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Kevin P. Myers
Bucknell University, kmyers@bucknell.edu

Marta Majewski
Bucknell University

Dominique Schaefer
Bucknell University

Alexis Tierney
Bucknell University

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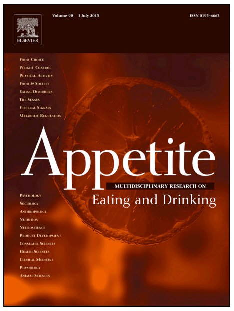

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Chronic experience with unpredictable food availability promotes food reward, overeating, and weight gain in a novel animal model of food insecurity

Kevin P. Myers*, Marta Majewski†, Dominique Schaefer†, Alexis Tierney†

Department of Psychology and Neuroscience Program

Bucknell University

* Corresponding author: kmyers@bucknell.edu, Department of Psychology, O'Leary Center,
Bucknell University, Lewisburg, PA 17837, USA.

† These authors contributed equally and are listed in alphabetical order.

Abstract

Ubiquitous, easy access to food is thought to promote obesity in the modern environment. However, people coping with food insecurity have limited, unpredictable food access and are also prone to obesity. Causal factors linking food insecurity and obesity are not understood. In this study we describe an animal model to investigate biopsychological impacts of the chronic unpredictability inherent in food insecurity. Female rats were maintained on a 'secure' schedule of highly predictable 4x/day feedings of uniform size, or an 'insecure' schedule delivering the same total food over time but frequently unpredictable regarding how much, if any, food would arrive at each scheduled feeding. Subgroups of secure and insecure rats were fed ordinary chow or high-fat/high-sugar (HFHS) chow to identify separate and combined effects of insecurity and diet quality. Insecure chow-fed rats, relative to secure chow-fed rats, were hyperactive and consumed more when provided a palatable liquid diet. Insecure HFHS-fed rats additionally had higher progressive ratio breakpoints for sucrose, increased meal size, and subsequently gained more weight during 8 days of ad libitum HFHS access. Insecurity appeared to maintain a heightened attraction to palatable food that habituated in rats with secure HFHS access. In a second experiment, rats fed ordinary chow on the insecure schedule subsequently gained more weight when provided ad libitum chow, showing that prior insecurity *per se* promoted short-term weight gain in the absence of HFHS food. We propose this to be a potentially useful animal model for mechanistic research on biopsychological impacts of insecurity, demonstrating that chronic food uncertainty is a factor promoting obesity.

Keywords: food insecurity, insecurity-obesity paradox, motivation, meal size, food reward, high-fat/sugar diet, overeating, obesity

Introduction

Research on the obesity epidemic often names food ubiquity as a defining attribute of the modern food environment. Easy access to varied, inexpensive, palatable food clearly does promote overeating and excess weight (e,g., Drewnowski & Darmon, 2005; Zobel et al., 2016; Hall, 2018; Cooksey-Stowers et al., 2017). However, food is not ubiquitous or easily available at all times for a substantial proportion of the population, even in relatively wealthy countries. Approximately 35 million Americans are food insecure (Coleman-Jensen, et al., 2020) meaning they lack consistent, reliable access to food from time to time. In recent decades prevalence of food insecurity in the USA and the UK has fluctuated between 10-15% of households annually (Coleman-Jensen, et al., 2020; Pool & Dooris, 2021), and this has been exacerbated during the COVID-19 pandemic (Fitzpatrick et al., 2021; Niles et al., 2020). Yet, paradoxically, despite being defined by limited food access, food insecurity is positively associated with overweight and obesity (Cheung et al., 2015; Dhurandhar, 2016; Larson & Story, 2011; Pan et al., 2012; Townsend et al., 2001).

Food insecurity differs from food insufficiency (i.e., chronic hunger) in that most people who are food insecure are able to maintain adequate energy intake over time and are not experiencing a caloric deficit relative to food secure individuals (Zizza, Duffy, & Gerrior, 2012). Rather their temporal pattern of intake differs, with reduced meal frequency, longer and more variable stretches of deprivation, and larger meals and snacks when food is available (Nettle & Bateson, 2019; Zizza, Duffy, & Gerrior, 2012). Insecurity is arguably as much a psychological state as a nutritional one, as chronic uncertainty means food insecure individuals are frequently worrying about food access (Maxwell, 1996). Food insecurity has nutritional implications as well, since it constrains purchasing decisions and food choice (Gregory, Mancino, & Coleman-

Jensen, 2019), leading to poorer overall diet quality including reduced consumption of fruits and vegetables, greater intake of sugar sweetened beverages, and substitution of more energy-dense processed foods (Hanson & Connor, 2014; Mei, et al., 2020; Morales & Berkowitz 2016).

In addition to obesity, food insecurity is a risk factor for a range of poor health outcomes, including cardiovascular disease, hypertension, diabetes, and increased mortality (Gundersen & Ziliak, 2015; Stuff, et al., 2004; Banerjee et al., 2021). It is also linked to poor mental health including depression, anxiety disorders, attentional disorders, and sleep disorders (Arenas, et al., 2019; Jones, 2017; Lu, et al., 2019; Myers, 2020; McLaughlin, et al., 2012), impulsive choice (Rodruiguez, 2021), eating disorders (Becker et al., 2017; Hazzard et al., 2020) and poorer cognitive and academic performance (Na et al., 2020; Phillips et al., 2018; Portella-Para & Leung, 2019).

Despite the prevalence of food insecurity and its many links to poor health outcomes, causal mechanisms through which food insecurity impacts different biological and behavioral processes at the individual level to influence eating, weight gain, and health are poorly understood. Mechanistic research on food insecurity is challenging given that it is a multifaceted social phenomenon which intersects with a host of social, demographic, economic, environmental, and lifestyle factors. Established survey instruments typically measure household-level insecurity, although individuals in a household may experience insecurity very differently (especially, for instance, parents and children) and comparison between food secure and insecure people is confounded by several socioeconomic variables. Longitudinal analyses of large data sets are better able to statistically model complex relationships but are ultimately still limited for causal inference.

We propose that an animal model would be useful for controlled, mechanistic experiments studying how unpredictable food availability affects various biological, psychological, and behavioral processes to alter appetitive behaviors and promote weight gain. While of course an animal model cannot capture the full complexity of the human social situation, animal models can test causal hypotheses about underlying biopsychological responses to manipulations of dietary conditions. Mechanistic research on the physiological and psychological drivers of overeating and obesity in animal models has largely ignored the existence of food insecurity until very recently (Andrews et al., 2021; Bateson, et al., 2021; Estacio, et al., 2021). Some prior animal work studied the effects of repetitive deprivation (e.g. Zhang et al., 2012), although that conflates the amount and schedule of food access. Other work has studied alternating cycles of deprivation and intermittent palatable food access (e.g. Ahn & Phillips, 2012; Corwin, Avena, & Boggiano 2011; Kreisler et al, 2018), but those models involve regular, predictable access schedules and do not incorporate the chronic unpredictability inherent in food insecurity.

In the present work, we modeled food insecurity by manipulating each rat's experience with highly predictable versus unpredictable food availability while equating total food intake over time, to test the hypothesis that chronic unpredictability itself produces lasting behavioral alterations that promote weight gain. To accomplish this, we used programmable feeders on the rats' home cages to deliver meals at scheduled feeding times. A predictable "secure" schedule routinely delivered a highly consistent amount of food at four set times every day. An "insecure" schedule used the same daily feeding times, but was made unpredictable by skipping one randomly selected feeding each day and also making the three remaining feedings smaller on some days and larger on other days. In this manner the secure and insecure schedules delivered the same total amount of food across every 4-day cycle, but the insecure schedule involved frequent uncertainty about food delivery, fluctuations in total daily intake, and more variation in deprivation level and inter-meal intervals. We devised these schedules to model the situation faced by most food insecure individuals in the United States who obtain adequate energy intake over time but face chronic uncertainty about food availability in the short term, who frequently skip meals, and experience unpredictable variation in periods of food deprivation (Zizza, Duffy, & Gerrior, 2012; Nettle & Bateson, 2019).

A second aim was to investigate whether chronically unpredictable food access exacerbates the behavioral impacts of a high-fat/high-sugar (HFHS) diet. This is of interest because food insecurity is associated with poorer diet quality, especially with respect to fruit and vegetable intake, sugary beverages, and energy-dense processed foods (Gregory, Mancino, & Coleman-Jensen, 2019; Hanson & Connor, 2014; Mei, et al., 2020; Morales & Berkowitz 2016). Thus, the link between food insecurity and obesity could be influenced by from diet quality independently of uncertainty, or a combination of the two. In the present work, rats on the secure and insecure schedules were further divided into subgroups receiving standard rodent chow or a HFHS chow (higher in energy density, higher in absolute and proportional content of fats and of simple sugars, and lower in protein) in order to investigate the separate and combined effects of predictability and diet quality.

