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### Title

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### Permalink

<https://escholarship.org/uc/item/6682k4w3>

### Journal

Appetite, 123

### ISSN

0195-6663

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### Publication Date

2018-04-01

### DOI

10.1016/j.appet.2017.12.009

Peer reviewed



Published in final edited form as:

*Appetite*. 2018 April 01; 123: 135–145. doi:10.1016/j.appet.2017.12.009.

## Pattern of access determines influence of junk food diet on cue sensitivity and palatability

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### Abstract

**Aims**—Like drug addiction, cues associated with palatable foods can trigger food-seeking, even when sated. However, whether susceptibility to the motivating influence of food-related cues is a predisposing factor in overeating or a *consequence* of poor diet is difficult to determine in humans. Using a rodent model, we explored whether a highly palatable ‘junk food’ diet impacts responses to reward-paired cues in a Pavlovian-to-instrumental transfer test, using sweetened condensed milk (SCM) as the reward. The hedonic impact of SCM consumption was also assessed by analyzing licking microstructure.

**Methods**—To probe the effects of pattern and duration of junk food exposure, we provided rats with either regular chow ad libitum (controls) or chow *plus* access to junk food for either 2 or 24 h per day for 1, 3, or 6 weeks. We also examined how individual susceptibility to weight gain related to these measures.

**Results**—Rats provided 24 h access to the junk food diet were insensitive to the motivational effects of a SCM-paired cue when tested sated even though their hedonic experience upon reward consumption was similar to controls. In contrast, rats provided restricted, 2 h access to junk food exhibited a *cue generalization* phenotype under sated conditions, lever-pressing with increased

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**Author contributions:** Alisa Kosheleff conducted the experiments with assistance from Jingwen Araki, Jennifer Hsueh, Andrew Le, Kevin Quizon. Experimental design, data analysis and interpretation, and writing were done by Alisa Kosheleff, Sean Ostlund, Nigel Maidment, and Niall Murphy.

**Conflicts of interest:** None to declare.

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vigor in response to both a SCM-paired cue, and a cue not previously paired with reward. Hedonic response was also significantly higher in these animals relative to controls.

**Conclusions**—These data demonstrate that the pattern of junk food exposure differentially alters the hedonic impact of palatable foods and susceptibility to the motivating influence of cues in the environment to promote food-seeking actions when sated, which may be consequential for understanding overeating and obesity.

### Keywords

cafeteria diet; junk food; reward; incentive motivation; palatability; obesity

## Introduction

Non-homeostatic eating behavior is strongly motivated by the rewarding effects of palatability and variety in flavor and texture [1]. Overindulgence in such foods has been heavily implicated in the obesity epidemic sweeping developed nations [2], in which obesity rates have more than doubled in the last 30 years [3,4]. Importantly, for many overweight and obese individuals, efforts to control their body weight prove challenging, with craving and compulsive consumption being major culprits [5]. In some cases, compulsive overeating can become so extreme that it has been compared with drug addiction, as they share many characteristics such as escalating intake over time despite negative consequences, such as foot shock in rats [6,7] or negative health or social consequences in humans [8–12].

A key component of maladaptive reward seeking is the acquisition of Pavlovian associations between the primary rewards themselves (e.g., food, drugs) and predictive stimuli in the environment (i.e., a context or discrete cue). With repeated pairings, such cues can come to trigger reward cravings [13,14] and drive efforts to procure reward [15–17]. This process, whereby reward-associated cues acquire motivational properties that allow them to become capable of eliciting reward seeking, is termed Pavlovian incentive motivation, frequently referred to in the literature as ‘wanting’. The motivational influence of drug-paired cues is well documented in the drug addiction literature, where drug-paired cues have been shown to potentiate drug seeking for alcohol [18], nicotine [19], cocaine [20], and morphine [21], and sensitivity to such cues is considered to underlie a vulnerability to craving, compulsive drug seeking and, consequently, addiction [22,23]. Importantly, food-paired cues also become highly salient, capable of producing strong physiological reactions (e.g., increased salivation) and cravings [24]. Food-paired cues are well known to potentiate non-homeostatic (i.e., hunger state-independent) feeding, in both rats [25–27] and humans [28,29], as well as maladaptive overeating [30] in both obese and normal-weight restrained eaters (i.e., such as when dieting) [31–33].

