Behavioural Brain Research 317 (2017) 163–178

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

The impact of a junk-food diet during development on 'wanting' and 'liking'

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HIGHLIGHTS

- Junk-food (JF) exposure during development reduces weight gain in two strains of rats.
- JF-induced weight gain has opposite effects on cue attraction in males and females.
- JF gainers work harder for cues and are more attracted to a JF context.
- JF exposure in Long-Evans, but not Sprague-Dawley rats, blunts sucrose 'liking'.
- JF exposure reduces anxiety-like behavior in males, but not females.

ARTICLE INFO

Article history:
Received 5 August 2016
Received in revised form
15 September 2016
Accepted 16 September 2016
Available online 17 September 2016

Keywords:
'Wanting'
'Liking'
Incentive salience
Junk-food
Obesity
Motivation

ABSTRACT

The global increase in obesity rates has been tied to the rise in junk-food availability and consumption. Increasingly, children are exposed to a junk-food diet during gestation and early development. Excessive consumption of junk-food during this period may negatively impact the development of brain motivation and reward pathways. In this study we investigated the effects of a chronic junk-food diet throughout development on cue-motivated behavior ('wanting'), hedonic 'liking' for sweet tastes, as well as anxiety and weight gain in male and female Long-Evans (LE) and Sprague-Dawley (SD) rats. Here we found that chronic exposure to a junk-food diet resulted in large individual differences in weight gain (gainers and non-gainers) despite resulting in stunted growth as compared to chow-fed controls. Behaviorally, junk-food exposure attenuated conditioned approach (autoshaping) in females, particularly in non-gainers. In contrast, junk-food exposed rats that gained the most weight were willing to work harder for access to a food cue (conditioned reinforcement), and were more attracted to a junk-food context (conditioned place preference) than non-gainers. Hedonic 'liking' reactions (taste reactivity) were severely blunted in LE, but not SD rats, and 'liking' for sucrose negatively correlated with greater weight gain. Finally, junk-food exposure reduced anxiety-like behavior (elevated plus maze) in males but not females. These results suggest that junk-food exposure during development may give rise to dissociable differences in 'liking' and 'wanting' neural systems that do not depend on weight gain and may not be detected through Body Mass Index monitoring alone.

1. Introduction

Obesity is a global health risk, and the rapid escalation of its prevalence suggests shifting environmental factors may have a role in its growth. As of 2012, over 15% of children and over 30% of adults in the United States are obese, while another 30% of the population is overweight [1]. These numbers are representative of a growing obesity epidemic [1–3]. The growing accessibility of inexpensive processed foods and their increasingly pervasive advertising may play a role in this alarming trend [4,5]. Many of these processed foods are saturated with sugar, salt, and fat. Yet they lack adequate protein and other nutrients that are important for day-to-day health and normal growth and development, categorizing them as "junk-food". In countries with high and rising obesity rates, daily

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http://dx.doi.org/10.1016/j.bbr.2016.09.041
0166-4328/

food intake is not exclusively driven by hunger or energy demand. It is suggested that for some individuals, the increased palatability and accessibility of junk-food has seized neural reward and motivation mechanisms and turned food-seeking into errant food craving, which may lead to diet-induced obesity [6,7].

When a new food is first ingested, its sensory qualities may trigger sensations of hedonic pleasure and 'liking', which in turn promote 'wanting' to consume that food again [6,8]. With repeated exposure, however, the environmental cues associated with the junk-food may gain more motivating power and incentive value. The salience of cues associated with food is facilitated through activity in mesocorticolimbic systems, which makes rewards and their cues desired and 'wanted' [9–11]. The neural systems for 'liking' and 'wanting' typically function in close synchrony, but data show that they can be changed independently. For example, with repeated consumption of a reward, such as palatable junk-food, 'wanting' becomes sensitized [11,12]. Sensitization of 'wanting' was first described in the 'incentive sensitization' theory of addiction, and can result in a dissociation of 'wanting' and 'liking' that leads to strong feelings of desire for particular rewards and their cues, despite no increase and sometimes a reduction in 'liking' [13]. Although it was initially applied to drugs and their cues, recent evidence suggests this theory also applies to food cues. Food cues can play a similar role by triggering visual attention and enhancing the desire to eat [14–17], particularly in obese individuals who might be hyper-responsive to the motivational properties of these cues [18–20].

However, susceptibility to (incentive) sensitization appears to show a large degree of individual variation, with marked sex differences [21]. For example, there is evidence for individual variation in the level of attraction and motivation to junk-food cues [12,22,23]. In particular, we recently demonstrated that animals that gained excessive weight on a junk-food diet (gainers) displayed greater cue-induced approach to food cues even before gaining access to the diet [12], and were also more willing to work for the presentation of those cues (conditioned reinforcement) after obesity onset. However, many of these studies were carried out in adults. The current ease of access and high palatability of these foods means that exposure to a junk-food diet may begin as early as childhood or even prior to birth through the mother's diet.

Childhood obesity has been implicated as a cofactor in a number of lifetime diseases such as depression, anxiety, diabetes, elevated blood pressure, orthopedic problems, and pulmonary complications [24–26], and has been associated with early mortality [27,28]. Previous studies have shown that a mother's diet during pregnancy alters the protein make-up of the offspring's cerebral cortex despite cross-fostering [29], while also producing changes in dopaminergic activity [30]. Developmental perspectives on the obesity epidemic are necessary to understand the increasing prevalence of childhood obesity across generations [1,25], and dissociations between 'liking' and 'wanting' could have a lasting impact when occurring within the plastic neural networks of a maturing brain. However, it is currently unclear whether overconsumption of junk-food is related to distortions of either 'liking' or 'wanting', or both, when exposure begins prenatally.

Here we examined the effect of lifetime exposure to junk-food on 'wanting' by measuring the degree to which food cues 1) elicit approach (autoshaping), 2) reinforce operant responding (conditioned reinforcement), and 3) by determining the attraction of a junk-food paired context (conditioned place preference). We also measured the impact of lifetime exposure to a junk-food diet on hedonic orofacial 'liking' reactions, using taste reactivity measures [31] in response to sucrose. In addition, since anxiety is often associated with increased consumption of fatty-sugary foods [32], we also evaluated individual differences in the impact of junk-food on levels of anxiety-like behavior using the elevated plus maze. Finally there are marked sex differences in the motivation for food [33,34]. Recent findings also show strain and sex differences for spatial learning [35,36], behavior toward unfamiliar foods [37] and metabolic responses [38]. Therefore measures of 'wanting', 'liking' and anxiety were determined in males vs. females, across two strains of rats, Long-Evans and Sprague-Dawley.

2. Materials & methods

2.1. Subjects

Long-Evans (LE) and Sprague-Dawley (SD) rats were bred in-house from breeding pairs purchased from both Harlan and Charles River. Rats were housed on a 12:12 h reverse light/dark cycle and had ad-lib access to food and water unless stated otherwise. All procedures were approved by the Institutional Animal Care and Use Committee for Wesleyan University.

2.2. Diet

Adult male and female rats were placed on either a standard chow and junk-food (JF) or a control diet (C; Teklad Rodent Lab Diet 2018 in pellet form; Envigo: 18.6% protein, 6.2% fat, 44.2% carbohydrates; 3.1 kcal/g) for seven days prior to assigning C and JF breeding pairs (Fig. 1A). The junk-food diet was a mash composed of a blend of Chips Ahoy chocolate chip cookies (260 g), Ruffles potato chips (80 g), Jiff creamy peanut butter (260 g), Nesquik chocolate flavored powder (260 g), powdered Rodent Lab Diet 2018 (400 g; 14% protein, 19.6% fat, 58% carbohydrates; 4.5 kcal/g), and water (355 ml). These foods contain a rich mix of sugars, salt, and fats, and were chosen as palatable representatives of what are commonly called 'junk-foods' implicated in human obesity. Breeding pairs were maintained on their designated diet (C or JF) until weaning of offspring (postnatal day (PND) 21). Litters were culled to a total of ten (five female; five male) and maintained on the same diet as their parents throughout the experiment, thus creating two groups: a control group that received only standard chow (C: N = 30: LE M/F = 10/10, SD M/F = 7/3), and an experimental group that received junk-food in addition to standard chow throughout gestation, post weaning and throughout the remainder of the experiment (JF: N = 60: LE M/F = 23/17, SD M/F = 8/12). After weaning, rats were housed by sex in groups of two or three. All tests were carried out in red light conditions during the dark cycle unless otherwise stated. Testing began at 12–14 weeks of age (i.e., adulthood). Animals were weighed once per week before weaning and twice per week post-weaning.

2.3. Autoshaping/conditioned reinforcement/extinction

2.3.1. Apparatus

All procedures were conducted in standard Med-Associates chambers equipped with two retractable levers (one CS (illuminated), one control) located on the front wall on either side of a recessed food cup, which delivered 45 mg sucrose pellets (Test-Diet). A speaker located at the top of the chamber delivered a 2.9 kHz tone. For the conditioned reinforcement session, the back wall was outfitted with nose-poke holes (one active, one inactive, location counterbalanced) located on either side of a central retractable lever. During this time the food cup on the front wall was covered with a custom metal plate. Med-PC software automatically collected lever responses, nose pokes, and food cup entries for all sessions. Chambers were placed in sound attenuating cabinets to reduce ambient light and noise. Red LED house lights were mounted to the wall of the cabinet and were turned on during all sessions.