Beginning 8 weeks after instituting the secure vs. insecure feeding schedules, rats were assessed on several measures of behavioral, appetitive, and cognitive function. Namely, we measured spontaneous locomotor activity levels, meal patterns during short term access to a palatable liquid diet, food reward value on a progressive ratio operant task, a novel object recognition test of short term memory, and finally, weight gain when provided ad libitum access to the obesogenic HFHS diet.

Methods

Ethics statement

All experimental procedures were approved by the Bucknell University IACUC and consistent with the *NIH Guide for the Care and Use of Laboratory Animals, 8th edition.*

Subjects

Subjects were 54 female Sprague-Dawley rats from 11 litters born in our colony to breeding stock originally derived from Envigo. We studied females exclusively for this initial study because several prior human studies have shown disproportionate adverse impacts among women for BMI, obesity, and mental health outcomes (Gooding et al., 2012; Hernandez, et al., 2017; Martin, et al., 2016; Townsend et al., 2001).

When rats were 41-45 days old, they were assigned to experimental conditions pseudo-randomly, constrained by body weight and litter of origin. Rats were allocated into home cages of three rats per cage, such that experimental conditions were equated for average initial body weight, and weight variation between rats was uniformly distributed across home cages within each condition. Cages were 8 × 16 × 10.5″ polycarbonate tub cages with corncob bedding and wire lids. Ad libitum water was available in the home cages at all times. Individual rats within each home cage were identified by ear notches. The colony room was on a reversed light-dark cycle (lights OFF 9:00AM-9:00 PM). Rats remained on ad libitum chow (LabDiet 5001) until experimental treatments began 18 days later, when rats were 60-64 days old.

Experimental conditions

Each home cage was outfitted with an automated carousel-style food dispenser assembled from 3D-printed parts actuated by a 12V stepper motor controlled by an Arduino Nano microcontroller with a real-time clock chip. The feeder carousel could be loaded with pre-weighed portions of chow to be dispensed at programmed times throughout the day.

The two independent variables were food availability schedule (secure vs insecure) and diet quality (standard chow vs high-fat/high-sugar chow). For the predictable, secure (“SEC”) feeding schedule, the automated feeders delivered a consistent amount of food at every one of four fixed feeding times each day. The feeding times spanned the lights-off period (9:00AM, 1:00PM, 5:00PM and 9:00PM). The unpredictable, or insecure (“INSEC”) schedule used the same four feeding times daily, with two modifications. First, every day one randomly selected feeding was omitted. Second, the remaining three feedings were all larger on some days and all smaller on other days, so that on different days the total food that INSEC rats received was either 75% or 125% of what the SEC rats received each day (which was constant). Precise feeding amounts are detailed in Table 1. These schedules were arranged so that SEC and INSEC rats received the same total amount of food across each 4-day period, yet for SEC rats every meal was uniform and highly predictable, while for INSEC rats the schedule involved unpredictable meal omissions and irregular variations in meal size and total daily intake.

The second independent variable was diet quality. On each feeding schedule, half the rats received standard rat chow (“CHOW,” LabDiet 5001) and the other half received a high-fat/high-sugar chow (“HFHS,” Research Diets D12451, 45% kcal from fat, 20% sucrose by weight). Amounts were devised to be approximately 90% of daily ad libitum chow intake in a pilot group of age-matched female rats, with the expectation that rats would habitually consume all food within a short time after delivery. Our frequent observations confirmed that to be the case. Amounts for HFHS conditions were adjusted to be calorically equivalent to CHOW conditions. Sample sizes were CHOW-SEC n = 15, CHOW-INSEC n = 12, HFHS-SEC n = 15, and HFHS-INSEC n = 12. Uneven sample sizes across experimental groups at the outset were due to rats being housed in groups of three. Four rats were removed from the study at various points when they developed mammary tumors (n = 2 CHOW-SEC, n = 1 HFHS-SEC, n = 1 HFHS-INSEC).

The experimental treatments began when rats were 60-64 days old and continued for 56 days before proceeding to behavioral testing, as follows.

Locomotor Activity Monitoring

Spontaneous activity levels were measured in standard operant chambers (Coulbourn Habitest, 12"W × 10"D × 12"H) with all stimulus/response modules (levers, etc.) removed, and a 6” diameter nesting cup partly filled with bedding placed on the wire grid floor. An infrared activity monitor (Coulbourn Instruments H24-61) was mounted in the ceiling. This monitor acts as a motion sensor and comparator that generates a signal during each 400 ms time bin during which motion is detected inside the chamber. Those signals were logged and tallied by Coulbourn GraphicState software. A white noise generator in the room masked background noise. Eighteen rats (4-5 rats from each experimental condition) were tested each day. Rats in both the SEC and INSEC conditions were fed the SEC schedule on the day leading up to activity measurement to equate recent intake and relative deprivation level. Rats were placed into the chambers at 10:00PM (one hour after their last feeding) and left overnight to acclimate. Beginning the next day at 8:00AM activity counts were collected continuously until 9:00PM. This measurement window was selected primarily to determine whether prior history of predictable or unpredictable food delivery would be reflected in food anticipatory activity around mealtimes. A drinking water bottle was available in the chamber, but rats were not fed during activity monitoring.

Meal patterns

After activity monitoring was completed for all rats, the SEC vs INSEC daily feeding schedules continued for another 8 days before proceeding to meal pattern testing. For this test, rats were provided a palatable liquid diet in lickometry chambers for 8 hours as intake patterns were monitored. The liquid diet (1.47 kcal/g) was a mixture of water (60% w/w), heavy cream (21% w/w), sucrose (15% w/w), and amino acid blend (4% w/w, hydrolyzed collagen, Great Lakes, Grayslake, IL). Rats were previously familiarized with the liquid diet by providing 40 mL in each home cage on two occasions. Rats were then familiarized with consuming the liquid diet in the lickometry chambers for a single 30-min session. In preparation for the critical test, rats in both the SEC and INSEC groups were fed on the SEC schedule on the entire preceding day and at 9:00AM on the test day to equate recent intake and deprivation level. Rats were placed into the lickometry chambers just before the customary 1:00PM feeding time and had ad libitum access to a bottle of liquid diet for the next 8 hours. Intake was monitored by contact lick sensors attached to the drinking spout and interfaced to a computer which recorded each lick. Bottles were weighed before and after the session to determine total intake. Licking records were subsequently processed to analyze meal patterns, tabulating the total number of licks, the size of each meal (in licks) and the number of meals initiated in 8 hours, using the criteria of 10 minutes without licking to define the end of each meal.

Progressive Ratio Operant Task

Following the meal pattern testing, the SEC vs INSEC feeding schedules continued for 26 days before proceeding to operant training. Rats were trained to lever press for 45mg sucrose pellets in Coulbourn Habitest operant chambers. Each chamber was outfitted on the front wall with a fixed response lever positioned to the left of a centrally located pellet trough, and was dimly illuminated by a house light on the rear wall. A multicolor-LED cue light (Coulbourn H11-02R) positioned immediately above the lever illuminated when the response lever was active. When a pellet was delivered, the house light and cue light both darkened for 2 sec and a white light recessed inside the pellet trough illuminated. During this phase of the experiment the SEC vs INSEC schedules were suspended and all rats were fed a daily ration (14 g/day/rat for CHOW or 10.1 g/day/rat HFHS) in their home cages after completing their daily operant session. This was to standardize deprivation level at the start of each daily training session.

Each rat was initially exposed to three consecutive 90-min magazine training sessions during which sucrose pellets were delivered freely on a 60-90 sec random ITI, and contacts with the lever would also trigger immediate pellet delivery. In subsequent sessions a lever press was necessary to obtain each sucrose pellet on a continuous reinforcement schedule. Daily sessions lasted 90-min or until 80 pellets were obtained. Rats that obtained <20 pellets in the first two sessions were given an extended overnight session, and thereafter all rats reliably earned 70-80 pellets in each 90-min session. At that point rats had three consecutive daily sessions of VR-3 schedule, then three sessions of VR-5. Finally, rats were tested for two consecutive days in a progressive ratio (PR-5) schedule. In these sessions, 5 lever presses were required to obtain the first pellet, and thereafter each time a pellet was earned the requirement for the next pellet increased progressively by 5. The session terminated when a rat ceased pressing for at least 15 minutes, and the lever press requirement of the last pellet obtained was considered the breakpoint. Each rat’s breakpoint value across two consecutive sessions was averaged.