Growing evidence from animal models suggests that, like drugs of abuse, poor diets (i.e., high-fat, high-sugar and refined foods) may have long-term consequences for behavior and cognition, making it difficult to determine whether a hypersensitivity to food-paired cues precedes maladaptive eating in humans, or emerges as a result of it. Indeed, poor quality diets have been shown to produce deficits in hippocampal-dependent learning and memory [34–36], promote a shift from goal-directed to habitual responding [37–39], and alter reward

liking and craving [6,40–46]. While specific mechanisms are unclear, growing evidence supports a role for mesolimbic dopamine dysfunction [6,47,48], though the hippocampus may also be preferentially vulnerable to the deleterious effects of junk foods [49–51]. As alluded to above, behavioral responses to reward can be dissociated into ‘wanting’ - attributing motivational salience to reward-related stimuli, and ‘liking’ - the hedonic pleasure experienced by consuming reward [52], and each has been shown to be impacted by such diets [6,40–46]. A recent study [48] supports a role for both pre-existing individual differences and junk-food-driven changes in reward seeking and liking: rats later identified as susceptible to junk-food-induced obesity show stronger pre-existing conditioned approach behavior than obesity-resistant rats, while junk food exposure, regardless of weight gain, dampened the hedonic impact of palatable foods. Other studies have shown that the *pattern* of consumption may also matter: sugar-binging rats display addiction-like behaviors not seen in rats with ad libitum sugar access or control rats [43,44]. These data indicate that factors such as *how* the diet is consumed, in addition to individual predisposition to weight gain, must be considered when investigating diet-induced changes in behavior.

Here, we investigated whether a junk food diet could alter reward seeking and liking. Because of the differences between continuous overconsumption and binge eating, we used both ad libitum (24 h) and restricted, intermittent (2 h) daily access to junk food. To probe these behavioral effects, we used the Pavlovian-to-instrumental transfer (PIT) paradigm (a test of cue-evoked incentive motivation, or *wanting*, for food) and microstructural analysis of licking behavior for a palatable solution (a measure of reward *liking*) [53,54]. PIT was employed because of the power of this approach to parse the incentive motivational impact of cues from their conditioned reinforcing effects [55–57]. Since cue-invigorated food-seeking and consumption *when hungry* may be considered adaptive, and we were specifically interested in *maladaptive* food-seeking behavior, i.e., eating in the absence of hunger, our focus was on tests conducted when rats were sated on home chow, although tests were also conducted under the more conventional hungry condition. Our focus on the sated condition was also based on reports that cues invigorate food consumption in humans in the sated state [29,58], and that this may contribute to overeating and obesity [59–61]. We also examined how diet-induced weight gain relates to cue-evoked reward ‘wanting’ and ‘liking’ by comparing across high and low weight-gainers. We hypothesized that the incentive motivational properties of reward-paired cues in the sated state would increase with junk food exposure, and that, based on the literature cited above, intermittent-fed rats would be particularly vulnerable to this effect.

## Materials and Methods

### Subjects and apparatus

Adult (10 weeks old) male Sprague-Dawley rats ( $n = 79$ ) were pair-housed for the duration of the experiment. Rats were food restricted to 85% of their free-feeding body weight during initial behavioral training. All behavioral training and testing took place in sound- and light-attenuating operant chambers (Med Associates, VT) equipped with a retractable lever, a white noise generator, a clicker audio generator, a food cup capable of delivering liquids, and a contact lickometer system capable of recording licking behavior. All experimental

procedures were approved by the UCLA Institutional Animal Care and Use Committee and were in accord with the National Research Council Guide for the Care and Use of Laboratory Animals. See Table 1 for a summary of the training and testing timeline described in detail below.

