*. 1998; 1:271–272.
- Hodos W. Progressive ratio as a measure of reward strength. *Science*. 1961; 134:943–944. [PubMed: 13714876]
- Ikemoto S, Panksepp J. Dissociations between appetitive and consummatory responses by pharmacological manipulations of reward-relevant brain regions. *Behavioral Neuroscience*. 1996; 110:331–345. [PubMed: 8731060]
- Jewett DC, Cleary J, Levine AS, Schaal DW, Thompson T. Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior. *Psychopharmacology*. 1995; 120:267–271. [PubMed: 8524973]
- Kaushik S, Rodriguez-Navarro JA, Arias E, Kiffin R, Sahu S, Schwartz GJ, Cuervo AM, Singh R. Autophagy in hypothalamic AgRP neurons regulates food intake and energy balance. *Cell Metabolism*. 2011; 14:173–183. [PubMed: 21803288]

- Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. *Journal of Neuroscience*. 2002; 22:3306–3311. [PubMed: 11978804]
- Kelley SP, Nannini MA, Bratt AM, Hodge CW. Neuropeptide-Y in the paraventricular nucleus increases ethanol self-administration. *Peptides*. 2001; 22:515–522. [PubMed: 11287109]
- Kohli R, Boyd T, Lake K, Dietrich K, Nicholas L, Balistreri WF, Ebach D, Shashidkar H, Xanthakos SA. Rapid progression of NASH in childhood. *Journal of Pediatric Gastroenterology and Nutrition*. 2010; 50:453–456. [PubMed: 20179647]
- Krashes MJ, Koda S, Ye CP, Rogan SC, Adams AC, Cusher DS, Maratos-Flier E, Roth BL, Lowell BB. Rapid reversible activation of AgRP neurons drives feeding behavior in mice. *Journal of Clinical Investigation*. 2011; 121:1424–1428. [PubMed: 21364278]
- Mennella JA, Pepino MY, Reed DR. Genetic and environmental determinants of bitter perception and sweet preferences. *Pediatrics*. 2005; 115:216–222.
- Myers KP, Sclafani A. Development of learned flavor preferences. *Developmental Psychobiology*. 2006; 48:380–388. [PubMed: 16770764]
- National Cancer Institute Applied Research Program. [Accessed 21 September 2011] Sources of calories from added sugars among the US Population, 2005–06. Updated 21 December 2010. 2010. Available from: http://riskfactor.cancer.gov/diet/foodsources/added_sugars/
- Nixon JP, Zhang M, Wang CF, Kuskowski MA, Novak CM, Levine JA, Billington CJ, Kotz CM. Evaluation of a quantitative magnetic resonance imaging system for whole body composition analysis in rodents. *Obesity*. 2010; 18:1652–1659. [PubMed: 20057373]
- Ogden CL, Carroll MD. Division of Health and Nutrition Examination Surveys. Prevalence of obesity among children and adolescents: United States, trends 1963–1965 through 2007–2008. *Health E-Stat*. 2010; 2010 Available from: <http://www.cdc.gov/nchs/fastats/overwt.htm>.
- Paxinos, G.; Watson, C. Atlas of the rat brain in stereotaxic coordinates. 5th. San Diego CA: Elsevier Academic Press; 2005.
- Richardson NR, Roberts DC. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *Journal of Neuroscience Methods*. 1996; 66:1–11. [PubMed: 8794935]
- Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM. Dopamine operates as a subsecond modulator of food seeking. *Journal of Neuroscience*. 2004; 24:1265–1271. [PubMed: 14960596]
- Rossi M, Kim M, Morgan D, Small C, Edwards C, Sunter D, Abusnana S, Goldstone A, Russell S, Stanley S, Smith D, Yagaloff K, Ghatei M, Bloom S. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. *Endocrinology*. 1998; 139:4428–4431. [PubMed: 9751529]
- Stanhope KL. Role of fructose-containing sugars in the epidemics of obesity and metabolic syndrome. *Annual Reviews of Medicine*. 2012; 63:329–343.
- Sturman DA, Mandell DR, Moghaddam B. Adolescents exhibit behavioral differences from adults during instrumental learning and extinction. *Behavioral Neuroscience*. 2010; 124:16–25. [PubMed: 20141277]
- Tracy AL, Clegg DJ, Johnson JD, Davidson TL, Woods SC. The melanocortin antagonist AgRP(83–132) increases appetitive responding for a fat, but not a carbohydrate, reinforcer. *Pharmacology Biochemistry, and Behavior*. 2008; 89:263–271.
- Vartanian LR, Schwartz MB, Brownell KD. Effects of soft drink consumption on nutrition and health: a systematic review and meta-analysis. *American Journal of Public Health*. 2007; 97:667–75. [PubMed: 17329656]

- Moderate high fat diet increases motivation for sucrose in adult rats.
- In this study, high fat diet increases sucrose motivation in peri-pubertal rats.
- Peri-pubertal rats had increased sucrose motivation compared with adults.
- The increased sucrose motivation may be mediated by hypothalamic AGRP.
- Conclusion: High fat diet drives motivation for sweets independent of obesity.