Novel Object Recognition

Following the completion of progressive ratio operant testing, rats were returned to the SEC vs INSEC feeding schedules for 8 days before starting the novel object recognition (NOR) test of working memory. Prior to testing, rats were familiarized with the empty test arena (40 cm L × 40 cm W × 46 cm H) for 10 minutes on two consecutive days. All rats were fed on the SEC schedule on the day prior to and morning of NOR testing to equate recent feeding and deprivation level. For the NOR test, the rat was placed into the arena with two identical objects positioned equidistant from the center and allowed to explore for 5 min. Objects used were plastic toys that were weighted so they could not be moved or overturned during exploration. After a 5-min exploration period the rat was removed to a holding cage for a 1-hr retention interval, and the arena and objects were cleaned with 71% isopropyl alcohol. When the rat was returned following the retention interval, the arena contained one of the original objects and a novel object. Memory for the original object is indicated by preferential exploration of the novel object during a 3-min test period. Identity and relative position of the original and novel objects were counterbalanced across rats. Overhead video recordings were scored for object exploration (defined as actively investigating the object by sniffing it or touching it with a forepaw) by two independent observers who were blind to rats' experimental conditions.

Weight gain on ad libitum HFHS diet

Following the completion of NOR testing, rats were maintained on the SEC vs INSEC feeding schedules for another 6 days. In the final phase all rats were provided with ad libitum HFHS diet to determine if groups were differentially prone to weight gain during ad libitum access. At the outset of this phase, all rats were fed on the SEC schedule for 2 days in order to equate recent intake prior to providing all rats with ad libitum HFHS diet. Each rat's weight was recorded when rats were provided ad libitum HFHS diet, and again after 8 days. Because rats remained group housed, only weight gain was measured as individual food intakes could not be assessed.

Results

Locomotor Activity

Total activity counts for the 13-hr measurement are depicted in Figure 1A and were analyzed with a 2 (Schedule: SEC vs INSEC) X 2 (Diet: CHOW vs HFHS) ANOVA. Prior insecurity produced hyperactivity, main effect of Schedule, $F\ (1, 49) = 6.36$, $p < .05$. However, the effect of insecurity interacted with maintenance diet, $F\ (1, 49) = 6.36$, $p < .05$ such that CHOW-INSEC rats were hyperactive relative to their secure counterparts, but HFHS diet were not. That is apparently because the HFHS diet itself produced hyperactivity, main effect of Diet, $F\ (1, 49) = 12.23$, $p < .01$. This interaction was confirmed by *post hoc* contrasts (CHOW-INSEC vs CHOW-SEC, t-test for unequal variances, $t\ (13.4) = 2.84$, $p < .05$, HFHS-INSEC vs HFHS-SEC, $t(25) = 0.03$, $p = 0.97$).

For descriptive purposes, activity counts across each 30-min block of the 13-hour measurement period are depicted in Figures 1B & 1C, though these data presentations were not analyzed further as the experiment is not powered for multiple comparisons across time. All groups showed a substantial increase in activity surrounding the 9:00AM lights-off, which would ordinarily be the first scheduled feeding. Thereafter, CHOW-SEC rats' activity remained relatively low and distributed uniformly across the measurement period, while CHOW-INSEC rats remained more active across the next several hours, showing some indication of cyclic activity peaking shortly prior to scheduled feeding times. HFHS-SEC and HFHS-INSEC groups were similar to each other across time, with a sharp peak at lights-off that immediately waned, followed thereafter by a gradual increase across time with no peaks evident at scheduled feeding times.

Meal Patterns

Providing rats with a palatable liquid diet for 8 hours revealed group differences in total intake and meal size, consistent with the hypothesis that prior food insecurity leads to greater consumption when food is available. Total 8 hr intakes are depicted in Figure 2A, and were analyzed with a 2 (Schedule: SEC vs INSEC) X 2 (Diet: CHOW vs HFHS) ANOVA. Prior insecurity promoted greater intake, as INSEC rats consumed significantly more than SEC rats, F (1, 48) = 7.31, $p < .01$. The CHOW rats also consumed more than HFHS rats F (1, 48) = 48.86, $p < .001$, presumably reflecting CHOW rats' evaluation of the liquid diet relative to their maintenance diet. However the effect of INSEC schedule on intake did not depend on prior diet, Schedule X Diet interaction, F (1, 48) = 0.006, $p = 0.94$.

The group differences in total intake were due to effects on meal size, not the number of meals initiated. Because all rats began the 8-hr session with a large meal followed by several smaller meals, the first meal was analyzed separately from subsequent meals (Figures 2B and 2C). Irrespective of prior diet type, INSEC rats ate a larger first meal than SEC rats, main effect of Schedule F (1, 48) = 9.71, $p < .01$. Moreover, the first meal was larger for CHOW rats than HFHS rats, main effect of Diet F (1, 48) = 5.68, $p < 0.05$, however the effect of INSEC schedule on first meal size did not depend on the maintenance diet, Schedule X Diet F (1, 48) = 0.04, $p = 0.85$.

A different pattern was observed in the subsequent meals across the rest of the session (Figure 2C), as the effect of insecurity was no longer seen in CHOW rats but persisted in HFHS rats. In this comparison there was no longer a main effect of Diet, F (1, 48) = 0.63, $p = 0.43$, nor of Schedule, F (1, 48) = 0.05, $p = 0.82$, but there was a significant Diet X Schedule interaction, F (1, 48) = 5.52, $p < .05$. Post hoc exploration of this interaction confirmed that meals were larger for INSEC rats than SEC rats in the HFHS condition, $t(25) = 2.25$, $p < 0.05$, with no difference between the CHOW groups, $t(25) = 1.27$, $p = 0.22$.

The number of meals initiated in 8 hrs (data not shown) was similar across all groups, Schedule F (1, 48) = 2.06, $p = 0.59$; Diet F (1, 48) = 0.06, $p = .90$; Schedule X Diet F (1, 48) = 0.26, $p = 0.61$. In sum, prior insecurity increased total intake in both insecure groups, and increased the size of the first meal in CHOW-INSEC rats, and increased the first and subsequent meal sizes in HFHS-INSEC rats.

Progressive Ratio Breakpoints

Lever-pressing to earn sucrose pellets on a progressive ratio schedule was measured to test the hypothesis that a history of food insecurity increases food reward value, at least for sucrose. Progressive ratio breakpoints (the last ratio requirement successfully completed, two-session averages) are depicted in Figure 3 and were analyzed with a 2 (Schedule: SEC vs INSEC) X 2 (Diet: CHOW vs HFHS) ANOVA. Overall, INSEC rats had higher breakpoints than SEC rats, main effect of Schedule, F (1, 48) = 5.98, $p = 0.018$, indicating that prior insecurity elevated the reward value of sucrose. There was no significant main effect of Diet, F (1, 48) = 2.35, $p = 0.13$. Although the ANOVA did not show a significant Schedule X Diet interaction, Diet, F (1, 48) = 0.54, $p = 0.46$, planned contrasts within diet conditions indicate that INSEC rats had higher breakpoints than SEC rats in the HFHS condition, $t(25) = 2.64$, $p = 0.014$, but not in the CHOW condition, $t(23) = 1.05$, $p = 0.30$. Overall these results indicate that prior insecurity promotes increased sucrose reward value, although this effect may be especially evident in individuals consuming a HFHS diet.

It is noteworthy that in the meal pattern test and the progressive ratio test, the HFHS-SEC group exhibited the lowest motivation and intake of all groups. This pattern suggests that an effect of insecurity is to maintain heightened responsiveness to palatable food that otherwise habituates when such food is reliably available.

Novel Object Recognition

Performance on the Novel Object Recognition test was first analyzed as a validity check verifying that rats behaved in the expected fashion by exploring the novel object more than the familiar object. A paired t-test including all rats irrespective of experimental condition confirmed the expected pattern, with significantly higher exploration time for the novel object than the familiar object, paired $t(49) = 5.74$, $p < 0.001$. A second preliminary analysis checked whether total exploration time varied across groups, finding that it did not (no main effect of Schedule, $F(1,46) = 0.02$, $p = 0.97$, Diet, $F(1,46) = 2.16$, $p = 0.15$, nor interaction, $F(1,45) = .54$, $p = 0.47$).

Potential effects of prior insecurity and diet type on memory performance were then analyzed using an Exploration Proportion, calculated as the time exploring the novel object as a proportion of total time exploring either object. A proportion of 0.5 represents equal time exploring each object and a higher value indicates preferential exploration of the novel object. One rat (INSEC-HFHS condition) was removed from the analysis because it explored neither object in the recognition test, yielding an undefined value.