### Initial behavioral training

To maximize detection of a facilitatory effect of junk food exposure on incentive motivation, we trained naïve rats using a sub-threshold PIT paradigm known to support minimal cue-evoked responding under normal home chow diet conditions [62–64]. A 50% sweetened condensed milk (SCM) solution was used as a reward stimulus during all training phases. After 1 day of magazine training, rats underwent 10 days of instrumental training during which they learned to press a lever to receive a 0.1ml infusion of SCM (delivered over 2 sec). Daily instrumental training lasted for 30 minutes or until 30 reinforcements were earned. Lever pressing was continuously reinforced for the first session, and was then shifted to a variable interval (VI) schedule, which was increased every day beginning with VI-5s on Day 1, then progressing each day to VI-10s on Day 2, VI-15s on Day 3, VI-25s on Day 4, VI-35s on Day 5, and VI-45 s on Days 6–10. Rats were then given 10 days of Pavlovian conditioning with the lever withdrawn, during which time the SCM delivery was paired with the offset of a 30-sec auditory cue (click or white noise; CS<sup>+</sup>). Daily Pavlovian conditioning lasted for 10 cue presentations (trials) per session, each separated by a variable 2.5 min interval. Analysis of initial behavioral training data is presented in Supplementary Materials (Supplemental Fig. 1).

### Junk food diet

Following initial training, rats were assigned to one of three diet groups: Control, Intermittent, or Ad Libitum, and one of three diet durations: 1, 3, or 6 weeks. During this time, all rats received unlimited access to chow and water, while the two treatment groups (Intermittent and Ad Libitum) also received access to two junk foods (one sweet, one savory) each day for either 2 h only (Intermittent) or for 24 h (Ad Libitum). The junk foods differed from day to day and consumption of each food type was measured daily along with body weight. Junk foods included cookies, chocolates, cheese, and hot dogs, among others (see Supplementary Materials for a full list). Animals were assigned to the various food exposure groups in a manner that ensured comparable levels of lever pressing across groups based on the initial behavioral training data. Consumption patterns during this phase of the study are presented in Supplementary Materials (Supplemental Fig. 3).

### Behavioral retraining

On the last day of the diet exposure phase, all food was removed and rats were given access to chow only for two hours a day for the remainder of the experiment. After three days of such food restriction, rats were briefly retrained, beginning with 3 days of instrumental retraining on a VI-45s schedule, then 1 day of Pavlovian conditioning. On the Pavlovian re-conditioning day, rats were trained in two sessions. In the first session, rats were presented with a new auditory sound (click or white noise) *not* previously paired with SCM, which would serve as the control stimulus (CS<sup>0</sup>). The CS<sup>0</sup> was presented in the same manner as the CS<sup>+</sup> only no SCM was delivered. Approximately 2 h after this session, rats were given a

Pavlovian conditioning session identical to that in the initial behavioral training phase (i.e., with the CS<sup>+</sup> and SCM deliveries). The following day, rats underwent 1 day of instrumental extinction, which involved 30 min of access to the lever *without* any SCM infusions, in order to suppress response rates. Analysis of behavioral retraining data is presented in Supplementary Materials (Supplemental Fig. 4).

### Pavlovian-to-instrumental transfer testing

In the PIT test, rats were given continuous access to the lever but no rewards were delivered. The CS<sup>+</sup> and CS<sup>0</sup> were presented non-contingently (i.e., cue onset and offsets occurred regardless of lever pressing) 4 times each for 30 sec at a time, in ABBA order, separated by 3.5-min intervals. As explained in the Introduction, we were specifically interested in how reward-paired cues might invigorate instrumental reward-seeking *when sated*, but since PIT tests are more commonly conducted in a hungry state, we ran two PIT tests (one under conventional food-deprived, hungry conditions, and one under sated conditions, order counterbalanced between diets and durations), separated by a day of rest (i.e., no behavioral training) and 5 days of behavioral retraining and extinction as outlined above. Immediately prior to each PIT test, rats were individually housed for 1 h in a new, clean homecage, where all rats had access to water, and rats undergoing their sated test also had ad libitum access to chow.