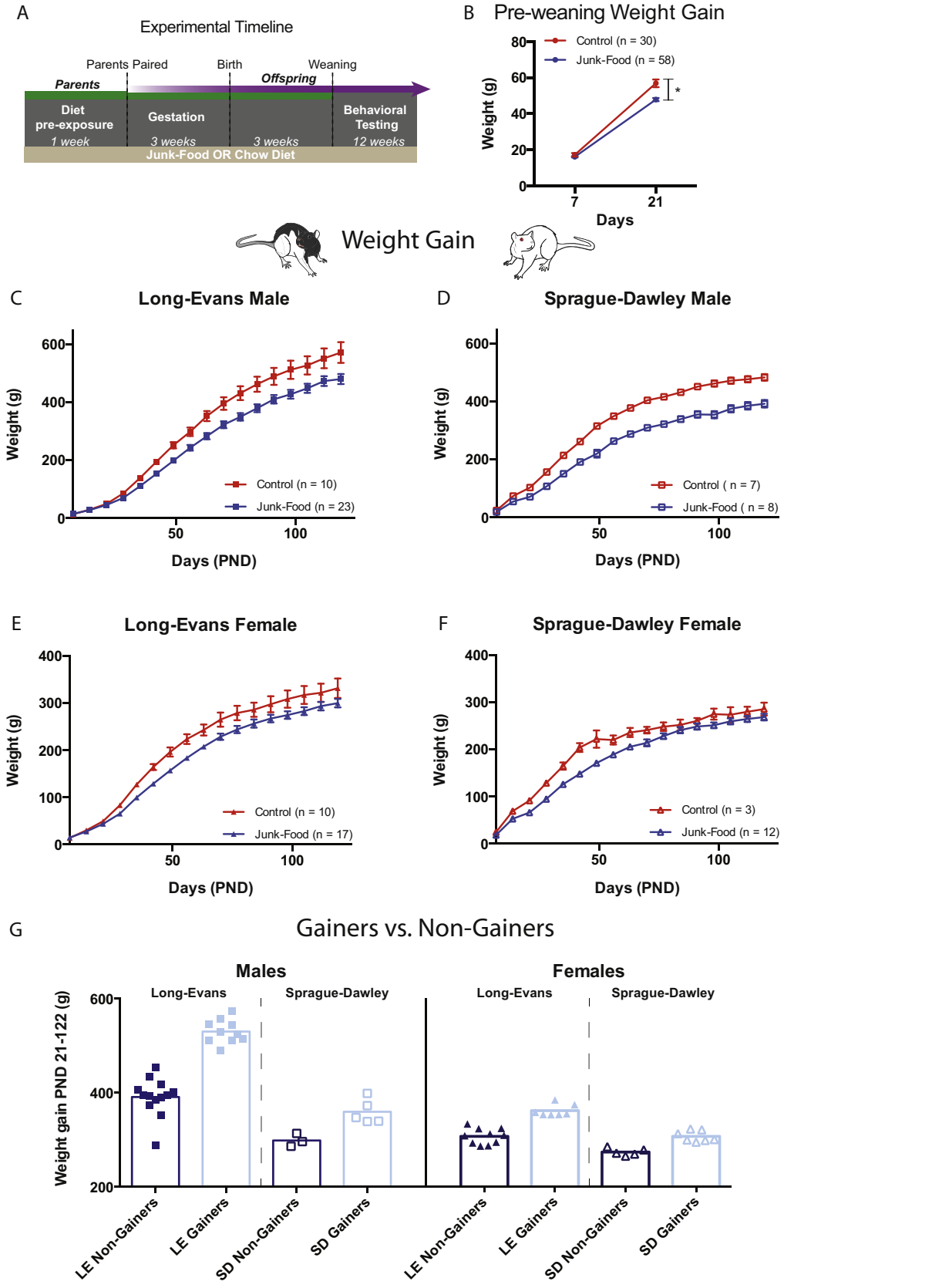

Fig. 1. Chronic exposure to junk-food during development reduces weight gain. (A) Timeline for junk-food exposure during development. (B) The impact of diet (chow or junk-food) on weight gain at day 7 and 21 postnatal. (C–F) Weight gain for male and female Long-Evans and Sprague-Dawley rats. (G) Weight gain for junk-food gainers and non-gainers across strains and sex.

2.3.2. Autoshaping procedure

Prior to testing, animals (JF: N = 34, M/F = 19/15; C: N = 16, M/F = 8/8) were exposed to sucrose pellets in their home cages for two days to reduce neophobia, followed by magazine training (30 UCS sucrose pellets; VI-45). Subsequent Pavlovian autoshaping training was conducted as previously described [12]. In brief, each session consisted of 30 CS presentations (extended illuminated lever + tone; VI-60) each lasting 8 s, immediately followed by delivery of one sucrose pellet, regardless of behavior. A control lever (no programmed consequence) was present throughout each session. Animals initially received nine days of Pavlovian autoshaping during which time lever presses and food cup entries were measured during cue presentation. Although reward delivery is non-contingent on the animal's behavior, animals typically interact (e.g. licking, biting, pressing) with the predictive lever (sign-tracking) or the location of food delivery (goal-tracking), which may be quantified as a measure of the incentive salience attributed to that cue, and reveal individual differences in cue attraction [39]. An animal was classified as a sign-tracker if it performed 3 times more lever press responses than food cup entries (lever presses $\geq$ 66% of lever presses + food cup entries) and a goal-tracker if it performed 3 times more food cup entries than lever responses during the CS presentations of the last day (Day 9) of Pavlovian autoshaping. An individual was classified as an intermediate if it directed between 33% and 66% of its responses to either the lever or the food cup [40]. All animals developed a conditioned response after initial training.

2.3.3. Conditioned reinforcement procedure

Following autoshaping, animals underwent a test for conditioned reinforcement (one session, 30 min) in order to measure to what extent the CS could act as a reinforcer in the absence of reward. A nose-poke into the active hole resulted in a 3-s presentation of the reward-related cue (lever + tone). A nose-poke into the inactive hole had no programmed consequence.

2.3.4. Extinction procedure

After conditioned reinforcement, animals were given three additional autoshaping training sessions (as described above) and extinction of conditioned responding was assessed. Extinction was identical to the autoshaping sessions (30 CSs), except that pellets were never delivered throughout the entire session.

2.4. Conditioned place preference

2.4.1. Apparatus

The apparatus was composed of two 29 × 29 × 31 cm chambers: one with black dots on white walls (box A) and one with black and white striped walls (box B; see Fig. 5A). The amount of black and white was matched for each context. The two conditioning compartments were separated by a third smaller connecting chamber (box C) that could be closed off by a guillotine door.

2.4.2. Procedure

To reduce the risk of neophobia, animals (LE: JF: N = 40, M/F = 23/17; C: N = 20, M/F = 10/10; SD: JF: N = 20, M/F = 8/12; C: N = 10, M/F = 7/3) in each group were exposed to 15 g of either standard chow or junk-food in their home cage for two consecutive days before testing. The CPP procedure consisted of three phases: pre-exposure (one day), conditioning (three days in each compartment in alternation, order counterbalanced, with 48 h between each day), and testing (two test days, separated by 48 h)[41]. During pre-exposure, animals were introduced via the small connecting chamber and allowed to explore the entire apparatus for twenty minutes. During conditioning, each animal was restricted to a single chamber on alternating days. Junk-food was always paired with the least preferred context (based on each animal's pre-exposure), and standard chow was paired with the preferred context. Following conditioning, rats were tested under conditions identical to pre-exposure on two separate days: once under homeostatic ad lib conditions and once under hunger conditions, with one day of food deprivation in between the tests. Behavior was recorded via an infrared camera placed above the apparatus.

2.5. Taste reactivity

2.5.1. Apparatus

The taste reactivity apparatus was composed of a plexiglass chamber (26.5 × 26.5 × 40.5 cm) with a plexiglass floor and an angled mirror below it to reflect the animal's orofacial reactions.

2.5.2. Surgery

To permit oral infusion of sucrose solutions, rats were anesthetized with ketamine (100 mg/kg, IP) and xylazine (7 mg/kg, IP), given atropine (0.04 mg/kg, IP) to protect respiration, and surgically implanted with bilateral intraoral guide cannulas. Oral cannulae entered the mouth in the upper cheek pouch lateral to the first maxillary molar, ascended beneath the zygomatic arch, and exited the skin at the dorsal head cap [31]. Animals were post-operatively treated with the antibiotic cefazolin (75 mg/kg, SC) and carprofen (5 mg/kg, SC) as an analgesic, and allowed 7 days to recover. Cannulas were checked for patency and cleaned with water every other day following surgery.

2.5.3. Procedure

On the first day, animals (JF: N = 39, M/F = 21/18; C: N = 18, M/F = 10/8) were allowed five minutes to explore the apparatus, followed by 1–2 days of habituation to the pump delivering water (1 ml/mn) remotely into the rat's mouth through oral cannula. Following habituation, orofacial reactions to water were recorded to obtain a baseline score. For the following three days, animals received either a 1%, 3%, or a 9% sucrose solution in random order. Orofacial reactions of appetitive 'liking', neutral, and aversive reactions were video recorded on each session for later analysis [31,42].

2.6. Elevated plus maze

The elevated plus maze (EPM) consisted of four arms measuring 40 cm in length and 15 cm in width, raised 97 cm above the ground. Two arms were "closed," restricted by walls of 40.5 cm in height on all three sides, and two arms were "open," with no walls. Each arm was located across from its matching arm with a 15 × 15 cm intersection in the middle of the maze.

Each animal (LE: JF: N = 40, M/F = 23/17; C: N = 20, M/F = 10/10; SD: JF: N = 20, M/F = 8/12; C: N = 10, M/F = 7/3) was introduced into the maze by being placed in the intersection facing the closed arm, and was allowed a single 15 min trial to explore the maze. Behavior was recorded via an infrared camera placed above the maze.

2.7. Video analysis & statistics

Behavior for the CPP, EPM, and taste reactivity tests were manually video scored by a scorer blind to experimental conditions. For the CPP, an animal was considered "in" a chamber based on head position [43]. For the EPM, an animal was considered "in" an arm if all four paws were in the arm. For the taste reactivity tests, the first sixty seconds of visible reactions were scored for each session. The reactions were categorized as "hedonic" (e.g. rhythmic and lateral tongue protrusions, paw licking), "neutral" (e.g. drinking, groom-

ing), or "aversive" (e.g. headshakes, forelimb flails, gapes), and were scored using frame-by-frame digital analysis [12,42].

Analyses between three or more groups were carried out using Repeated Measures ANOVA followed by post-hoc tests or One-Way ANOVA (SPSS). Analyses between two groups were carried out using two-tailed paired and unpaired *t*-tests. Significance level was set at $p < 0.05$. K-means clustering based on weight gain from postnatal day 21 to 122 for animals exposed to a junk-food diet was used to identify individuals susceptible (JF-Gainer) vs resistant to diet-induced obesity (JF-Non-Gainer) as in [12,44,45]. This statistical method provides an unbiased separation that can be applied uniformly across studies [46].