Exploration Proportions are depicted in Figure 4 and were analyzed with a 2 (Schedule: SEC vs INSEC) X 2 (Diet: CHOW vs HFHS) ANOVA. No effects of prior insecurity or diet type were evident in this test, main effect of Schedule, $F(1,45) = 2.24$, $p = 0.14$, main effect of Diet, $F(1,45) = 0.642$, $p = 0.427$, and Schedule X Diet interaction, $F(1,45) = .643$, $p = 0.428$. In fact, contrary to expectations, INSEC rats on average showed marginally higher values (suggesting better recognition memory for the familiar object) than SEC rats, though no group differences were significant.

Weight gain during ad libitum HFHS diet access

At the conclusion of the experiment all rats were provided ad libitum HFHS chow for 8 days in the home cages, revealing that prior insecurity promoted more rapid weight gain during food availability, though that effect was mostly confined to rats previously exposed to the combination of HFHS and INSEC. Body weights at the start and end of the 8-day ad libitum HFHS access are depicted in Figure 5A and were analyzed in a 2 (Prior Schedule: SEC vs INSEC) X 2 (Prior Diet: CHOW vs HFHS) X 2 (Time: Day 0 vs Day 8) repeated measures ANOVA. Rats previously maintained on HFHS weighed more at both time points than rats maintained on CHOW, main effect of Prior Diet, $F(1,46) = 17.88$, $p < 0.01$. The baseline weight difference was unexpected given that all four feeding schedules were matched to deliver equivalent energy content. This may reflect effects of chronic HFHS vs CHOW intake on metabolism. However baseline weight difference depended only on diet and not secure vs insecure schedule, no effect of Prior Schedule, $F(1,46) = 1.91$, $p < 0.17$ or Prior Schedule X Prior Diet interaction $F(1,46) = 0.42$, $p = 0.52$. The crucial finding is that rats previously maintained on the INSEC schedule gained weight more rapidly across 8 days than rats previously maintained on the SEC schedule, as evidenced by a significant Prior Schedule X Time interaction, $F(1,46) = 6.25$, $p = 0.016$. This is further supported by the planned post hoc contrast of weight gain, which was higher for INSEC than SEC, $t(23) = 2.52$, $p < .05$. Although no interactions involving both Prior Diet and Time were significant, it does appear most of the impact of prior insecurity is explained by weight gain among HFHS-INSEC rats. Planned post hoc contrasts on Day 8 body weights within groups show that HFHS-INSEC weighed more than

HFHS-SEC, $t(23) = 2.42$, $p < .05$, but CHOW-INSEC did not weigh more than CHOW-SEC, $t(23) = 1.04$, $p = 0.31$.

Because initial weights differed between rats previously maintained on CHOW and HFHS, we further analyzed each rat's 8-day weight gain as a percentage increase from its starting weight, Figure 5B, in a 2 (Prior Schedule: SEC vs INSEC) X 2 (Prior Diet: CHOW vs HFHS) ANOVA. This confirmed that prior insecurity resulted in larger proportional weight gain, main effect of Prior Schedule, $F(1,46) = 5.04$, $p < 0.05$. Although neither the effect of Prior Diet, $F(1,46) = 2.56$, $p = 0.12$, nor Prior Diet X Prior Schedule interaction $F(1,46) = 0.58$, $p = 0.45$ were statistically significant, again it appeared that the effect of schedule is more evident in the HFHS-INSEC group, as planned contrasts show that HFHS-INSEC gained more weight that HFHS-SEC, $t(23) = 2.44$, $p < .05$, yet CHOW-INSEC and CHOW-SEC did not significantly differ.

Experiment 2

Experiment 1 revealed that after long term experience with an unpredictable feeding schedule, rats gained more weight when provided ad libitum HFHS diet, consistent with observations in people that food insecurity is associated with opportunistic eating when food is available (Nettle, et al., 2019; Stinson, et al., 20180) and higher BMI (Cheung et al., 2015; Dhurandhar, 2016; Larson & Story, 2011; Pan et al., 2012; Townsend et al., 2001). However an effect on weight gain was only clearly evident in rats who previously experienced the combination of insecurity and HFHS maintenance diet. It may be that insecurity promotes weight gain by exacerbating effects of long-term HFHS consumption on appetite control. However, Experiment 1 may have underestimated the potential impact of prior insecurity in the CHOW-

INSEC group, for whom the final phase involved shifting from scheduled feedings to ad libitum access, but also a switch from chow to HFHS. The switch to HFHS *per se* presumably stimulated hyperphagia in both CHOW groups, which may have masked a separate but subtle effect of the prior INSEC schedule. Therefore Experiment 2 replicated the CHOW-SEC and CHOW-INSEC conditions in a new cohort of rats, to test if the insecure schedule would promote subsequent weight gain during a period of ad libitum access to standard chow. Moreover, since some evidence indicates that insecurity promotes metabolic conservation (Bateson et al., 2021), it could make individuals more resistant to weight loss during periods of restriction. Therefore, in Experiment 2, the ad libitum chow phase was interrupted by an occasional period of 24-hr food deprivation to measure weight loss. This was intended to provide a more realistic model of challenges to weight maintenance for food insecure people, who may experience frequent alternation between periods of deprivation and access.

Methods

Subjects and Experimental Treatment

Subjects were 18 young adult female Sprague-Dawley rats from 6 litters born in our breeding colony. All housing conditions, the procedures for allocating rats to CHOW-SEC (n = 9) and CHOW-INSEC (n = 9) experimental groups, and the experimental feeding schedules were carried out as described for Experiment 1. Rats were maintained on the CHOW-SEC vs CHOW-INSEC feeding schedules for 12 weeks.

Weight changes during alternating access periods

For the last two days of the daily programmed feedings, both groups were fed according to the SEC schedule to equate recent feeding before transitioning to a phase where weight loss during restriction and weight gain during ad lib feeding were monitored. Baseline body weights

(Day 0) were obtained 4 hr after the last programmed meal delivery, and again 24 hours later (Day 1). No chow was available during that time. Ad libitum chow was then provided and body weights were measured after 8 days (Day 9). That cycle repeated for another 24 hr restriction (Day 10) and 8 days ad libitum chow (final weights taken on Day 18).

Results

For descriptive purposes, body weights across the two cycles are depicted in Figure 6A. An initial analysis using only baseline weights (Day 0) and final weights (Day 18) in a 2 (Group) X 2 (Time) repeated measures ANOVA demonstrated larger increases in body weight for CHOW-INSEC rats. Although groups were similar on Day 0, INSEC rats diverged from SEC rats, as reflected in the Condition X Time interaction, $F(1,16) = 19.43$, $p < 0.001$. As expected, the simple main effect of Time was also significant, $F(1,16) = 69.30$, $p < 0.001$ as all rats gained weight, but the simple main effect of Group was not, $F(1,16) = .63$, $p = 0.44$. By Day 18, INSEC rats had a net gain of 32.1 ± 11.75 g (M ± SD), significantly more than SEC rats' gain of 9.8 ± 9.5 g, t (16) = 4.41, $p < .001$.

The INSEC rats' larger net gain by Day 18 could reflect both a resistance to weight loss during food restriction and a more rapid weight gain during food access. Weight lost by each group during two separate periods of food restriction (Day 1 and Day 10, Figure 6B) was compared in a 2 (Group) x 2 (Day) repeated measures ANOVA which showed some evidence that INSEC rats were more resistant to weight loss, main effect of Group, $F(1,16) = 7.21$, $p < 0.05$. Although there was no effect of Day, $F(1,16) = 3.49$, $p = .08$ nor Group X Day interaction, $F(1,16) = 1.76$, $p = .20$, post hoc tests indicate INSEC rats lost less weight than SEC during the first deprivation day, t (16) = 3.29, $p < .01$ but not the second, t (16) = 0.08, $p = .93$.

INSEC rats gained more weight than SEC rats when chow was available. Weight gain during each of the two ad libitum periods (Figure 6C, Days 2-9 and Days 11-18) was compared in a 2 (Group) x 2 (Cycle) repeated measures ANOVA, demonstrating a significant main effect of Group, $F(1,16) = 8.12$, $p < 0.05$. There was no effect of Cycle $F(1,16) = 2.94$, $p = 0.10$, nor Group X Cycle interaction, $F(1,16) = 0.002$, $p = 0.97$. Ultimately the INSEC rats exhibited a larger net increase in weight across the two cycles of restriction and access, as shown in Figure 6D, $t(16) = 4.41$, $p < .01$.

These results extend Experiment 1 by demonstrating that the weight gain seen in that experiment after the combination of HFHS diet and INSEC schedule does not require prior HFHS intake, as it was seen in Experiment 2 among CHOW-INSEC rats transitioned to ad libitum chow.