\$watermark-text

\$watermark-text

\$watermark-text

Figure 1.

PR motivated responding for sucrose rewards is increased in peripubertal rats fed a 31.8% fat diet (n=8). 1a. Across the FR sessions, there was no effect of diet, but the diet effect is manifest when rats are switched to the PR paradigm. 1b. Data are expressed for each parameter as percent of the respective mean value in the chow-fed cohort (n=8). Both number of active lever presses and # of sucrose rewards are significantly elevated ($p<0.05$) with no change in 'Stop Time'.

Figure 2.

Effect of 31.8% fat diet on medial hypothalamic peptide mRNA expression. Data are normalized for high fat-fed rats (n=17) vs. that of chow controls (n=16). AGRP mRNA is significantly elevated ($p<0.05$).

Figure 3.

Activation of AGRP neurons at the onset of sucrose self-administration. 3a. Co-localization of cFos and AGRP in arcuate nucleus neuron, 60x magnification. 3b. Number of activated (cFos-immunopositive) AGRP-immunopositive neurons in the mediobasal hypothalamus of peri-pubertal rats trained to self-administer sucrose (n=10), or behavioral controls (handled only; n=6). Both the total number of activated AGRP neurons (left bars) or the number of activated AGRP neurons as a percent of total AGRP neurons (Total = AGRP-positive with or without cFos-immunopositive co-staining) are significantly increased in the rats trained to self-administer sucrose. Timing of euthanasia would reflect activation of the AGRP in anticipation of, or at the start of, the actual self-administration session.

Figure 4.

Third ventricular (ICV) AGRP (0.01 nmol) stimulates sucrose self-administration in the PR paradigm, but has no effect upon daily food intake across the study period (PR Days 2—10, with injections on Days 2, 5, and 8). AGRP (n=9) data are expressed as percent of the aCSF mean data (n=8).

LEVER PRESSES

Pubertal vs. Adult

Figure 5.

Juvenile rats have increased motivation for sucrose rewards compared to adult rats. 5a. Sucrose preference tests for juvenile (peri-pubertal, $n=15$) and young adult ($n=14$) rats. Rats had 30 min to drink from the range of concentrations (0-20% sucrose). Data are shown as number of rats that preferred each specific concentration. 5b. PR motivated responding for sucrose is increased in peripubertal rats ($n=15$) compared with adult rats ($n=14$). Number of active lever presses (upper) and number of inactive lever presses (lower) for each of the PR paradigm days are shown as mean $\pm$ SEM. Overall across PR days, $p=0.018$, pubertal vs. adult rats.

Table 1
Experimental Protocols

	Diet Intervention	Self-administration	Additional Measurements
Experiment 1.			
1a (chow vs. 31.8% fat)	5-8 wk	5-8 wk	Wk 9, body composition and IVGTT; fasting plasma metabolic measurements; nucleus accumbens catecholamines; hypothalamic peptide mRNA
1b (chow vs. 31.8% fat)	5-8 wk	9-12 wk	
Experiment 2.			
Chow vs. 31.8% fat	5-8 wk	5-8 wk	cFos and AGRP ICC
Experiment 3.			
Chow and ICV AGRP/CSF	N/A	5-8 wk	
Experiment 4.			
Chow or 31.8% fat	5-8 wk	5-8 wk	
	10-13 wk	10-13 wk	

Table 2
Effect of Peri-Pubertal High Fat Diet on Progressive Ratios Performance for Sucrose

	Active Lever Presses	#Sucrose Rewards	Stop Time	Inactive Lever Presses
Diet and Behavioral Testing, 5-8 wk				
Chow (n=8)	104 ± 10 *	8.5 ± 0.4 *	109 ± 14	16 ± 3
31.8% Fat (n=8)	175 ± 25	10 ± 0.4	108 ± 11	18 ± 2
Diet, 5-8 wk, Behavioral Testing 9-12 wk				
Chow (n=8)	104 ± 14	8 ± 0.5	114 ± 8	11 ± 2
31.8% Fat (n=8)	135 ± 12	9 ± 0.3	133 ± 9	18 ± 2