### Licking microstructure

To quantify reward ‘liking’, immediately after each PIT test, rats were given an opportunity to lick, non-contingently, from a spout in the operant chamber delivering 50% SCM for 5 min in order to assess their licking microstructure. A contact lickometer (Med Associates, VT) was used to measure individual licking responses. The program timer controlling session length did not begin until each rat initiated licking, allowing a full 5 min of access from when the spout was first licked. All licks were recorded and parsed into bouts, defined as any continuous series of licks separated by less than 1 sec [65].

### Weight gain and abdominal adipose tissue measurement

To determine the impact of junk food consumption on body composition, body weights were taken daily and abdominal white fat was collected and weighed following euthanasia by isoflurane overdose the day after the final PIT test.

### Data analysis

Data were analyzed by ANOVA using SPSS (IBM, Armonk, NY). Effects were defined as statistically significant when  $p < 0.05$ , and significant interactions were further assessed via multiple pairwise comparisons using a logical extension of Fisher’s protected least significant difference procedure for controlling family-wise Type I error rates [66]. Outliers were detected using Extreme Studentized Deviate (criterion  $p < 0.01$ ). All data are expressed as means  $\pm$  standard error of the mean (SEM). As noted above, and in the Introduction, we were specifically interested in the capacity of cues to alter reward wanting and liking in the *sated* state. Therefore, sated PIT and licking data were analyzed, *a priori*, independently of those from the hungry state tests, and are presented in detail in the Results section. Brief

descriptions of the results of the hungry tests are also provided in the Results, but readers are referred to the Supplementary Materials for a full description of the analyses of these hungry tests (see Supplementary Fig. 6 and 7).

**Weight gain as a factor in PIT and licking analysis**—As recent reports link individual susceptibility to obesity with cue-sensitivity [48] and striatal neuroadaptations [47], we divided rats into low and high ‘weight-gainers’ (irrespective of diet or duration) using 2-group K-means clustering [48] based on the percentage of weight gained during the experiment (weight gained / start weight). This weight-gain factor was included in PIT and lick analyses.

**PIT analysis**—Because high baseline responding can obscure the expression of PIT by engaging ceiling effects [67,68], we employed a targeted analysis of pre-CS response rates to ensure homogenous baseline reward-seeking. Two outliers were removed on the basis of their pre-cue (i.e., baseline) lever presses: one from the Controls, 3-week group, and one from the Ad Libitum, 1-week group. These animals were also excluded from analyses of food cup entries. A univariate ANOVA confirmed that pre-cue, baseline response rates did not differ significantly between diets or durations, nor was there any interaction between these two factors (all  $F$ ’s < 1.00, all  $p$  > 0.05 ns; mean rate of lever pressing per 30 sec:  $0.60 \pm 0.07$  SEM). Therefore, data are presented as elevation scores from baseline responding wherein the number of lever presses during the 30 sec immediately preceding the cue period (i.e., pre-cue baseline responding) was subtracted from the total number of lever presses during the 30 sec cue period. Cue, diet, duration and weight-gain effects were analyzed using repeated-measures analyses of variance (rmANOVA), with paired- and independent-sample post-hoc  $t$ -tests where appropriate. Time in the food cup was analyzed in the same manner.

**Lick analysis**—Immediately after the PIT test, rats were given a 5-min SCM exposure test, during which all licks were recorded. When drinking palatable solutions, rodents take occasional pauses of varying lengths, resulting in distinct bouts of licking behavior [69]. The average bout length, in particular, is considered to reflect the experienced palatability/hedonic impact of the solution, especially during periods of short access, such that involvement of post-ingestive processes is precluded [65,70]. Thus, in addition to total number of licks, we also assessed the average bout length, where a bout is a series of licks in which each lick is separated by 1 second or less. One statistical outlier with significantly lower total licks (due to equipment malfunction) was removed from this analysis (Intermittent, 6-week group). As for PIT data, cue, diet, duration and weight-gain effects were analyzed using repeated-measures analyses of variance (rmANOVA), with paired- and independent-sample post-hoc  $t$ -tests where appropriate.