Discussion

This research studied the effects of chronically unpredictable food access on food motivation, ad libitum intake patterns, cognition, and weight gain, as a step towards developing an animal model of food insecurity. The protocol was designed to maximize food uncertainty without undernutrition, by making feeding amounts persistently unpredictable while equating total food intake over time. We also included a high-fat/high-sugar diet condition to study potential interactions between chronic unpredictability and the poorer diet quality commonly seen among food insecure individuals. We found several behavioral changes resulting from insecurity.

Insecure chow-fed rats showed more locomotor activity in the absence of food. Insecure chow-fed and insecure HFHS-fed rats showed increased intake of a palatable liquid diet. Among HFHS-fed rats, insecurity also increased motivation to earn sucrose pellets on a progressive ratio task, as well as lasting increased meal size during liquid diet access. Insecurity also promoted subsequent weight gain. No weight differences emerged during maintenance on the controlled feeding schedules which equated the amounts that secure and insecure rats received over each 4-day cycle. However, when switched to ad libitum food, previously insecure rats gained weight more rapidly. This was true for insecure HFHS-fed rats switched to ad lib HFHS, and for insecure chow-fed rats when given ad lib chow. Thus, weight gain after insecurity does not necessarily depend on poor diet quality.

The so-called "insecurity-obesity paradox" refers to the frequent observation that insecurity is positively associated with higher BMI and obesity despite limited access to food. However, the links between insecurity and higher BMI are complex and likely to be multiply determined, since the association is not always observed (Franklin, et al., 2012). Our present findings show that a history of uncertain, unreliable food access *per se* may be a causal factor contributing to the insecurity-obesity link.

Furthermore, the present results show several commonalities with behavioral phenotypes associated with food insecurity in humans. Though observations of dietary self-selection and meal patterns among food insecure people under controlled conditions are sparse, one such study made a wide variety of foods available ad libitum after equating recent intake and found food insecure women selected larger meals, and selected more fat and carbohydrate but not protein (Stinson, et al., 2018). Another found positive associations between current insecurity and intake of test foods in a contrived 'taste test' paradigm (Nettle, et al., 2019). Food insecure individuals also report more habitual binge-like eating behaviors and are at increased risk of binge eating disorder (Stinson, et al., 2018; Rasmusson et al., 2019) and exhibit heightened food reinforcement value (Crandall & Temple, 2018). Given parallels in our observations of meal patterns, food motivation, and weight gain in rats with unpredictable feeding schedules, we propose this to be a useful model for further mechanistic studies of the physiological, psychological, and behavioral impacts of food insecurity.

When activity levels were measured for an extended period in the absence of food, rats fed ordinary chow on the insecure schedule were hyperactive compared to secure controls. This is noteworthy given evidence that food insecurity may be a predisposing or exacerbating factor in attentional and behavioral regulation deficits in children and adolescents (Lu et al 2019; Melchior, et al., 2012; Poole-Di Salvo, et al., 2016). Food insecure women also behave more impulsively in a food-reinforced delay discounting task (Rodruiguez, et al., 2021). An effect on hyperactivity was not detected in rats receiving the combination of insecurity and HFHS diet, because the HFHS diet on its own also promoted hyperactivity relative to chow, in agreement with previous literature (Marwitz, et al., 2015). While it is unclear whether insecurity and the HFHS diet independently promote hyperactivity through similar impacts on the brain, our results provide experimental evidence that behavioral hyperactivity can be caused by insecurity independent of poor diet quality or other circumstantial variables.

Prior insecurity increased rats' intake during 8 hours of access to a palatable liquid diet. It is important to note that in this and all other behavioral tests, the secure and insecure groups were fed identically for some time prior to the test to minimize differences in recent deprivation, so results are attributable to lasting effects of insecurity. Both insecure groups (chow- and HFHS-fed) consumed more than their secure counterparts, although some differences in 8-hr meal patterns depended on maintenance diet. Insecure rats in both diet conditions had larger initial meals but only rats in the insecure HFHS condition showed persistently larger meals throughout the 8-hour test. However, comparing across CHOW and HFHS conditions may have been affected by the contrast between the liquid diet offered in the test and the rats' maintenance diet. That is, the sweet, highly palatable liquid diet was presumably much more attractive to the chow groups than their customary diet, and less so for the HFHS groups. This is consistent with the observation that both CHOW groups consumed more of the palatable liquid diet, and larger meals, than both HFHS groups. Thus, while the test clearly shows impacts of insecurity regardless of maintenance diet, the additional interaction between HFHS maintenance and insecurity should be considered preliminary. Further work will also be useful for investigating the mechanisms underlying this effect, as increased meal size could reflect heightened palatability perception or blunted sensitivity to satiation signals, or both. Data on these processes in food insecure people is limited and mixed. Children in food insecure families are described as enjoying food more and also showing reduced satiety responsiveness (McCurdy, et al., 2021) and more eating past satiation (Kral, Chittams, & Moore, 2017). Another study found that food insecure adults did give higher liking ratings for chocolate, but not for other snack foods, and liking ratings did not explain the correlation between insecurity and test food intake (Nettle, et al., 2019). The inter-relationships between food insecurity, hedonic evaluation, and satiation processes require further investigation, for which our model is well suited.

Food insecurity in humans is correlated with poorer cognitive function broadly speaking, including cognitive decline in adulthood (Na et al 2020; Phillips et al., 2018; Portella-Para & Leung, 2019) and poorer academic performance throughout schooling (e.g. reviews by Shankar, Chung, & Frank, 2017 for children, and Bruening, et al., 2017 for university students). However, it is difficult to isolate food insecurity from other interrelated factors inherent in poverty and economic inequality, and cognition is impacted by diet quality even in the absence of insecurity (Francis & Stevenson, 2013). An animal model will be useful for identifying ways food insecurity itself may directly impact cognitive systems in the brain. Here we used a relatively simple test of short-term memory – the novel object recognition test with a 1-hour retention interval – and found no effect of insecurity. This test was selected for its simplicity as a preliminary screening tool, given the exploratory nature of this experiment and its extended timeline. This initial result suggests no gross deficits in low-level attentional and encoding mechanisms mediating short-term recognition memory under low cognitive load, though additional work should focus on more challenging cognitive tasks and those involving long-term storage and retrieval processes. Of the few prior animal studies of food insecurity, one has reported that a feeding schedule that varies unpredictably between under- and over-feeding did produce deficits in novel object recognition (Estacio, et al., 2021) using a more challenging 24-hour retention interval that makes the test more sensitive to hippocampally-mediated consolidation processes. That study also used aged female mice, and it may also be that insecurity exacerbates age-related deficits (at least with regard to recognition memory) or that impairments are evident in circumstances where cognitive load is particularly high. Further work with our model should explore a broader range of tasks assessing different aspects of cognition, as different neural circuits are differently susceptible to nutritional and environmental factors.

Both of the present experiments demonstrated that prior insecurity can promote more rapid weight gain when transitioned to ad libitum food access. Although this effect was not seen among CHOW-INSEC rats switched to ad libitum HFHS food in Experiment 1, again that may reflect the confounding effect of the switch from chow maintenance to ad libitum HFHS in both chow groups. In Experiment 2, insecurity during chow maintenance diet did in fact promote weight gain on ad libitum chow. That suggests that the link between weight gain and insecurity does not depend on a prior history of poor diet quality nor is it a byproduct of relying on high-energy dense foods, once again highlighting the potential causal role of the insecure feeding schedule itself.

Weight gain may be influenced by increased food intake, or metabolic adaptations that favor energy storage and reduced energy expenditure, or both. The “insurance hypothesis” which is grounded in behavioral ecology (Nettle, Andrews, & Bateson, 2017) proposes that the obesity-insecurity link reflects adaptive responses to environmental uncertainty that promote energy storage as a buffer against frequent shortfalls in food availability. Support for the idea that insecurity impacts weight primarily via energy storage rather than intake comes from diet record studies showing average daily calorie intakes of food secure and insecure people are generally similar (Shinwell, et al., 2021; Zizza, et al., 2012). On the other hand, an experimental study of food choice did observe increased ad libitum intake among food insecure women compared to food secure women during a 3-day free access period (Stinson, et al., 2018). Food insecure women are also more likely to be overweight when receiving monthly government food assistance (Townsend, et al., 2001) which has been hypothesized to reflect a so-called “benefit cycle” of over- and under-consumption, implicating eating behavior during access periods in the long-term accumulation of excess weight. Given the complex links between insecurity and weight status, an animal model will be useful for further investigating these mechanisms experimentally.