3. Results

3.1. Weight gain and food intake

There were no differences in weight on postnatal day (PND) 7 between offspring born to mothers maintained on a control (C; standard chow) or junk-food (JF) diet during gestation and weaning (Day 7: $F_{(1,87)} = 1.669$, $p = 0.200$; Fig. 1B). However, although both groups gained weight over time (Effect of Day: $F_{(1,86)} = 3343.184$, $p = 0.000$), they did so at different rates (Day by Diet: $F_{(1,86)} = 41.225$, $p = 0.000$). Surprisingly, by PND 21, body weight was on average 15% lower in offspring from the junk-food group than in chow-fed controls (Day 21: $F_{(1,87)} = 21.810$, $p = 0.000$). This difference in body weight was maintained for the duration of the experiment regardless of gender or strain (Junk-Food vs Chow: Male LE: $F_{(1,31)} = 9.497$, $p = 0.004$; Female LE: $F_{(1,23)} = 7.738$, $p = 0.011$; Male SD: $F_{(1,13)} = 71.42$, $p = 0.000$; Female SD: $F_{(1,13)} = 14.112$, $p = 0.002$; Fig. 1C–F), although males gained more weight than females ($F_{(1,86)} = 104.336$, $p = 0.000$). There were no strain differences in weight gain in males or females, although female Long-Evans trended towards displaying greater body weight than Sprague-Dawleys (Females: $F_{(1,38)} = 3.924$, $p = 0.055$; Males: $F_{(1,46)} = 0.542$, $p = 0.465$). The reduced weight gain in junk-food-fed as opposed to chow-fed animals occurred despite no overall difference in food consumption (PND 21–77: $F_{(1,66)} = 0.54$, $p = 0.465$). In fact, junk-food-fed animals consumed on average 5% more food than chow-fed animals (Mean = 105.16%, SE = 4.35). It is therefore unlikely that the difference in total weight gain could be explained by differences in the amount of food intake, and instead may result from differences in macronutrient content of the two diets, notably a lower level of protein in the junk-food diet (14% vs. 18.6%), which could in turn alter metabolism.

By the end of the study, when animals were four months old, junk-food-fed males still possessed a lower body weight than their chow-fed counterparts (Junk-Food vs Chow: Male LE: $F_{(1,32)} = 6.718$, $p = 0.014$; Male SD: $F_{(1,14)} = 27.697$, $p = 0.000$). In contrast, females had achieved a similar weight as their chow-fed counterparts (Junk-Food vs Chow: Female LE: $F_{(1,24)} = 3.073$, $p = 0.093$; Female SD: $F_{(1,14)} = 1.184$, $p = 0.296$).

Animals exposed to a junk-food diet were separated by sex and strain into 'gainers' (JF-G) and 'non-gainers' (JF-NG) based on individual weight gain from PND 21 to PND 122. JF-Gainers (N = 22) had significantly greater body weight than the JF-Non-Gainers (N = 30) as early as PND 7 ($F_{(1,57)} = 4.654$, $p = 0.035$), and showed overall greater body weight over the course of the study ($F_{(1,56)} = 14.09$, $p = 0.000$) regardless of strain or gender (F's > 13.308, $p < 0.012$; Fig. 1G). Animals were group housed to reduce stress and anxiety making it impossible to establish individual levels of junk-food consumption. However it has previously been shown that gainers tend to eat more than non-gainers [44] (personal communication CRF).

3.2. Autoshaping

To examine the impact of diet on the degree of attraction to reward-related cues, animals underwent 9 days of Pavlovian Conditioned Approach training (autoshaping), where an 8 s lever + tone conditioned stimulus (CS) predicted delivery of a sucrose pellet into a food cup.

3.2.1. Sign-tracking vs. goal-tracking

During the presentation of a food cue, individual animals differ in their tendency to direct attention to either the predictive lever + tone CS (sign-tracking) or the food cup where the reward will be delivered (goal-tracking), or in some instances, a combination of the two (intermediates) [39]. There was a notable difference between strains, whereby Sprague-Dawley rats showed a greater propensity towards sign-tracking than Long-Evans (Effect of Strain: $F_{(1,79)} = 12.454$, $p = 0.001$; Fig. 2A), but no effect of diet or sex (Diet: $F_{(1,79)} = 0.017$, $p = 0.898$; Gender: $F_{(1,79)} = 0.586$, $p = 0.446$). In fact, no Sprague-Dawley rats displayed a goal-tracking phenotype. Furthermore, among those exposed to a junk-food diet, development of a gainer/non-gainer phenotype did not appear to predict or influence whether an animal was more likely to display sign- or goal-tracking behavior (G vs. NG: $F_{(1,53)} = 0.125$, $p = 0.726$). Due to these strain differences, and in the absence of any diet differences in ST/GT phenotype, Pavlovian autoshaping data was collapsed, using total conditioned approach behaviors (i.e. the sum of CS induced lever presses and food cup entries) to evaluate effects of a junk-food diet on behavior.

3.2.2. Junk-food reduces cue attraction, primarily in Long-Evans

Animals exposed to a junk-food diet displayed blunted attraction to the sucrose predictive lever cue and food cup (Diet: $F_{(1,78)} = 6.00$, $p = 0.017$; Fig. 2B). However, this effect did not appear to be uniform across strains and seemed to be driven by the Long-Evans. Specifically, exposure to a junk-food diet reduced conditioned approach behaviors in LE (Diet: $F_{(1,48)} = 11.517$, $p = 0.001$; Fig. 2C), but not SD (Diet: $F_{(1,28)} = 0.905$, $p = 0.350$; Fig. 2D) rats.

3.2.3. Junk-food blunts cue 'wanting' in females

When maintained on a chow diet, female rats showed significantly greater approach behavior than their male counterparts ($F_{(1,24)} = 7.238$, $p = 0.013$; Fig. 3A–B). However, exposure to a junk-food diet significantly lowered levels of approach behavior in junk-food females (Female JF vs. C: $F_{(1,36)} = 6.848$, $p = 0.013$; Fig. 3B) to a level similar to that of their male counterparts (JF Female vs. C Male: $F_{(1,52)} = 0.402$, $p = 0.529$). In fact, females fed a junk-food diet failed to increase their conditioned responding with training (Female JF: $F_{(8,200)} = 1.751$, $p = 0.089$), unlike chow-fed females (Female C: $F_{(8,80)} = 3.017$, $p = 0.005$).

3.2.4. Weight gain has opposite effects on cue attraction in males and females

When animals were clustered by gainer/non-gainer phenotype, there were no overall differences between them in conditioned approach behavior across strains (G/NG: $F_{(1,52)} = 0.034$, $p = 0.854$). However, in males exposed to a junk-food diet, gainers showed a reduction in approach behavior when compared to controls and non-gainers (G vs. C: $F_{(1,25)} = 4.3$, $p = 0.049$; G vs. NG: $F_{(1,25)} = 0.4.305$, $p = 0.048$; NG vs. C: $F_{(1,28)} = 0.037$, $p = 0.849$; Fig. 3C). In contrast, in females the impact of gainer phenotype on approach behavior was reversed. The non-gainers displayed a significant decrease in approach behavior compared to controls (NG vs. C: $F_{(1,24)} = 8.378$, $p = 0.008$; Fig. 3D), although here there was no difference when compared to the gainers (NG vs. G: $F_{(1,25)} = 2.071$, $p = 0.163$).

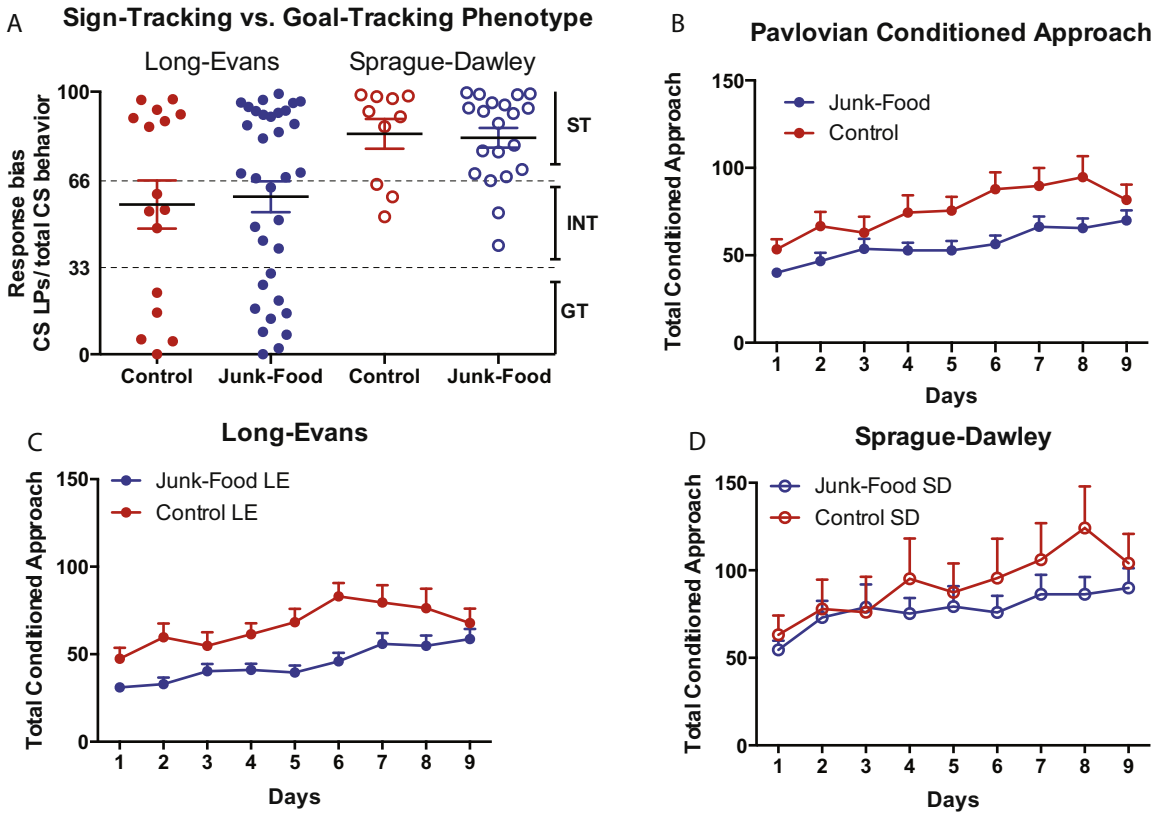

Fig. 2. Chronic exposure to a junk-food diet reduces conditioned approach behavior, particularly in Long-Evans. A) Distribution of sign-trackers and goal-trackers in Long-Evans and Sprague-Dawley rate exposed to chow or junk-food. B–D) Total conditioned approach behavior during CS presentation for all animals (B), Long-Evans (C), and Sprague-Dawley (D) rats exposed to junk-food or chow across 9 days of autoshaping. Data are Mean and SEM.