## Results

### Weight gain and adipose tissue content

Full details of these measures are provided in Supplementary Materials; only features potentially pertinent to interpretation of the results of the main focus of the study, namely

effect of diet on cue-induced food seeking and palatability, are described here for clarity. Multivariate ANOVAs showed no difference among the various diet and duration groups in starting weight (mean 299.68 g,  $\pm$  1.72 SEM), but significant differences were apparent in weight gained by the day of euthanasia as a percentage of starting weight (Fig. 1). As expected, rats exposed to all diets for longer periods prior to euthanasia gained more weight (main effect of duration  $F(2,70) = 22.36$ ,  $p < 0.001$ ). Specifically, 6 week rats gained more weight than 1 week ( $t(49) = 5.89$ ,  $p < 0.001$ ) or 3 week ( $t(53) = 4.20$ ,  $p < 0.001$ ) rats, and 3 week rats gained more weight than 1 week rats ( $t(50) = 2.81$ ,  $p < 0.01$ ). There was also a significant main effect of diet ( $F(2,70) = 3.52$ ,  $p < 0.05$ ), where Ad Libitum rats gained more weight than Controls ( $t(49) = 2.05$ ,  $p < 0.05$ ). There was no diet  $\times$  duration interaction. Abdominal adipose tissue content, expressed as a percentage of total body weight, was significantly higher in Ad Libitum rats compared with the other two diet groups at 6 weeks (Supplementary Materials Fig. 2B).

**Incorporation of weight gain as a factor in PIT and licking analyses**—Using 2-group K-means clustering [48] based on the percentage of weight gained during the experiment (weight gained / start weight), we divided rats into low versus high “weight-gainers” (Fig. 2). A total of 20 High Weight Gainers were identified (Control  $n = 4$ , Intermittent  $n = 5$ , Ad Libitum  $n = 11$ ) and 59 Low Weight Gainers (Control  $n = 19$ , Intermittent  $n = 23$ , Ad Libitum  $n = 17$ ). Weight gain status (High vs. Low) was included as a factor in both PIT and licking microstructure analyses.

### Pavlovian-to-instrumental (PIT) testing

To determine the impact of junk food exposure on *sated* cue-evoked reward seeking, we sated rats on home chow for 1 h (consumption data presented in Supplementary Materials Fig. 5), then presented the  $CS^+$  and  $CS^0$  noncontingently, allowing rats the opportunity to lever press in the absence of any reward deliveries.

An ANOVA of lever-press activity with factors: cue ( $CS^+$  vs.  $CS^0$ , repeated measure), diet (Controls vs. Intermittent vs. Ad Libitum), duration (1 vs. 3 vs. 6 weeks), and weight gain (High vs. Low) revealed a significant main effect of cue ( $F(1,62) = 6.03$ ,  $p < 0.05$ ), and a significant cue  $\times$  diet interaction ( $F(2,62) = 3.49$ ,  $p < 0.05$ ), but no main effect of, or interactions with, duration or weight gain. Further analyses were therefore conducted on data collapsed across duration and weight gain (Fig. 3A). One-sample t-tests (versus 0) revealed that Control rats significantly increased their responding during the  $CS^+$  ( $t(21) = 5.54$ ,  $p < 0.001$ ), but not the  $CS^0$  (as expected), and paired t-tests confirmed that responding during the  $CS^+$  was significantly higher from that during the  $CS^0$  ( $t(21) = 4.43$ ,  $p < 0.001$ ). Intermittent rats significantly increased their responding for both the  $CS^+$  ( $t(27) = 3.50$ ,  $p < 0.01$ ) and the  $CS^0$  ( $t(27) = 3.68$ ,  $p < 0.01$ ) to a similar degree, and a paired t-test showed no significant difference between the two cues, suggesting a nonspecific food-seeking effect of both cues. In contrast, Ad Libitum rats failed to significantly increase lever pressing in response to either cue and there was no significant difference between the cues. Independent samples t-tests revealed that Control rats increased their lever-pressing in response to the  $CS^+$  more than Ad Libitum rats ( $t(47) = 3.35$ ,  $p < 0.01$ ), but not more than Intermittent rats, while Intermittent rats increased responding to the  $CS^0$  more than Controls ( $t(48) = 2.48$ ,  $p <$