In our model we found only limited indication of metabolic shifts promoting weight gain, namely the observation in Experiment 2 that insecure rats lost less weight than secure rats during the first 24-hour period of food deprivation. This could not have been due to differences in recent food intake immediately prior to the deprivation since both groups were fed the identical schedule for 48 hours prior. However, the effect was rather transient since it was not seen in a second 24-hour food restriction 8 days later. Moreover, long term maintenance on the secure or insecure schedules – during which rats were fed equivalent total energy over each 4-day cycle – did not itself produce differences in weight. Unexpectedly, both HFHS groups weighed more than both CHOW groups, presumably reflecting a long-term influence of the metabolic or epigenetic impacts of the HFHS diet (which has been documented previously, e.g., De Meijer, et al., 2010; Lomba, et al., 2009; Lomba, et al., 2010; Lomba, et al., 2010; Moretto, et al., 2017). However, in no condition did rats on an insecure schedule begin to weigh more than their secure counterparts until food access became unlimited.

Although individual measures of food intake were not collected during the ad libitum access period due to group housing, hyperphagia is a more likely explanation than differences in energy expenditure for the more rapid weight gain we observed following insecurity, since hyperphagia was also evident in other measures. In Experiment 1, insecurity promoted increased intake via larger meal size during 8-hr access to a palatable liquid diet, and that effect was most persistent in HFHS-INSEC rats who also subsequently gained the most weight in the ad libitum phase. There was no evidence that physical activity levels would account for the increased weight gain since, in Experiment 1, the HFHS-INSEC group gained more weight than their HFHS-SEC counterparts but did not differ in the physical activity assay earlier in the study. However, the evidence for resistance to weight loss during deprivation warrants additional investigation. Moreover, we did not investigate body composition, and it remains possible that this model produced subtler effects on energy metabolism and storage that were not revealed by monitoring total body weight. Other animal studies suggest unreliable food access favors increased metabolic efficiency. In experiments with European starlings afforded either temporally predictable or unpredictable access to an operant foraging task, birds in unpredictable conditions gained more weight per gram of food consumed, and assimilated more energy from the food (excreting less in waste) and had increased fat stores (Andrews, et al., 2021; Bateson et al., 2021).

A recurring pattern in the present results was that reliable long-term HFHS access on its own tended to reduce the motivational significance of highly palatable food, but those reductions were prevented by insecurity. Namely, in Experiment 1 the HFHS-SEC rats who had consistent, predictable access to HFHS food subsequently consumed the least and had the smallest meals in the ad libitum liquid diet test, had the lowest progressive ratio breakpoints for sucrose, and gained the least weight during ad lib HFHS access. This apparent habituation to the value of highly palatable food is consistent with previous work showing that high-energy diets initially increase food motivation in the short term (3-5 weeks, e.g., La Fleur, et al., 2007; Figlewicz, et al., 2013) but ultimately attenuate food motivation over the long term (>8 weeks, Davis et al., 2008; Finger, et al., 2012; Tracy, et al., 2015; Íbias, et al, 2016). The novel finding here is that insecurity apparently prevents that long-term attenuation, preserving a high responsiveness to palatable rewards. People coping with food insecurity tend to rely on highly processed, energy dense foods, and it is often assumed this reflects economic necessity and availability. One implication of our present results is that insecurity may actively promote poor diet quality by maintaining a heightened reinforcing value of palatable foods.

Our insecurity protocol does have some resemblance to established “intermittent access” protocols in which alternating cycles of food restriction and brief access to highly palatable food induce a pattern of binge-like consumption and other behavioral changes sometimes described as ‘addiction-like’ (reviewed by Corwin, Avena, Boggiano, 2011). However, our current results are not explained by the same mechanisms since those protocols require habitual limited-access to highly palatable food. Cyclic intermittent access to ordinary chow does not produce the binge-like phenotype (and in fact serves as a control condition in those protocols). In the present experiments, we observed changes in activity, intake, and weight gain in rats chronically maintained on unpredictable access to ordinary chow. Further, limited-access protocols alter meal patterns and motivation, but do not typically promote long-term weight gain, whereas in our experiments the insecure schedule promoted subsequent weight gain, even in Experiment 2 when insecure access and ad libitum access were both plain chow. We therefore argue that the behavioral phenotype we have documented and its underlying neural mechanisms are largely separate from previously established rodent binge-eating models, and are useful as a model of behavioral impacts of insecurity.

One interpretive caveat arising from the experimental design is that the several behavioral tests comprising Experiment 1 began after rats had been on the experimental feeding schedules for 8 weeks and spanned the subsequent 2.5 months, although the order of the tests was not counterbalanced. Complete counterbalancing would have been impractical given the differences in time needed (in some cases days and others weeks) to carry out each of the dependent measures, and weight gain during *ad libitum* intake necessarily came last. From this design we are unable to conclude how long animals must be on the insecure feeding schedule before behavioral effects are detectable, or whether the various effects we observed arise contemporaneously. Conceivably any of the effects may have been weaker or stronger if measured at a different point in the experiment. Further work will be necessary to understand the time course of these effects, and how enduring they are if animals with a history of food insecurity are switched to secure food access.

Another interpretive issue is that both the SEC and INSEC schedules represented chronic mild food restriction, with feeding schedules devised to provide ~90% of typical ad libitum intake. This was to ensure that total food intake by SEC and INSEC groups was closely matched over the long term, and also that SEC rats were truly eating in discrete meals tightly bound to the feeding schedule, and not accumulating a surplus of food they could eat ad libitum throughout the day. But in actuality, in the modern food environment (and in research on people who are food insecure) the secure 'control' condition is more akin to ad libitum access. We do not propose the SEC schedule in this design represents typical free-living conditions, but rather that it is the appropriate experimental comparison for isolating and characterizing the effects of unpredictability inherent in food insecurity. This also makes it more challenging to directly compare effects in this model to other animal experiments using ad libitum feeding conditions. For instance, the previously mentioned studies showing bi-phasic impacts of HFHS diet history on progressive ratio responding (La Fleur, et al., 2007; Figlewicz, et al., 2013; Davis et al., 2008; Finger, et al., 2012; Tracy, et al., 2015; Íbias, et al, 2016) are part of a much larger literature on behavioral and metabolic impacts of high energy diets, yet among that work, studies using limited daily portions of high-energy diets are relatively rare. In our model, especially in the interaction between insecurity and HFHS diet history, imposing persistent mild restriction may yield smaller effect sizes and may sometimes fail to find effects in a secure control condition that is considered well-established for HFHS diets based on studies that employ unlimited access.

Additional research with this model will be useful for further exploring how animal sex, age, and social status interact with insecurity. This initial study focused on female rats exclusively because of documented disproportionate impacts on women. Prevalence rates of food insecurity are higher in women (Broussard, 2019; Matheson & McIntyre, 2014) and insecurity is associated with higher BMI in women but not men in several studies (Gooding et al., 2012; Hernandez, et al., 2017; Townsend et al., 2001). The association between insecurity and mental illness is present in both sexes but significantly stronger in women (Martin, et al., 2016). However, there is currently no clear explanation for those discrepancies, and they could reflect both physiological and cultural factors. Our current study finds several effects on chronic food insecurity on appetitive behaviors and weight gain in female rats. Comparing male and female rats in this model can help elucidate whether physiological sex differences, such as those in HPA axis function or interactions between gonadal hormones and energy regulation, mediate the impacts of insecurity.

Our study also began the insecurity treatment in early adulthood. Additional work can investigate whether insecurity is more or less impactful at different ages. Household food insecurity is a significant risk factor for poor child health (Drennen et al., 2019) and poor academic and social functioning (Jyoti, Frongillo, & Jones, 2005). However, extremely limited data exists on children transitioning from insecurity to security at different ages that would allow conclusions about special vulnerabilities at particular developmental stages (Gallegos, et al., 2021). Further, existing data on food insecurity in childhood is especially challenging to interpret, as caregivers may vary widely in attempts to protect children from insecurity, thus children in households labeled insecure may have more heterogenous experiences than adults. Nonetheless, one study measuring adults' recent and childhood experiences of insecurity found evidence for lingering consequences of childhood insecurity, such that adults who are no longer food insecure may continue to behave in some ways as if they still are (Nettle., et al., 2019).

For practical reasons and animal welfare best practices, in the present work all rats were housed in groups of three. Other animal studies of unpredictable food access have also used group housing (Andrews, et al., 2021; Bateson, et al., 2021; Estacio, et al., 2021). That factor would also be useful for additional parametric research. In humans, the experience of food insecurity may be mediated by subjective social status (Dhurandhar, 2016). In an animal model, group housing could potentially exacerbate or attenuate the impacts of the insecure feeding schedule. On the one hand, group housing may create intra-cage competition at feeding times, possibly promoting behavioral changes like habitual rapid eating. Alternatively, group housing may have buffered against the stressful effects of insecurity. Social isolation affects HPA axis function and stress reactivity, neural plasticity, and motivation in animal models (Stevenson, et al., 2019; Weintraub, et al., 2010; Westenbroek, et al., 2004; Westenbroek, et al., 2019) often in a sex-specific manner. This model could be useful for further study of social variables in insecurity.