3.3. Conditioned reinforcement

Following Pavlovian autoshaping, animals were given the opportunity to learn a new instrumental behavior (nose-poking) to gain momentary access to a brief three-second presentation of the CS (lever + tone) that had previously been paired with food delivery. By selectively responding in the active (rather than inactive) nose-poke hole, resulting in CS presentation, but no sucrose UCS delivery, animals demonstrated the degree to which the CS had acquired conditioned reinforcing properties and is itself attractive in the absence of a UCS. Overall, rats displayed conditioned reinforcement by preferentially making more active than inactive nose-pokes ($t_{(74)} = 4.815$, $p = 0.000$). This preference occurred irrespective of diet (JF: $t_{(53)} = 3.844$, $p = 0.000$; Control: $t_{(20)} = 2.947$, $p = 0.008$; Fig. 4A), and the degree of preference for the active hole was not affected by diet (Nose poke x Group: $F_{(1,73)} = 0.029$, $p = 0.866$), nor did it influence overall levels of responding (Diet: $F_{(1,73)} = 2.356$, $p = 0.129$).

3.3.1. Gainers, but not non-gainers, will work harder for food cues

When junk-food fed rats were examined by diet-induced phenotype, gainers displayed a significant preference for the active nose-poke (Gainers: $t_{(23)} = 4.080$, $p = 0.000$; Fig. 4B), similar to chow-fed control rats, and the effect was the same in both strains (SD G: $t_{(11)} = 2.249$, $p = 0.046$; LE G: $t_{(11)} = 4.045$, $p = 0.002$; Fig. 4B). However, the non-gainers overall failed to preferentially respond on the active nose-poke (Non-gainers: $t_{(29)} = 1.932$, $p = 0.063$) although this was largely due to a lack of effect specifically in the SDs (SD NG: $t_{(7)} = 0.129$, $p = 0.901$; LE NG: $t_{(21)} = 2.306$, $p = 0.031$). Attribution of conditioned reinforcing properties to the CS in gainers occurred despite lower levels of general activity when compared to controls (G vs. C: $F_{(1,42)} = 5.836$, $p = 0.020$). In fact, Sprague-Dawley rats performed overall more nose-pokes than Long-Evans rats (Strain: $F_{(1,63)} = 13.856$, $p = 0.000$), as measured by total nose-poking performance, suggesting a greater level of activity.

3.4. Extinction

3.4.1. All animals are sensitive to changes in reward contingency, irrespective of diet

Following conditioned reinforcement, animals underwent three days of Pavlovian autoshaping training before being tested under extinction conditions (CS no UCS). This allowed examination of the persistence of the CS attraction when the UCS reward was omitted. A single extinction session consisting of 30 non-reinforced CS presentations showed that extinction resulted in a decrease in conditioned approach behavior (Extinction: $F_{(1,78)} = 23.598$, $p = 0.000$) that was similar between diet conditions (Extinction x Diet: $F_{(1,78)} = 2.544$, $p = 0.115$; Fig. 5A), suggesting that animals were sensitive to the change in reward conditions irrespective of diet. The same was true when animals were split by their propensity to gain weight (Non-Gainers: $t_{(29)} = 2.212$, $p = 0.035$; Gainers: $t_{(23)} = 3.357$, $p = 0.003$; Fig. 5B).

3.5. Conditioned place preference

3.5.1. Junk-food diet attenuates the attraction of a junk-food paired context

Animals were given three conditioning pairings where one of two distinct contexts was paired with a palatable junk-food treat, while the opposing context was paired with access to standard chow. Their preference was then assessed on two separate occasions in the absence of any food reward, first under homeostatic

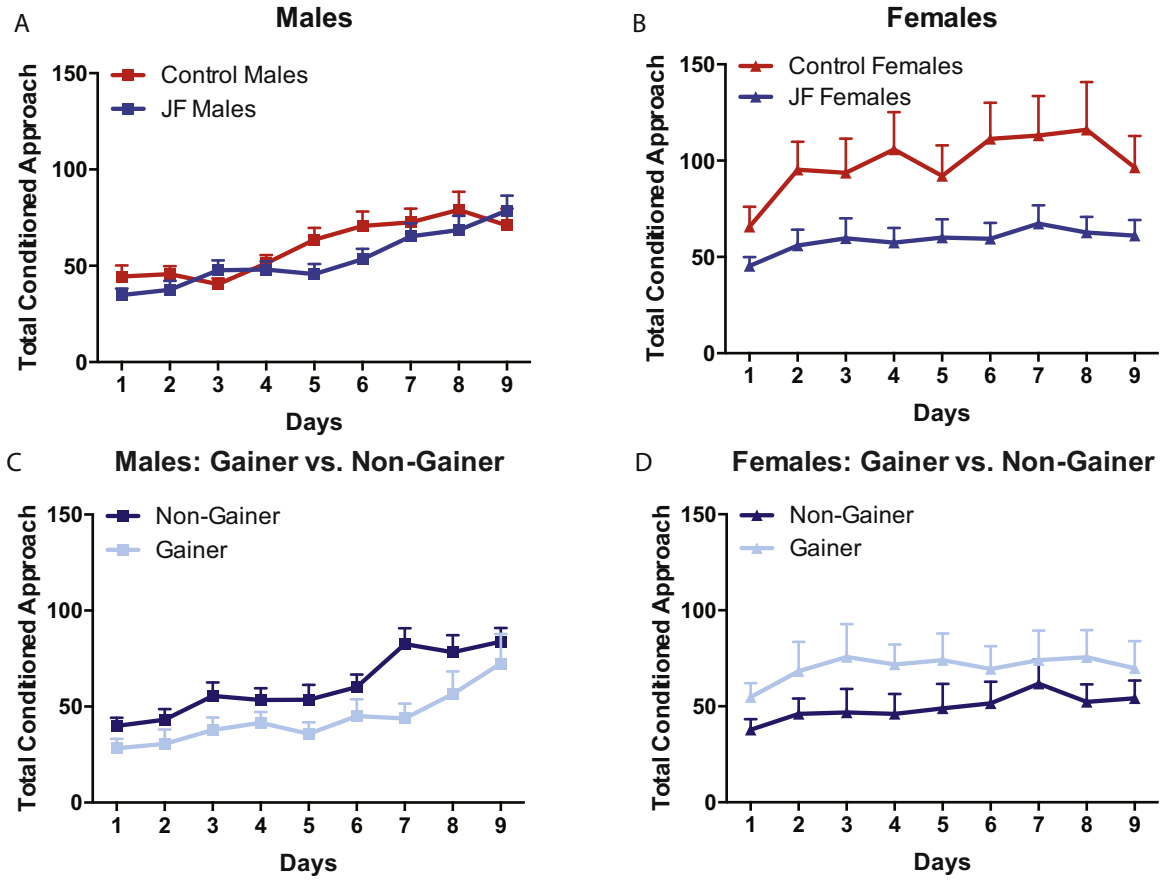

Fig. 3. Junk-food reduces conditioned approach behavior in females but not males. Conditioned approach behavior during autoshaping for male (A) and female (B) rats chronically exposed to chow or junk-food. Conditioned approach behavior for male (C) or female (D) junk-food gainer and non-gainer rats chronically fed a junk-food diet. Data are Mean and SEM.

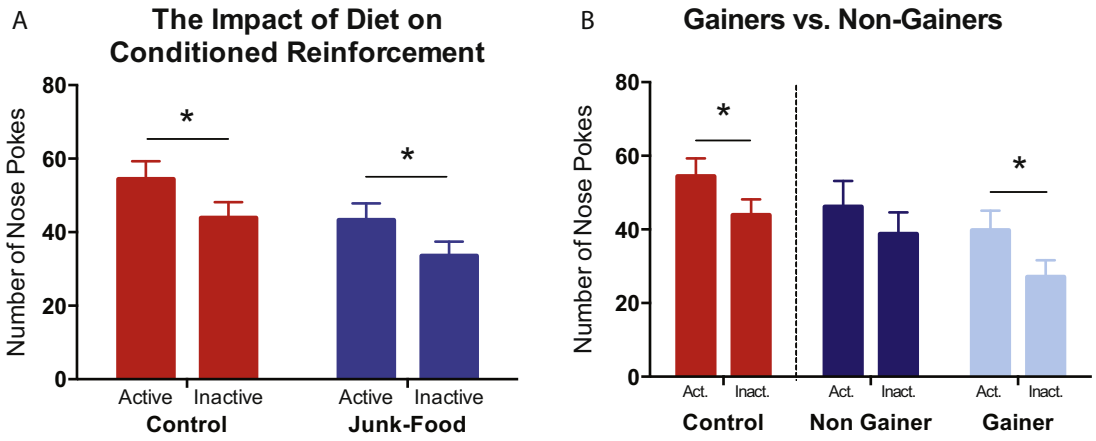

Fig. 4. Non-gainers fail to show conditioned reinforcement for a food cue. (A) The impact of a junk-food or chow diet on active or inactive nose pokes (conditioned reinforcement) for a food-paired cue. (B) The degree of conditioned reinforcement for gainers and non-gainers. Data are Mean and SEM, * $p < 0.05$.

conditions (ad-lib food access), and then 48 h later following 36 h of food deprivation to assess the impact of hunger on cue attraction (Fig. 6A).

During conditioning, animals ate progressively more food each day (Day: $F_{(2,164)} = 47.649$, $p = 0.000$; Fig. 6A), and there was a strong preference for the junk-food (Food Type: $F_{(1,82)} = 144.635$, $p = 0.000$). This was specifically the case for chow-fed animals that consumed more food overall (Diet: $F_{(1,82)} = 72.168$, $p = 0.000$) and displayed a strong preference for the junk-food diet over chow (Food Type: $F_{(1,26)} = 87.003$, $p = 0.000$). Junk-food-fed animals also consumed more junk-food than chow (Food Type: $F_{(1,50)} = 29.024$, $p = 0.000$), however there was no difference in the amount of junk-food consumed between gainers and non-gainers (GNG: $F_{(1,52)} = 0.026$, $p = 0.873$).