0.05) and Ad Libitum rats ( $t(53) = 2.86, p < 0.01$ ). In summary, intermittent junk food exposure potentiates food seeking even in response to a neutral cue, suggesting a generalization of  $CS^+$ -enhanced reward-seeking to less predictive, but otherwise similar, stimuli, while Ad Libitum junk food exposure abolishes cue-invigorated reward-seeking.

An ANOVA conducted on time in the food cup revealed no main effects or interactions. However, in order to permit comparison with lever-press data, food cup entry data were similarly collapsed across duration and weight gain (Fig. 3B). While this analysis revealed no significant effects of any factor, trends are apparent. Similar to lever pressing, cues elicited minimal food cup approach in Ad Libitum rats. In contrast to lever pressing, however, Intermittent (and Control) rats appeared to selectively increase their time at the food cup in response to the  $CS^+$  versus the  $CS^0$ . This suggests that Intermittent rats are not impaired in their ability to discriminate the cues.

An identical analysis of lever pressing during the hungry test failed to reveal a statistically significant effect of cue, diet or diet duration, although trends similar to the statistically significant results of the sated test are apparent i.e. greater cue differentiation in controls than in the Intermittent and Ad Libitum groups and lower general responses to cues in the Ad Libitum group (Supplementary Materials Fig. 6A). There was a significant main effect of cue on cue-invigorated food cup entries, with more time spent in the food cup during the  $CS^+$  versus the  $CS^0$  across all groups, but again, no significant effects of diet or diet duration (Supplementary Materials Fig. 6B). Full statistical analyses are presented in Supplementary Materials.

### Lick analysis

Immediately after the PIT test, rats were given a 5-min SCM exposure test, during which all licks were recorded. We conducted a multivariate (total licks and bout length) ANOVA with the factors diet (Controls vs. Intermittent vs. Ad Libitum), duration (1 vs. 3 vs. 6 weeks), and weight gain (High vs. Low).

**Total Licks**—This analysis revealed a significant effect of diet ( $F(2,63) = 10.61, p < 0.001$ ), a significant effect of weight gain ( $F(1,63) = 4.49, p < 0.05$ ), and a significant diet x weight gain interaction ( $F(2,63) = 5.05, p < 0.01$ ), but no effect of, or interaction with, duration. Data, collapsed across duration, are shown in Fig. 4A. Follow-up comparisons revealed that High Weight Gainers licked more than Low Weight Gainers within Controls ( $t(20) = 2.79, p < 0.05$ ) and Intermittent ( $t(26) = 3.42, p < 0.01$ ) groups, an effect that was noticeably absent in the Ad Libitum rats. Intermittent rats licked more than Controls whether they were Low Weight Gainers ( $t(39) = 2.35, p < 0.05$ ), or High Weight Gainers ( $t(13) = 2.18, p < 0.05$ ). High weight gaining Intermittent rats also licked more than high weight gaining Ad Libitum rats ( $t(14) = 3.81, p < 0.01$ ). Post-hoc comparisons on the simple main effects revealed that Intermittent rats licked more than Controls ( $t(48) = 2.98, p < 0.01$ ) and Ad Libitum rats ( $t(53) = 3.21, p < 0.01$ ), while High Weight Gainers licked more than Low Weight Gainers ( $t(76) = 1.75, p < 0.05$ ).