Finally, we propose this model can be useful for controlled investigations of physiological and neural mechanisms. Since food insecurity in humans is correlated not only with alterations in eating behavior and weight status, but also cognition, decision making, and psychological disorders, there are presumably diverse impacts on brain circuitry, yet there is only limited work linking insecurity with physiological variables and none measuring brain function. Some areas we expect to be informative are the involvement of the HPA axis and dopaminergic reward pathways. Among low-income individuals, those coping with food insecurity self-report higher levels of stress, and food insecure children show elevated cortisol (Ling, Robbins, & Xu, 2019). Experienced distress and using food to cope each mediate the relationship between insecurity and BMI (Keenan, Christiansen, & Hardman, 2021). Considering the many interactions between chronically elevated glucocorticoids and central circuits for energy homeostasis and reward (Dallman, et al., 2003; Adam & Epel, 2007; Rutters, et al, 2012), our model may be useful for experimentally characterizing the role of the HPA axis in food insecurity. Further, some of our behavioral results, namely locomotor hyperactivity and elevated progressive ratio responding for sucrose, implicate central dopamine signaling and forebrain processes of goal-directed behavior and reward valuation. Similarly, a rodent model of compulsive gambling has found that chronic exposure to outcome uncertainty sensitizes locomotor responsiveness and increases striatal D_2 receptor binding to a similar degree as serial amphetamine dosing (Fugariu, et al., 2020). In Pavlovian appetitive conditioning, outcome uncertainty promotes sign-tracking behavior towards the conditioned stimulus, regarded as a behavioral indicator of dopamine-mediated incentive salience attribution (Anselme, Robinson, & Berridge, 2013). Our protocol was designed to make eating opportunities unpredictable, and adds additional evidence that environmental uncertainty alters reward processing, consistent with the view that the experience of chronic uncertainty, over and above differences in nutrition or diet quality, is an important dysregulatory influence in food insecurity

In summary, we have shown here in a controlled animal model that making food access chronically unpredictable in the absence of undernutrition produces significant changes in eating behavior and food motivation, and increased weight gain when food becomes available. Thus, the condition of chronic uncertainty itself may be a factor in the obesity-insecurity link, altering eating behavior, food choice, and health, over and above the role of diet quality and the many confounding social and demographic factors inherent in economic inequality. Despite the relatively high prevalence of food insecurity in economically developed nations, and the well-established links between food insecurity and poor health outcomes, it has been largely overlooked by behavioral neuroscience research using animal models, which has instead mainly focused on the notion of ubiquitous, easy access to calories. Insecurity itself may amplify or exacerbate aspects of the modern food environment that are already known to promote obesity, and mechanistic studies of those interactions will contribute to a more complete understanding of the obesity epidemic.

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Table 1
Details of feeding amounts[1]

	Schedule		**Total food per 4 days**
	SEC ***every day***	***INSEC*** ***twice each every 4 days***	
CHOW (LabDiet 5001)	4 feedings x 3.8g each (total 15.3 g)[2]	1 omitted and 3 x 3.8g (total 11.4g) 1 omitted and 3 x 6.4g (total 19.2g)	61.2 g/rat (206 kcal)
HFHS (RD12451)	4 feedings x 2.8g each (total 11.1 g)[3]	1 omitted and 3 x 2.8g (total 8.4g) 1 omitted and 3 x 4.6g (total 13.8g)	44.4 g/rat (206 kcal)

[1] All amounts are per rat, with three rats per cage.
[2] This amount represents ~90% of typical daily ad libitum intake observed in age-matched rats.
[3] HFHS rations were calculated to match kcal/day to CHOW group.

Figure Captions

Figure 1: (A) Mean ± SEM total locomotor activity counts during the 13-hr observation period. *$p < 0.05$. Panels (B) and (C) depict those same results separated into consecutive 30-min bins. The solid and dashed regression lines indicate the linear trend for SEC and INSEC rats, respectively. In panels (B) and (C) the times on the X-axis indicate the start time of that 30-minute bin. Dark arrows indicate the customary feeding times (though no food was delivered during activity monitoring sessions). The fourth customary feeding time would have coincided with the end of the 13-hr measurement.

Figure 2: Mean ± SEM total intakes (g) and meal sizes (number of licks) during 8 hrs ad libitum access to a palatable liquid diet. *$p < 0.05$, **$p < 0.01$

Figure 3: Mean ± SEM breakpoints when rats lever-pressed to earn sucrose pellets on a PR-5 schedule. The breakpoint was the last ratio requirement successfully completed before the rat ceased lever pressing for >15 min. Each rat's result is the average of two sessions on consecutive days. *$p < 0.05$

Figure 4: Mean ± SEM Exploration Proportion scores in the Novel Object Recognition test phase. Exploration Proportion is the time spent exploring the novel object as a proportion of time exploring either object. The dashed line at 0.5 indicates equivalent exploration of the familiar and novel objects. A higher proportion indicates better memory for the familiar object. All groups' scores are > 0.5 however no differences between groups are statistically significant.

Figure 5: (A) Mean ± SEM body weights (g) at the beginning (Day 0) and end (Day 8) of an 8-day period of ad libitum access to the HFHS diet. On Day 0 the HFHS groups weighed more than the CHOW groups though there were no differences according to insecurity. After 8 days the HFHS-INSEC group gained more weight than the HFHS-SEC group. (B) Mean ± SEM

proportional weight gain in each group, with each rats' 8-day weight gain expressed as a percentage increase of its Day 0 weight. $*p < 0.05$

Figure 6: Experiment 2. (A) Mean ± SEM body weights (g) during two cycles alternating between 24 hrs food deprivation followed by 8 days ad libitum chow access. Day 0 and Day 1 represent rats' weight at the start and end of the first 24-hr food deprivation. Day 9 represents rats' weights after 8 days of ad libitum chow, at which point the cycle repeated. (B) Mean ± SEM amount of weight lost during each of the two 24-hr food restriction periods. (C) Mean ± SEM amount of weight gained during each of the two 8-day ad libitum access periods. (D) Mean ± SEM of each group's net weight change across the two cycles. $*p < 0.05$, $**p < 0.01$

Figure 1

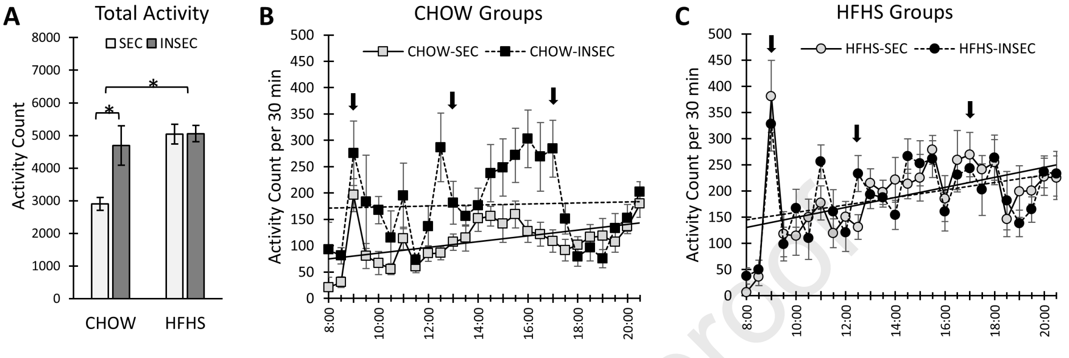

A
Total Activity
SEC INSEC
Activity Count
8000
7000
6000
5000
4000
3000
2000
1000
0
*
*
CHOW
HFHS
B
CHOW Groups
CHOW-SEC
CHOW-INSEC
Activity Count per 30 min
500
450
400
350
300
250
200
150
100
50
0
8:00
10:00
12:00
14:00
16:00
18:00
20:00
C
HFHS Groups
HFHS-SEC
HFHS-INSEC
Activity Count per 30 min
500
450
400
350
300
250
200
150
100
50
0
8:00
10:00
12:00
14:00
16:00
18:00
20:00