Contextual cues repeatedly paired with a palatable junk-food treat became attractive and made animals spend more time in that environment (Context: $F_{(1,89)} = 34.817$, $p = 0.000$). Both control and junk-food exposed rats showed a preference for the junk-food paired context on both tests (Pre-exposure – Test: t's > 3.628, $p < 0.002$), but this preference was not

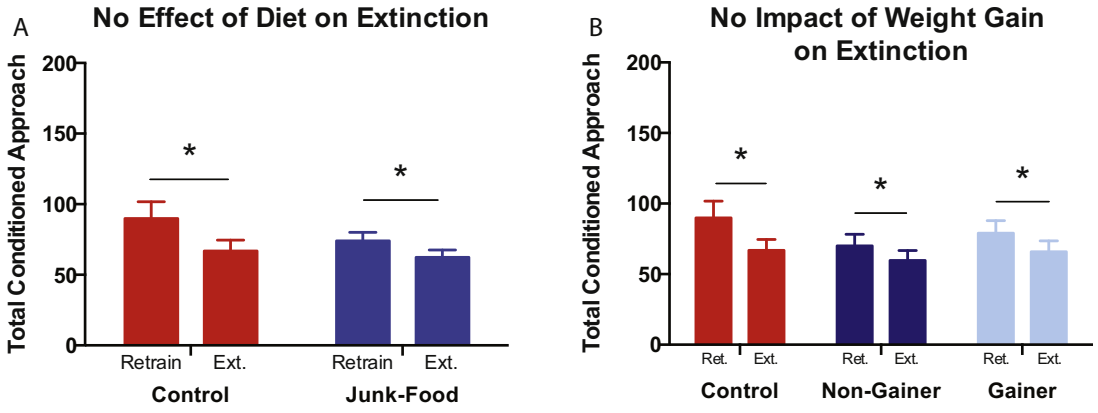

Fig. 5. Chronic exposure to a junk-food diet does not affect extinction. (A) The effect of a junk-food diet on extinction of conditioned approach behavior. (B) The impact of gainer or non-gainer phenotype on extinction in junk-food fed rats. Data are Mean and SEM, *p < 0.05.

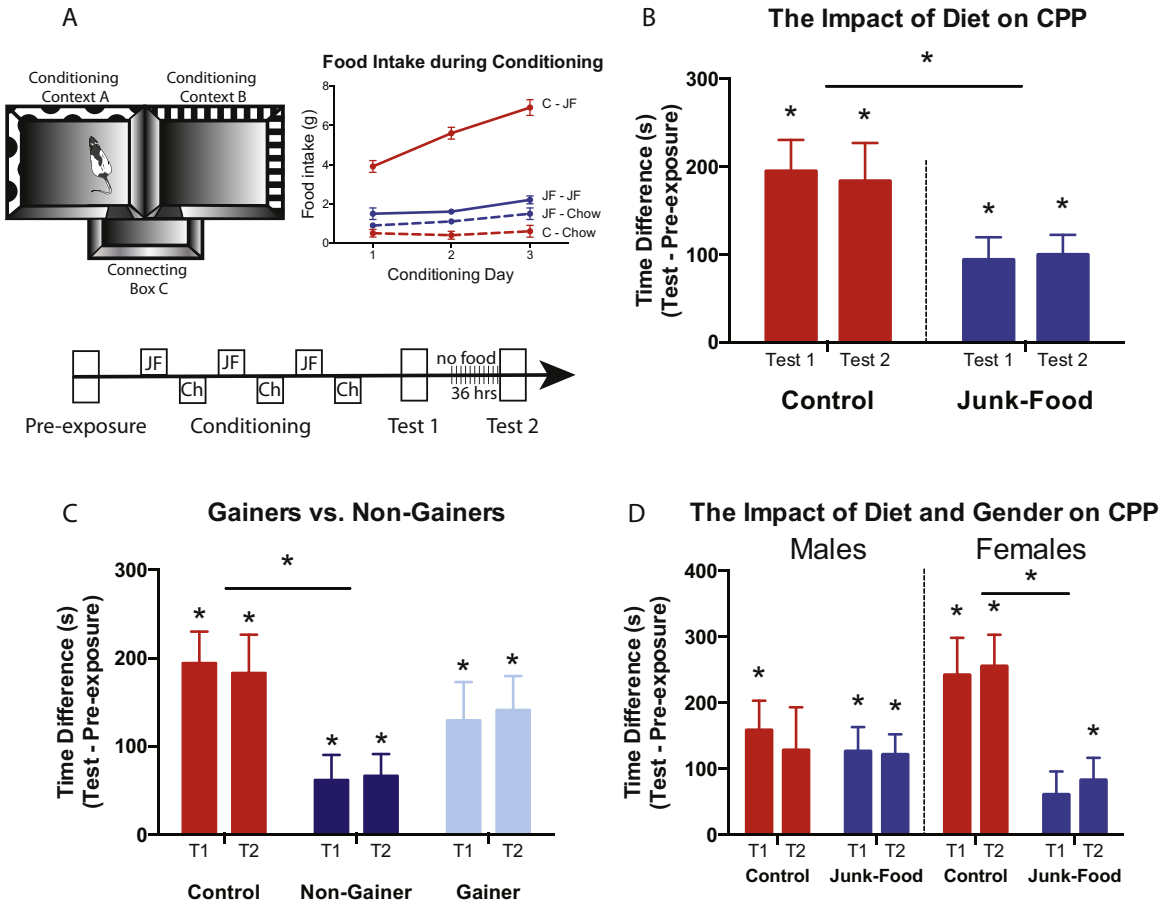

Fig. 6. Chronic junk-food exposure attenuates preference for a junk-food context, particularly in non-gainers. (A) Diagram of the conditioned place preference apparatus; timeline of the conditioning procedure; amount of chow or junk-food consumed during six alternating conditioning days. (B) The impact of a junk-food diet on the preference (time difference between test and initial pre-exposure) for the junk-food paired context during Test 1 (homeostasis) and Test 2 (after 36 h of food restriction). (C) Time preference for the junk-food context for gainers and non-gainers. (D) Time preference for the junk-food context within male and female rats chronically exposed to junk-food or chow. Data are Mean and SEM, *p < 0.05.

altered by food deprivation (C T1-T2: $t_{(29)} = 0.365$, $p = 0.718$; JF T1-T2: $t_{(59)} = 0.367$, $p = 0.715$). However, animals chronically exposed to a junk-food diet displayed a significantly weaker place preference across both tests than their chow-fed counterparts (Diet: $F_{(1,88)} = 4.165$, $p = 0.044$; Fig. 6B), suggesting a greater attraction for the junk-food paired context in control animals.

3.5.2. Non-gainers display a weaker preference for a junk-food context

Both gainer and non-gainer phenotypes showed a preference for a junk-food paired context on both tests (t's > 2.484, $p < 0.020$; Fig. 6C) but there was no impact of food deprivation on either group (Food deprivation: $F_{(1,58)} = 0.136$, $p = 0.714$; Food deprivation x Group: $F_{(1,58)} = 0.025$, $p = 0.875$). There was no significant differences in preference between gainer and non-gainer rats (G

vs. NG: $F_{(1,58)} = 2.757$, $p = 0.102$). However, non-gainers displayed a significantly weaker attraction to junk-food cues than chow-fed animals ($F_{(1,59)} = 8.689$, $p = 0.005$), which was not true of gainers ($F_{(1,57)} = 1.036$, $p = 0.313$).

3.5.3. Chronic junk-food reduces the attraction of a junk-food context in females

Male and female rats displayed a preference for the junk-food paired context irrespective of diet (t's > 2.140, $p < 0.042$; Fig. 6D) except for chow-fed males under physiological hunger ($t_{(16)} = 1.956$, $p = 0.068$) and junk-food-exposed females under homeostatic state ($t_{(28)} = 1.851$, $p = 0.075$), which nevertheless both trended towards significance. However, chronic exposure to junk-food during development significantly reduced the size of the preference for the junk-food context compared to control females ($F_{(1,40)} = 10.109$, $p = 0.003$), but not in males ($F_{(1,46)} = 0.118$, $p = 0.733$).

3.6. Taste reactivity

Varying concentrations of sucrose (1%, 3%, 9%), as well as water, were passively delivered to the animals' mouths via intraoral cannula. Hedonic and aversive orofacial reactions were video scored to quantify the amount that each concentration was 'liked' and 'disliked'. Overall, animals performed a greater amount of hedonic reactions to each sucrose concentration than to infusions of water alone (F's > 29.603, $p < 0.001$; Fig. 7A–B) and were sensitive to the increase in sucrose concentration (Concentration: $F_{(1,168)} = 45.781$, $p = 0.000$). Similarly, all animals reduced the number of aversive reactions in response to each sucrose concentration in comparison to water, and did so in a concentration sensitive manner (F's > 17.086, $p < 0.001$; Concentration: $F_{(1,168)} = 32.701$, $p = 0.000$).

3.6.1. Sprague-Dawley rats 'like' sucrose more

Interestingly, there were large strain differences in the amount of hedonic responding to sucrose solutions. Sprague-Dawley rats displayed a significantly greater increase in the amount of hedonic (but not aversive) reactions to sucrose than Long-Evans rats, irrespective of which diet they were exposed to (Strain Hedonic: $F_{(1,55)} = 57.853$, $p = 0.000$; Aversive: $F_{(1,55)} = 2.361$, $p = 0.130$). Sprague-Dawleys also displayed a difference in their sensitivity to sucrose concentration for hedonic reactions (Strain x Concentration: $F_{(2,110)} = 4.167$, $p = 0.018$). As a result, Long-Evans and Sprague-Dawley rats were analyzed separately.

3.6.2. Junk-food blunts hedonic and aversive reactions to sucrose in Long-Evans

Long-Evans rats chronically fed a junk-food diet displayed an overall blunted increase in the amount of hedonic 'liking' reactions to sucrose in contrast to chow-fed rats (Diet: $F_{(1,28)} = 11.137$, $p = 0.002$), and unlike chow-fed rats, failed to increase their hedonic reactions despite the 9-fold increase in concentration (1% → 9%) (Effect of Concentration: JF LE: $F_{(2,38)} = 1.710$, $p = 0.195$; Control LE: $F_{(2,18)} = 8.795$, $p = 0.002$). Nonetheless, both junk-food and chow-fed animals showed significantly greater hedonic 'liking' reactions to sucrose compared to water, at each concentration (LE JF: t_{19}'s > 2.331, $p < 0.032$; LE C: t_9's > 2.409, $p < 0.040$). Long-Evans rats exposed to a junk-food diet also showed less of a decrease in aversive reactions to sucrose than their chow-fed counterparts (Diet: $F_{(1,28)} = 4.945$, $p = 0.034$), despite showing progressively less aversive reactions with increasing sucrose concentration, unlike the control group (Effect of Concentration: JF LE: $F_{(2,38)} = 6.850$, $p = 0.003$; Control LE: $F_{(2,18)} = 2.535$, $p = 0.107$). Specifically, the 1% sucrose solution failed to decrease aversive reactions in junk-food exposed Long-Evans (JF 1%: $t_{19} = 1.606$, $p = 0.125$), whereas all other solutions decreased aversive reactions in LEs (t's > 2.533, $p < 0.033$).