Data represent mean +/- standard error of the mean

*
p<0.05 vs. 31.8% fat cohort

Table 3
Metabolic Parameters¹

	Chow (n=7-8)²	31% Fat Diet (n=8)
Body Weight (gm)	289 ± 8	294 ± 5
Body Fat (gm)	26.8 ± 1.2	27.4 ± 1.6
Chow/Diet Intake (kcal)	3045 ± 67	2573 ± 53 *
Fat Intake (kcal)	411 ± 9	653 ± 11 *
Triglycerides (mg/dl)	50.6 ± 3.0	43.3 ± 3.3
Plasma Leptin (ng/ml)	2.27 ± 0.11	2.14 ± 0.09
Fasting Glucose (mg/dl)	84 ± 2	80 ± 4
Fasting IRI (nM)	0.16 ± 0.02	0.15 ± 0.00
HOMA	0.96 ± 0.14	0.86 ± 0.17
IVGTT:		
5 min Glucose	311 ± 17	300 ± 14
5 min IRI	2.36 ± 0.37	2.61 ± 0.45
AUC Glucose (0-30 min)	2609 ± 162	2561 ± 146
AUC IRI (0-30 min)	25.2 ± 3.7	27.8 ± 4.5

¹Rats had diet intervention wks 5—8. Body fat and IVGTT data were obtained within the week after behavioral study (8-9 weeks). Other plasma measures were obtained in fasting animals at time of euthanasia, at approximately 9-10 weeks of age.

²IVGTT data were only obtained in 7 subjects, as one animal had a non-patent catheter. Terminal fasting insulin and glucose were obtained in all 8 subjects.

* p<0.05 vs. chow-fed rats.

Table 4
Nucleus Accumbens Amine Metabolites

	Chow (n=4)	31.8% Fat Diet (n=4)
DOPAC	13.06±1.18	17.13±5.08
DA	79.01±4.58	107.29±33.0
DOPAC/DA	0.165±0.007	0.161±0.002
HVA	6.83±0.83	9.95±2.74
MHPG	0.17±0.02	0.25±0.05
NE	20.81±4.04	45.76±10.89
HVA/NE	0.37±0.08	0.23±0.05
MHPG/NE	0.009±0.002	0.006±0.001
5HIAA	6.61±0.35	9.32±1.76
5HT	7.60±0.83	9.64±1.96
5HIAA/5HT	0.89±0.06	0.98±0.015

Table 5
Agrp Neuron Activation: Diet and Behavioral Treatment

	Active Lever Presses	AGRP neurons	cFos+AGRP neurons
Self-administration (n=5/gp)			
Chow	196 ± 29	204 ± 36	48 ± 9
31.8% Fat Diet	158 ± 21	209 ± 18	44 ± 10
Handling control (n=3gp)			
Chow	N/A	166 ± 11	16 ± 5
31.8% Fat Diet	N/A	159 ± 7	16 ± 12

Data represent mean +/- standard error of the mean

Table 6
Effect of ICV AGRP vs. aCSF on Progressive Ratios Performance for Sucrose

	Active Lever Presses	#Rewards	Stop Time ^a	Inactive Lever Presses
aCSF (n=8)				
PR Days 2-10	80 ± 4 *	7.5 ± 0.3 *	111 ± 6	11 ± 3
Injection Days (2,5,8)	73 ± 8	7.1 ± 0.3	114 ± 9	10 ± 3
Non-Injection Days	89 ± 5 *	7.8 ± 0.3	109 ± 5 *	11 ± 3
AGRP (n=7)				
PR Days 2-10	114 ± 14	8.5 ± 0.2	129 ± 9	21 ± 6
Injection Days (2,5,8)	93 ± 13	7.8 ± 0.4	125 ± 13	16 ± 6
Non-Injection Days	124 ± 16	8.5 ± 0.5	134 ± 8	23 ± 6

Data represent mean +/- standard error of the mean

^a time in minutes

* p<0.05, aCSF vs. AGRP

Table 7
Effect of age on on Progressive Ratios Performance^a for Sucrose

	Peripubertal Rats (n=15)	Adult Rats (n=14)
Active Lever Presses	162 ± 15 *	108 ± 14
# Rewards	9.6 ± 0.3 *	8.3 ± 0.4
Stop Time (min)	122 ± 7	107 ± 11
Inactive Lever Presses	19 ± 2 *	9 ± 1

Data represent mean +/- standard error of the mean

^a Averaged across PR Days 1--10

*
p<0.05

Table 8
Experimental Protocols: Kcal Consumed

Experiment	Chow	31.8% Fat
Diet+Self-Administration	3045±67 *	2573±53
Diet Followed by Self-Administration	4865±76 p=0.08	4642±92
mRNA Measurement	3030±44 *	2636±36
ICV AGRP	3980±121	
ICV CSF	4042±71	
AGRP/cFos	2948±84 *	2286±61
Peri-pubertal Rats	3126±42 *	2791±65
Young Adult Rats	3630±58 *	3390±69

*
p<0.05 for Chow vs. 31.8% fat comparison