Figure 2

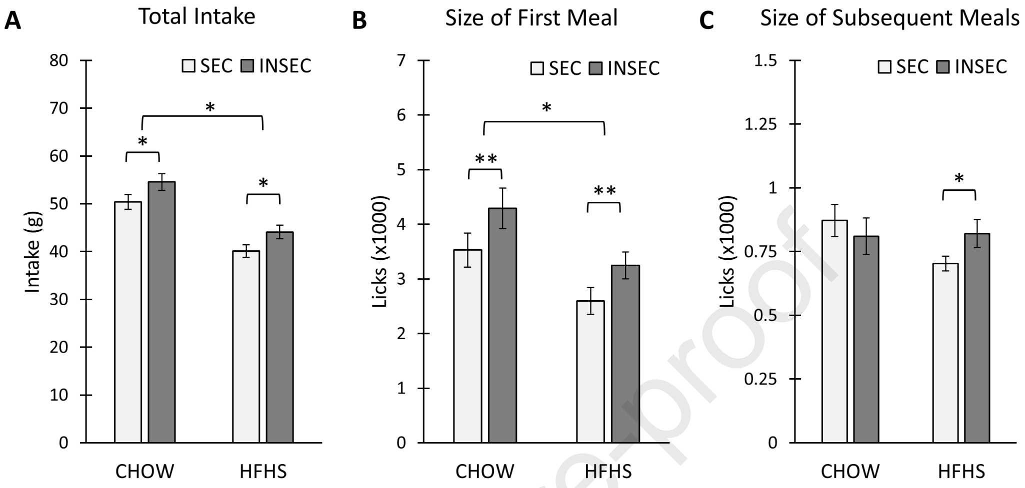

A
Total Intake
□ SEC ■ INSEC
Intake (g)
80
70
60
50
40
30
20
10
0
*
*
*
CHOW
HFHS
B
Size of First Meal
□ SEC ■ INSEC
Licks (x1000)
7
6
5
4
3
2
1
0
*
**
**
CHOW
HFHS
C
Size of Subsequent Meals
□ SEC ■ INSEC
Licks (x1000)
1.5
1.25
1
0.75
0.5
0.25
0
*
CHOW
HFHS

Figure 3

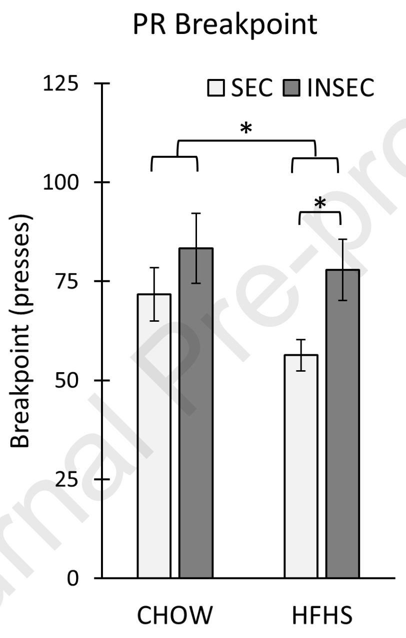

PR Breakpoint
☐ SEC
■ INSEC
125
100
75
50
25
0
Breakpoint (presses)
CHOW
HFHS
*
*

Figure 4

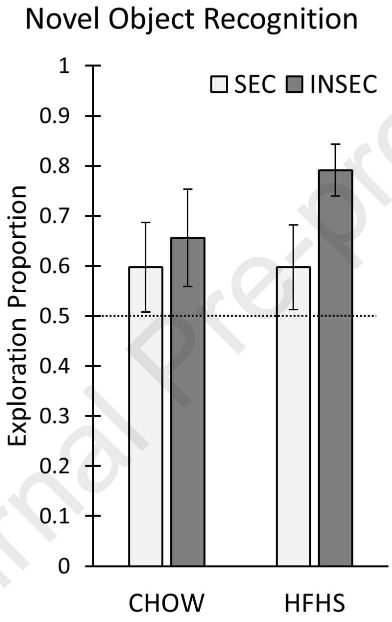
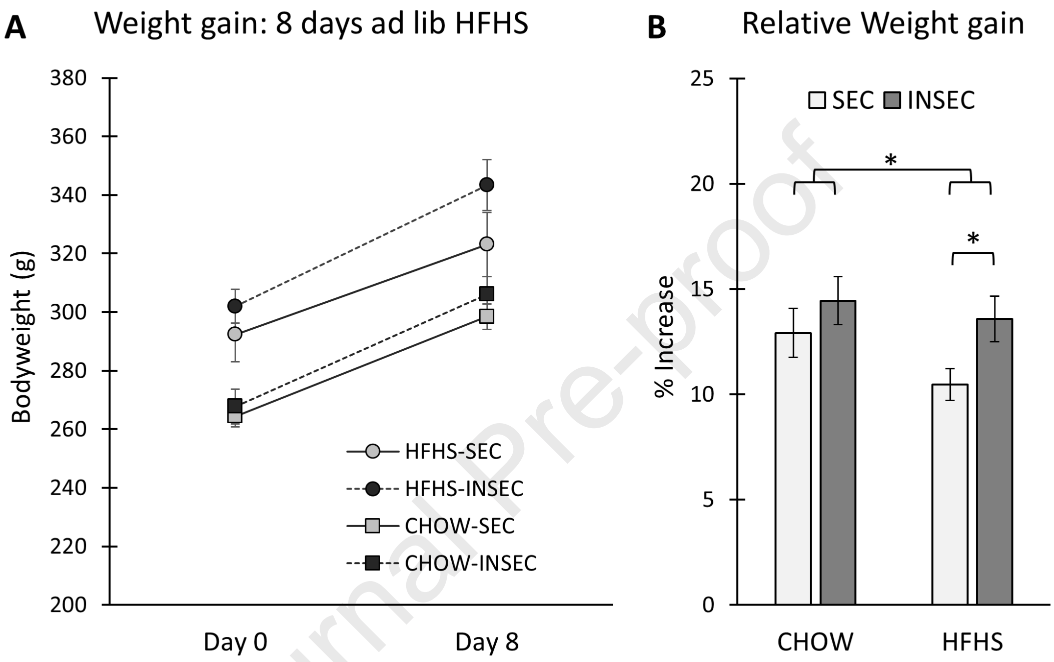

Novel Object Recognition
☐ SEC
▨ INSEC
Exploration Proportion
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
CHOW
HFHS

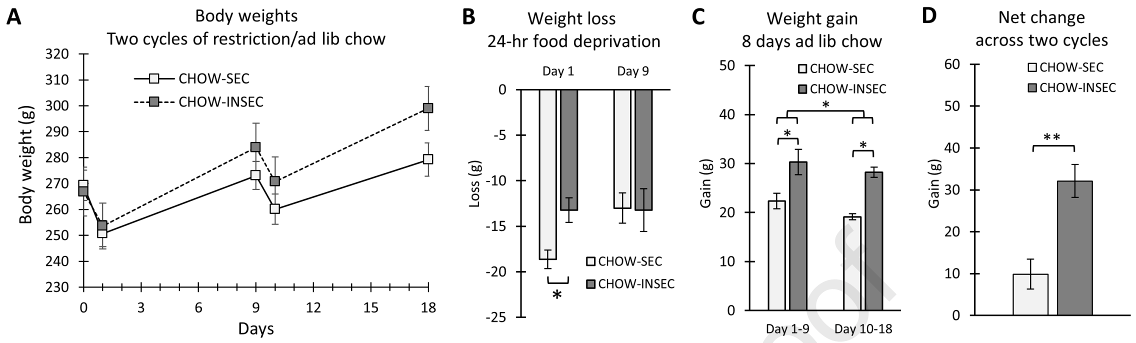
Figure 5



A Weight gain: 8 days ad lib HFHS

B Relative Weight gain

Figure 6


A
Body weights
Two cycles of restriction/ad lib chow
Body weight (g)
320
310
300
290
280
270
260
250
240
230
CHOW-SEC
CHOW-INSEC
0
3
6
9
12
15
18
Days
B
Weight loss
24-hr food deprivation
Day 1
Day 9
Loss (g)
0
-5
-10
-15
-20
-25
*
CHOW-SEC
CHOW-INSEC
C
Weight gain
8 days ad lib chow
Gain (g)
50
40
30
20
10
0
CHOW-SEC
CHOW-INSEC
*
*
*
Day 1-9
Day 10-18
D
Net change
across two cycles
Gain (g)
60
50
40
30
20
10
0
CHOW-SEC
CHOW-INSEC
**

Ethical disclosures for the paper “*Chronic experience with unpredictable food availability promotes food reward, overeating, and weight gain in a novel animal model of food insecurity*” by Kevin P. Myers, Marta Majewski, Dominique Schaefer, and Alexis Tierney:

- All animal experiments were approved by the Bucknell University IACUC and consistent with the *NIH Guide for the Care and Use of Laboratory Animals, 8th edition*. A statement attesting to this is included in the article Methods section per journal requirements.
- The authors declare no conflicts of interest, financial or otherwise.
- This is an original work by the named authors. It has not been previously published nor is it under consideration for publication elsewhere.