Among Sprague-Dawley rats, there was no difference in the overall amount of hedonic or aversive reactions between chow and junk-food-fed animals (Diet: Hedonic: $F_{(1,25)} = 1.654$, $p = 0.210$; Aversive: $F_{(1,25)} = 0.077$, $p = 0.784$). For hedonic responses, Sprague-Dawley animals all showed an increase at each sucrose concentration (SD JF: t_{18}'s > 3.898, $p < 0.002$; SD C: t_7's > 5.010, $p < 0.003$) and were sensitive to increasing sucrose concentration (Effect of Concentration: JF SD: $F_{(2,36)} = 11.672$, $p = 0.000$; Control SD: $F_{(2,14)} = 6.991$, $p = 0.008$). In contrast, for aversive reactions, only junk-food exposed SDs were sensitive to increasing sucrose concentration (Effect of Concentration: JF SD: $F_{(2,36)} = 4.427$, $p = 0.019$; Control SD: $F_{(2,14)} = 1.226$, $p = 0.323$), despite not showing a significant decrease following 1% sucrose (JF SD 1%: $t_{18} = 2.065$, $p = 0.054$; JF SD 3% & 9%: t_{18}'s > 3.617, $p < 0.003$). Surprisingly, chow-fed SDs did not show a significant decrease in aversive reactions following 9% sucrose (C SD 9%: $t_7 = 1.447$, $p = 0.191$; C SD 1% & 3%: t_7's > 2.459, $p < 0.044$).

3.6.3. No impact of weight gain or sex on hedonic or aversive reactions

Among animals exposed to a junk-food diet, development of significant weight gain had no impact on the overall amount of hedonic and aversive reactions (Hedonic: $F_{(1,37)} = 0.079$, $p = 0.780$; Aversive: $F_{(1,37)} = 0.885$, $p = 0.353$), and the same was true within strains (F's < 2.466, $p > 0.133$).

Overall there was no difference in hedonic or aversive reactions between males and females, although there was a trend towards greater hedonic reactions in females (Hedonic: $F_{(1,55)} = 3.145$, $p = 0.082$; Aversive: $F_{(1,55)} = 2.551$, $p = 0.116$).

3.6.4. Sucrose is less 'liked' with greater weight gain

We examined the relationship between weight gain and hedonic or aversive reactions for sucrose solutions. Interestingly, there was a negative correlation between weight gain and hedonic reactions at all sucrose concentrations in junk-food exposed animals, suggesting that with greater weight gain, sucrose produced less 'liking' reactions (1–9%: R^2's > 0.113; $F_{(1,38)}$'s > 4.778, $p < 0.036$; Fig. 7C). The same was true of chow-fed animals at 1% and 3% (1–3%: R^2's > 0.347; $F_{(1,17)}$'s > 8.532, $p < 0.011$), but not 9% ($R^2 = 0.046$; $F_{(1,17)} = 0.768$, $p = 0.394$). In contrast, there was no correlation between the amount of weight gained and the reduction in aversive reactions following sucrose administration after either diet (Junk-Food & Chow: R^2's < 0.064; F's < 2.484, $p > 0.123$). Among junk-food exposed animals, those who gained the most weight showed a strong negative correlation with hedonic reactions at all sucrose concentrations (Gainers 1–9%: R^2's > 0.175; $F_{(1,24)}$'s > 4.921, $p < 0.038$; Fig. 7D), while non-gainers only showed an effect at 3% sucrose (3%: $R^2 = 0.296$; $F_{(1,13)} = 5.035$, $p = 0.044$). Again no effect was seen on aversive reactions (Aversive 1–9%: R^2's < 0.113; $F_{(1,38)}$'s < 2.894, $p > 0.101$).

3.7. Elevated plus maze

3.7.1. Junk-food reduces anxiety-like behavior in the elevated plus maze

Each animal's anxiety level was assessed using the elevated plus maze, where greater time spent in the open arms (OA) of the maze was taken as an indication of low levels of anxiety, and shorter durations signified higher anxiety (Fig. 8B). Overall, exposure to a junk-food diet resulted in lower anxiety than chow-fed animals (Open Arm Time C vs JF: $F_{(1,89)} = 4.367$, $p = 0.040$; Fig. 8A). However there was no difference in anxiety between Long-Evans and Sprague-Dawley rats ($F_{(1,89)} = 0.153$, $p = 0.697$).

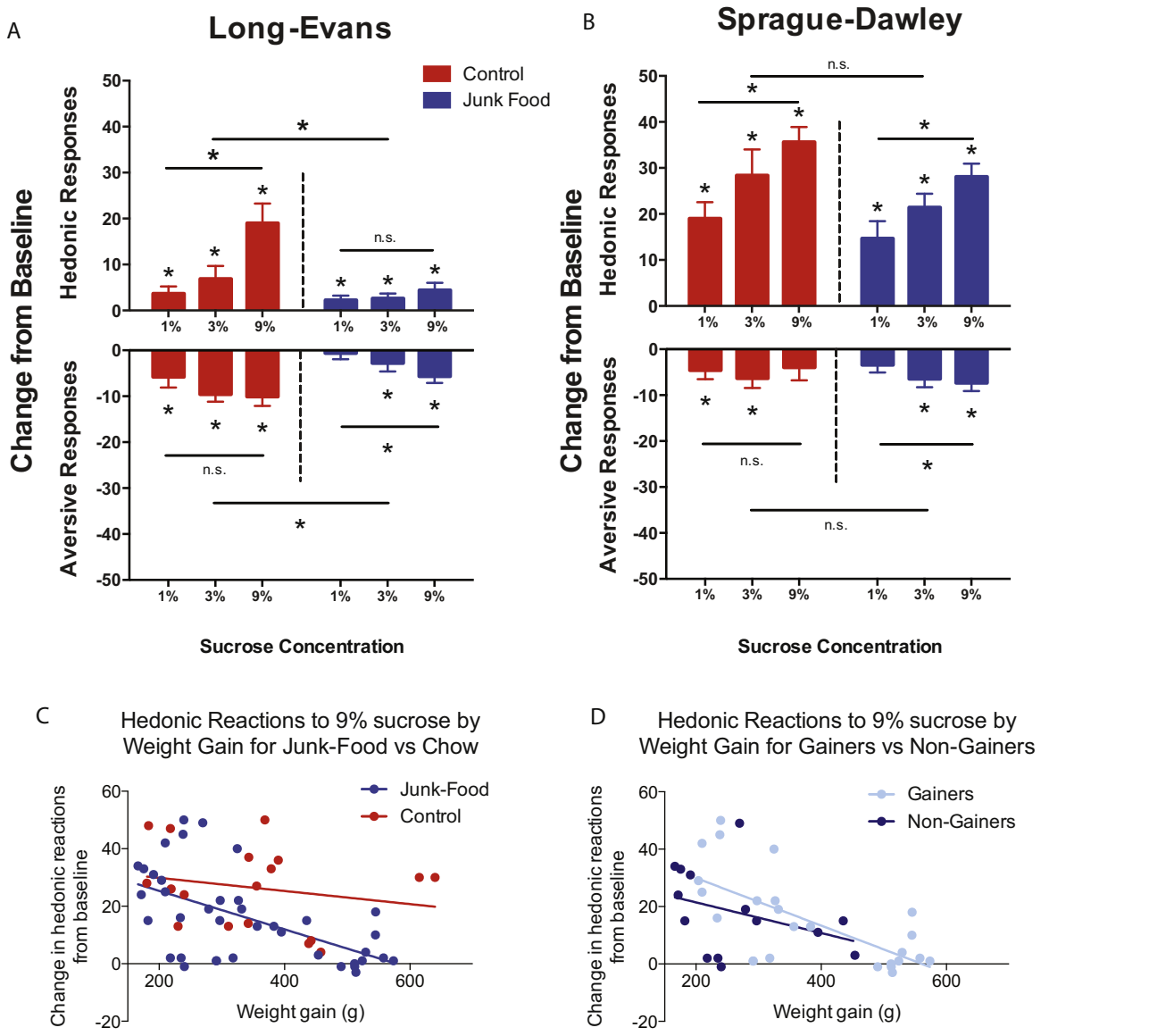

Fig. 7. Junk-food exposure blunts hedonic reactions to sucrose only in Long-Evans rats. Changes in hedonic and aversive reactions to 1%, 3%, and 9% sucrose from baseline water reactions in Long-Evans (A) and Sprague-Dawley (B) rats for animals chronically exposed to junk-food or chow. (C) Correlation between weight gain and hedonic reactions to a 9% sucrose solution for animals chronically exposed to junk-food ($R^2 = 0.326$; $F_{(1,38)} = 17.917$, $p = 0.000$) or chow ($R^2 = 0.046$; $F_{(1,17)} = 0.768$, $p = 0.394$), or (D) gainers ($R^2 = 0.459$; $F_{(1,24)} = 19.545$, $p = 0.000$), and non-gainers ($R^2 = 0.126$; $F_{(1,13)} = 1.738$, $p = 0.212$). Data are Mean and SEM, $^*p < 0.05$.

3.7.2. Junk-food reduces anxiety in males, but not females

As has been previously shown, control males were more anxious than their female counterparts (Male vs Female C: $F_{(1,29)} = 18.273$, $p = 0.000$; Fig. 8C). However exposure to a junk-food diet abolished this difference between males and females (Male vs Female JF: $F_{(1,59)} = 2.059$, $p = 0.157$) and significantly reduced anxiety in males fed a junk-food diet in comparison to their chow-fed counterparts (Male JF vs C: $F_{(1,47)} = 11.854$, $p = 0.001$). In contrast, junk-food appeared to have no effect in females (Female JF vs C: $F_{(1,41)} = 0.202$, $p = 0.656$).

3.7.3. Amount of weight gain does not impact anxiety

Greater weight gain as a result of exposure to a junk-food diet had no impact on anxiety when compared to those who did not gain as much weight, irrespective of sex (F's < 1.563, p > 0.221). However in males, both gainers and non-gainers displayed a reduction in anxiety in contrast to chow-fed males (Male G vs C: $F_{(1,31)} = 11.522$, $p = 0.002$; Male NG vs C: $F_{(1,32)} = 7.534$, $p = 0.010$; Fig. 8D).

3.7.4. Junk-food diet disrupts normal anxiety patterns in females

We then examined the relationship between weight gain and anxiety separately for each diet. Within the control animals, greater weight gain correlated with more anxious behavior ($R^2 = 0.362$; $F_{(1,28)} = 15.910$, $p = 0.000$; Fig. 8E). However, exposure to a junk-food diet mitigated this correlation between weight gain and anxious behavior ($R^2 = 0.034$; $F_{(1,58)} = 2.049$, $p = 0.158$; Fig. 8F). More specifically, females rather than males exposed to a standard chow diet were more anxious with greater weight gain (Females: $R^2 = 0.553$; $F_{(1,11)} = 13.61$, $p = 0.004$; Males: $R^2 = 0.004$; $F_{(1,15)} = 0.054$, $p = 0.820$; Fig. 8E), whereas the effect was not apparent in animals chronically exposed to junk-food (Females: $R^2 = 0.037$; $F_{(1,27)} = 1.050$, $p = 0.315$; Males: $R^2 = 0.000$; $F_{(1,29)} = 0.003$, $p = 0.960$; Fig. 8F). Finally, there was no correlation between weight gain and anxiety for either gainers or non-gainers (Gainers: $R^2 = 0.028$; $F_{(1,27)} = 0.767$, $p = 0.389$; Non-Gainers: $R^2 = 0.034$; $F_{(1,29)} = 1.027$, $p = 0.319$).

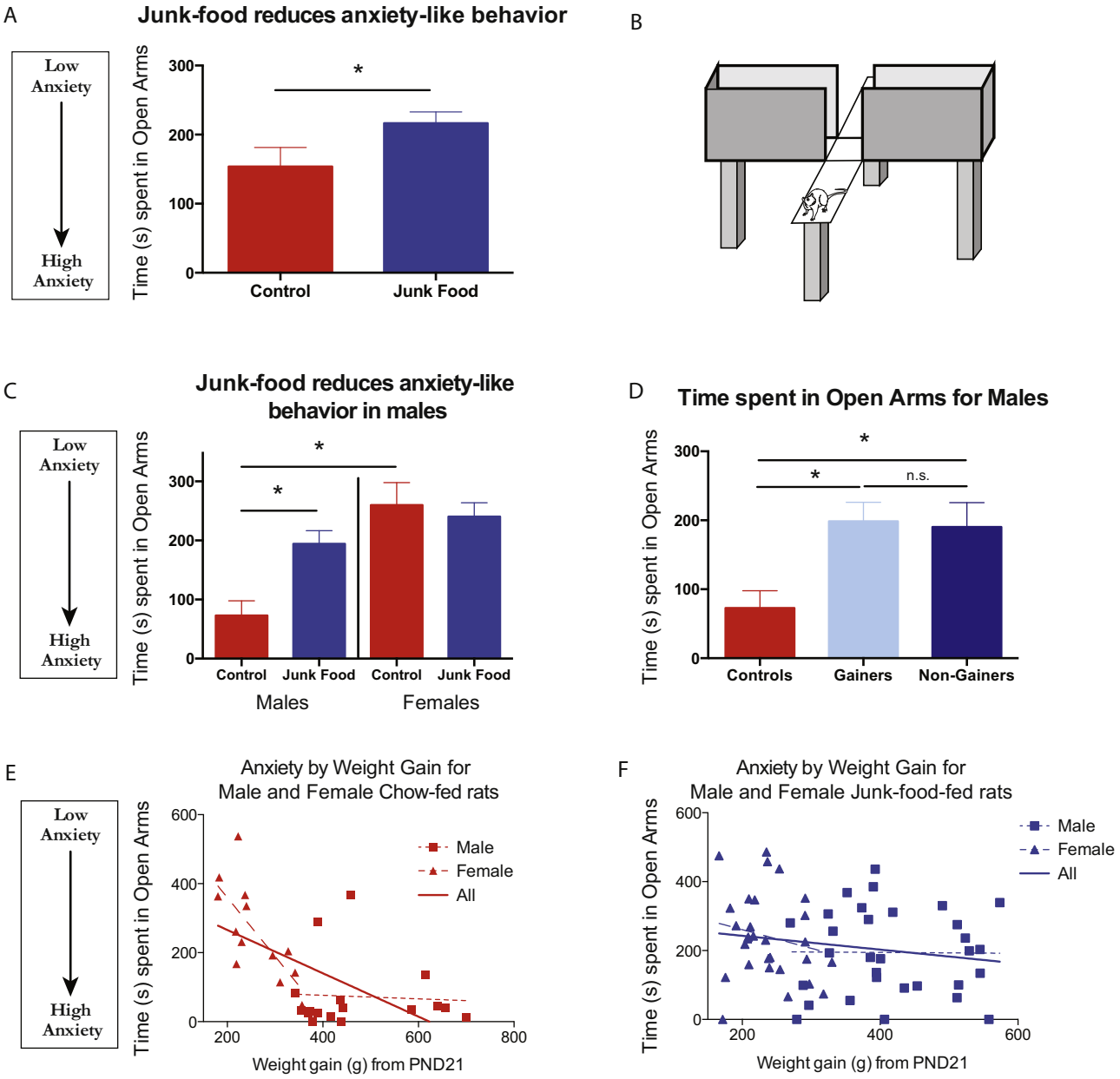

Fig. 8. Chronic exposure to a junk-food diet reduces anxiety-like behavior, particularly in males. (A) Time spent in the open arms for animals chronically exposed to junk-food or chow. (B) Diagram of the elevated plus maze (EPM). Time spent in the open arms for males and females exposed to junk-food or chow (C) or male gainers and non-gainers (D). Correlation of weight gain to time spent in open arms for males and females chronically exposed to chow (E) or junk-food (F). Data are Mean and SEM, *$p < 0.05$.

4. Discussion

4.1. Developmental effects of a prenatal and lifetime junk-food diet

This study was conducted to investigate the influence of lifetime exposure to a junk-food diet on motivated 'wanting', hedonic sucrose 'liking', and anxiety in Long-Evans (LE) and Sprague-Dawley (SD) rats of both sexes. A previous study using the same junk-food diet recipe found that while some rats gained excessive weight on the diet, others maintained a body weight similar to animals that ate standard chow; these rats were termed "gainers" and "non-gainers" [12]. In contrast, the junk-food exposed offspring in this study not only failed to gain excessive weight, but were lighter than offspring on a standard chow diet. There was no difference in weight between control and junk-food fed animals by 7 days of age, suggesting that gestational exposure to junk-food did not substantially influence birth weight. However, by 21 days of age (weaning), at which point animals had begun eating independently from their mother, junk-food animals displayed a substantially lower body weight. In both strains, this difference was more evident in males than females. In fact, the females no longer displayed any detectable weight difference due to diet by four months of age. The reduced body weight could be attributed to inadequate nutrition or insufficient protein in the diet during critical periods of development. In the current study, our junk-food diet contained levels of protein (14%) that were only marginally less than the level of protein in the standard chow (18.6%), and were still higher than protein levels in most studies examining the impact of a low protein diet (8–9%) [47–49]. Several of these studies have reported lower body weight for animals raised on a low protein diet. In humans, the Body Mass Index (BMI) is the primary method for diagnosis of obesity. How-

ever, consumption of nutritionally deficient junk-food diets during critical periods of development could result in reduced body weight along with metabolic and cognitive impairments [50,51]. These impairments may go under-reported if the reduced body weight evades BMI criterion for obesity.

In contrast, previous diet-induced obesity studies typically introduce the diet post-weaning or cross-foster high-fat pups to standard chow dams during nursing, which may account for the stark difference in body weight observed in this study [12,52,53]. In humans, larger maternal BMI is generally associated with larger birth weights [54,55], so future studies need to examine birth weight along with the degree of nutrition in the mother's prenatal diet. However in the present study, parents were only exposed to the junk-food diet for one week prior to being paired for mating. Although junk-food fed dams gained significantly more weight during that week than chow-fed counterparts, it may not have been sufficient time to develop excess body weight that would be similar to greater BMIs in humans.

Despite an overall lower body weight in animals exposed to a junk-food diet, there were large individual differences in the amount of junk-food-induced weight gain over the course of the study. Clustering the animals into "gainers" or "non-gainers" showed large weight gain disparity within strains and offered insights into the individual differences in motivation and reward seen in response to a junk-food diet [12].

4.2. Lifetime exposure to junk-food dampens reactivity to food cues differentially between strains

During Pavlovian conditioned approach, animals were repeatedly presented with an 8 s lever + tone cue that predicted a sugar pellet reward. Animals typically focus their attention primarily on either the retractable lever cue (sign-trackers; ST), the food dish (goal-trackers; GT) or a combination of both [39,56]. Here, exposure to a junk-food diet had no impact on ST/GT phenotype, and as previously reported, nor did weight gain between gainers and non-gainers [12]. However, previous research has reported large disparities in approach phenotype depending on the vendor [57], and here we found large disparities due to strain. Sprague-Dawley rats primarily displayed an ST phenotype and contained no GTs, whereas Long-Evans rats exhibited a more even distribution. As a result of these strain differences and in a similar manner to previous reports [12], cue-induced approach was examined by the total number of approach behaviors. Our results suggest that lifetime exposure to junk-food causes a dampening of cue-induced approach, specifically in LEs. Female rats also appeared to be more sensitive to the motivational dampening effects of junk-food and failed to increase their conditioned approach behavior with training, although this may in part be due to far greater levels of conditioned approach behavior in females exposed to a chow diet. Here we report no overall difference in approach behavior between gainers and non-gainers, although male gainers displayed a blunted conditioned approach response, whereas in females, non-gainers were the most visibly impaired. Previous findings using the same junk-food mash showed that male rats that gained excessive weight on the diet during adulthood displayed more cue-induced approach behavior prior to junk-food exposure than their counterparts who gained normal amounts of weight on the same junk-food diet [12]. It is possible that individuals that later go on to develop excessive weight gain show enhanced attraction to food related cues, but that a lifetime of chronic junk-food exposure may have blunted these effects and results in a somewhat amotivational syndrome overall, despite retaining large individual differences in weight gain. The long-term nature of the study may have also contributed to the lack of motivation observed in the animals that had access to junk-food [58]. Previous research has shown that in some cases length of time on a high-fat diet inversely corresponds to motivation, as measured by a progressive ratio operant task ([59]; although see [60]). Similarly, sugar overconsumption during adolescence, but not adulthood, has been reported to reduce motivation for a food reward [61]. In the present study, animals were tested in adulthood, but were exposed to a junk-food diet throughout their lifetime, including adolescence. It is possible that adolescence is a critical time period for the development of motivation, and exposure to a junk-food diet specifically during that period is responsible for the attenuated motivation seen here.

In the present study, despite lower conditioned approach behavior during autoshaping, when animals were given the opportunity to seek and acquire brief presentations of the food-paired cue (conditioned reinforcement) by performing a novel action (nose-poking), chronic junk-food exposure did not interfere with rat's willingness to selectively work for access to the cue. In fact, within junk-food exposed animals, gainers displayed stronger conditioned reinforcement than their non-gainer counterparts. In humans, evidence suggests that obese individuals are hyper-responsive to food cues [18–20] and previous reports in animals suggests that those who gain the most weight will work harder for food cues [12]. These animal and human studies focused on adolescence and adulthood. It should be noted here that Sprague-Dawley rats performed more nose-poke responses overall, but that this can likely be accounted for by the greater propensity for them to be sign-trackers, which are known to display more robust conditioned reinforcement [62].

Although the food cue acquired strong motivational properties, animals were still sensitive to changes in contingency (CS no UCS) and displayed the cognitive flexibility to reduce responding when it no longer predicted a reward (extinction). During extinction conditions, each diet group reduced total conditioned approach responses at a similar rate. The extinction sessions were used in order to compare the rate of decrease of incentive salience in cues that lose previously-attributed predictive importance, but extinction may also be a measure of learning—specifically learning new, less rewarding rules within a single context. In either case, diet did not modulate the rate of lever presses or magazine entries during the cue presentation. This finding is consistent with past extinction research done in animals that were fed high fat rodent food for two weeks prior to testing [63].

4.3. Reduced attraction to a junk-food context

Particular environments, especially those paired with palatable food, may become excessively attractive and drive some individuals to over-consume food. The conditioned place preference paradigm is frequently used as a model for relapse in substance abuse [41,64], because although there is no reward during the test sessions, animals tend to continue to display reward-seeking behavior by spending greater time in the context that was paired with the favored reward. Rats were exposed to two distinctive contexts paired with either chow or junk-food. Irrespective of their regular daily diet, all animals consumed more of the junk-food than the chow during conditioning, although this effect was strongest in controls. Both junk-food and chow-fed animals showed a preference for the junk-food paired context on the test day. However, chow-fed animals showed a significantly stronger preference for the junk-food context than the junk-food group. This may be due to their greater consumption of junk-food during conditioning, which likely results from the chow-fed animals only ever getting junk-food in the conditioning chamber. Because the junk-food diet animals had ad-lib access to the same junk-food mash within their home cages, its association with a context was likely less salient. Alternatively, it has been shown that a juvenile high-fat diet can impair hippocampal function and relational memory [65], which would disrupt acquisition and expression of the task in junk-food-

fed animals even in adulthood. Overall, our findings are consistent with past studies on the influence of palatable food on context preferences [66]. This study is unique, however, in examining animals that have had unrestricted access to junk-food in their home cages throughout life.

Interestingly, when clustered by weight gain, junk-food gainers and control animals displayed a similar pattern of greatly increased reactivity to the junk-food context, whereas non-gainers displayed a significantly less robust shift in context preference. It might be expected that a lifetime of chronic ad-lib access to the particular target food treat would result in a dampened place preference for junk-food (particularly for gainers who may be consuming more of it) compared to their chow-fed counterparts for whom junk-food is a highly palatable treat. Evidence of a similarly strong context preference suggests that junk-food gainers might instead possess a hyperreactive reward pathway that is here being masked by a lifetime of chronic ad-lib access to the particular target food treat.

It should also be noted that gainers show stronger cue attraction than non-gainers for conditioned place preference and conditioned reinforcement, but not for Pavlovian conditioned approach. It is possible that the presence of a food reward during Pavlovian conditioned approach, and the absence of any reward during conditioned place preference and conditioned reinforcement tests enhances cue attraction specifically in gainers.

4.4. Insensitivity to physiological manipulations

Altered physiological states can modulate the level of attraction or desire elicited by a cue [67,68]. Although food cues, especially cues for palatable foods, can be attractive under normal homeostatic conditions, hunger can invigorate the attraction of cues and cause more intense craving. Testing behavior under different physiological states may be an important tool in obesity research as it enables a distinction between food-seeking due to craving and food-seeking due to physiological need. In addition, if non-gainers display a blunted attraction to the junk-food context due to free access to junk-food in their home cages, denying access to that food for 36 h could result in stronger physiologically-induced craving.

However, physiological manipulation did not affect the extent to which the junk-food paired context was preferred. Animals in each group spent close to the same amount of time in each context during the ad-lib and hunger tests. This null result is consistent with previous findings [69]. It is possible that any physiological enhancement was masked by some degree of extinction that occurred during the first test, where animals were exposed to both contexts in the absence of food reward. This however also suggests that there were no detectable differences in the rate of extinction of the behavior between groups.

4.5. Junk-food animals display a pattern of blunted 'liking'

Obesity and weight gain have been linked with excessive 'wanting' and craving for (unhealthy) food and with heightened sensitivity and 'liking' to the pleasurable qualities of junk-foods that are typically high in sugar. A passive taste reactivity test was used in this study to examine 'liking'. Fluid was orally delivered to the animals regardless of behavior so as to avoid confounding observations due to motivated 'wanting'. The orofacial reactions elicited arise as reflexes and may therefore be taken as pure measures of hedonic 'liking' and 'disliking', independent of behavior. Taste reactivity reactions are highly consistent and can be observed across species – in humans, non-human primates, and rats [42]. Previous studies have suggested that chronic exposure to a junk-food diet or sugar water during adulthood or adolescence, respectively, results in a blunted reaction to sweet solutions [12,58]. Here we show that reduced 'liking' and blunted sensitivity to high sucrose concentrations occurs in animals with lifetime exposure to a junk-food diet. Surprisingly, however, this effect was restricted to Long-Evans rats, as Sprague-Dawley rats not only showed no impact of junk-food diet on taste reactivity responses, but also displayed a larger amount of hedonic reactions to sucrose water irrespective of diet. Long-Evans rats also showed a blunted reduction in their aversive reactions to sucrose. This trend toward blunted sensitivity to different concentrations of sucrose water in LE junk-food rats but not standard chow rats suggests a diet-induced alteration in neural circuits that contribute to hedonic 'liking' and 'disliking'. Interestingly, there were no differences in hedonic or aversive reactions in either Long-Evans or Sprague-Dawley gainers and non-gainers, suggesting that differences in weight gain phenotype due to diet did not differentially influence sensitivity to sweetness. When both strains were combined, the data showed that there was a strong inverse correlation between individual weight gain and the amount of hedonic reactions. Specifically, increased weight gain across both strains predicted lower sensitivity to sweetness, and this was particularly marked in animals exposed to a junk-food diet. The same was true between gainers and non-gainers, suggesting that animals that gained the most weight exhibited less 'liking' to sucrose, especially for higher sucrose concentrations (9%). Previously we found no difference in hedonic responses prior to junk-food exposure in adult male rats later identified as gainers and non-gainers; both gainers and non-gainers increased hedonic 'liking' with increasing concentrations of sucrose. However, following exposure to junk-food and weight gain, these same animals displayed a blunted sensitivity to different concentrations of sucrose and 'liked' each concentration nearly equally [12]. However, one study demonstrated that overweight human adolescents displayed significantly more 'liking' orofacial reactions (as measured by lip sucking) to images of high-fat food and high and low fat odorants than their healthy weight counterparts [70]. The difference between these findings is that while the rats showed a blunted response to the actual sweet reward, the studies in humans measured the response to reward cues, which might be anticipated as more pleasant and attractive than they are when experienced.

4.6. Lifetime exposure to junk-food reduces anxiety specifically in males

In humans, childhood obesity has been associated with higher levels of anxiety (Rofey DL, 2009), and perinatal exposure to a high-fat diet in non-human primates has been linked to increased anxiety to novel objects in female offspring [71], while exposure to a high fat diet during early development in rats impairs neuroendocrine responses to repeated stress [72]. In contrast, adolescent rats exposed perinatally to a high-fat diet showed decreased anxiety and selective alterations of glucocorticoid receptors in the hippocampus and amygdala [73] and exposure to a cafeteria diet in juvenile rats also reduces anxiety [74]. In the present study we found that lifetime exposure to a junk-food diet reduced anxiety. Males were typically more anxious than females in both strains, but chronic junk-food exposure during development greatly reduced anxiety-like behavior to a point where it was equivalent to that of females. Although we might have expected that since anxiety is often associated with increased consumption of fatty-sugary foods [32,75], gainers would display greater anxiety than the non-gainers, surprisingly that was not the case here. Interestingly, animals exposed to a regular chow diet were more anxious the greater their weight gain. This was primarily the case in females and not males, but was abolished by chronic consumption of a junk-food diet. Notably, there was no correlation between weight gain and anxiety in gainers or non-gainers. It is possible that while a junk-food diet reduces general anxiety, it renders individuals more susceptible to stress-induced anxiety, particularly in the absence of their

junk-food diet. Further research is needed to clarify the conditions that might explain these opposing findings.

It should be noted that several of the behaviors examined in the current study could be influenced by the estrous cycle in females. This includes anxiety-like behavior [76,77] and certain rewarded behaviors [78,79], but not Pavlovian conditioned approach behavior [80]. In the current study, female rats were naturally cycling and therefore likely in different phases during testing, making it unlikely that any effects could be driven by a specific estrous phase. Nonetheless, future studies should examine the interaction between hormonal changes and a junk-food diet on 'wanting' and 'liking'.

5. Conclusion

The current study examines the impact of a lifetime exposure to a junk-food diet in male and female rats of two outbred strains. Our results highlight how an unhealthy highly palatable diet that induces obesity in adults, instead results in stunted weight gain during development. If the same is true in humans, this could result in diet-induced health issues that may not be detected by measures such as the body mass index (BMI), which is currently used as the primary diagnosis tool for childhood obesity.

Despite lower body mass, we found that animals still displayed individual differences in weight gain. Those that gained the most weight exhibited greater attraction to cues and contexts predictive of food reward than their less weight-gaining counterparts, but no increase when the food reward was immediately present. We also found that junk-food exposure severely blunted 'liking' and sensitivity to sucrose solutions particularly in Long-Evans rats. This is consistent with a dissociation of 'wanting' and 'liking', and human data showing enhanced responsivity to food cues, but reduced responses to food itself [81]. Nonetheless, chronic junk-food exposure did at times reduce behavioral output overall, although this is to be expected considering the continuous free access to junk-food in their home environment. It will therefore be important to examine 'wanting' and 'liking' responses in food restricted or junk-food deprivation scenarios. Overall, these findings stress the importance of focusing on individuals with a lifetime exposure to an unhealthy diet starting even before birth, rather than strictly focusing on the phenomenology of an increased BMI and obesity.

Acknowledgements

We would like to thank Dr Carrie Ferrario for helpful comments on the manuscript, along with Melanie Schaffler, Caroline Mead, Louise Lyu, Cornelia Channing, Olivia Lofaro, Rebecca Tom, Jeremy Levit, Adam Fischer and Charlotte Freeland for their technical assistance. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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