**Bout Length**—Bout length showed a similar pattern to total licks across groups. There was a significant effect of diet ( $F(2,63) = 20.79, p < 0.01$ ), a significant effect of weight gain

( $F(1,63) = 15.31$ ,  $p < 0.01$ ) and a significant diet  $\times$  weight gain interaction ( $F(2,63) = 18.02$ ,  $p < 0.01$ ). Again, there was no main effect of, or interaction with, duration, and data collapsed across this variable are presented in Figure 4B. Follow up comparisons revealed that bout length was longer in High Weight Gainers than Low Weight Gainers in both Control ( $t(21) = 2.41$ ,  $p < 0.05$ ) and Intermittent rats ( $t(25) = 3.69$ ,  $p < 0.01$ ), an effect that was noticeably absent in the Ad Libitum group. Among Low Weight Gainers, Ad Libitum ( $t(33) = 2.21$ ,  $p < 0.05$ ) and Intermittent ( $t(33) = 2.16$ ,  $p = 0.04$ ) rats exhibited longer bouts than Controls. Among High Weight Gainers, Intermittent rats exhibited longer bouts than Ad Libitum rats ( $t(14) = 3.57$ ,  $p < 0.01$ ). Post-hoc comparisons on the simple main effects revealed that Intermittent rats had longer bouts than Controls ( $t(48) = 2.52$ ,  $p < 0.05$ ), while High Weight Gainers had longer bouts than Low Weight Gainers ( $t(76) = 2.49$ ,  $p < 0.05$ ).

An identical analysis of licking microstructure during the hungry test failed to reveal any group differences in total licks (Supplementary Fig. 7A). There was, however, a significant main effect of diet on bout length, but follow-up tests narrowly ( $P = 0.07$ ) failed to support evidence of longer bouts in Intermittent rats relative to controls (Supplementary Fig. 7B). Full statistical analyses are presented in Supplementary Materials.

## Discussion

We probed how a junk food diet influences cue-evoked reward seeking and reward palatability, using the PIT test and licking microstructure analysis, respectively. Our focus was on the results of tests conducted under sated conditions because of their relevance to maladaptive food-seeking behavior, i.e. over-eating. We found that junk food consumption resulted in the emergence of different patterns of behavior under sated conditions depending on the schedule of junk food exposure (intermittent versus ad libitum access). We also demonstrated that the hedonic impact of the SCM reward varied with both the schedule of diet exposure and weight gain. Contrary to our initial hypothesis, animals provided ad libitum access to junk food were insensitive to the instrumental invigorating effect of SCM-paired cues observed in their chow-fed counterparts (Fig. 3A). Moreover, their Pavlovian conditioned approach to the food cup also appeared to be suppressed under sated conditions (Fig. 3B). This was apparent despite the fact that the hedonic impact of the reward was similar to chow-fed animals, particularly when the weight gain factor is ignored (Fig. 4B). Animals with restricted daily access to junk food, on the other hand, pressed the lever more vigorously over baseline in response to the reward-paired cue, as predicted, but contrary to our initial hypothesis, this was also the case in the presence of the neutral cue, suggesting a generalization of the excitatory effects of the  $CS^+$  to other, similar stimuli (Fig. 3A) despite a trend towards a reward-paired-cue-specific food cup approach response (Fig. 3B). Interestingly, palatability responses were highest of all among high-weight-gaining intermittent access rats (Fig. 4). While some similar trends were apparent under hungry conditions these generally failed to attain statistical significance, perhaps reflecting increased variability in responses associated with the heightened behavioral state.

### Ad libitum junk food exposure decreases responsiveness to reward-paired cues under sated conditions

Rats provided ad libitum access to varied, highly palatable foods, in addition to regular chow, were generally not susceptible to the instrumental invigorating effects of reward-paired cues seen in their chow-fed counterparts, when sated (Fig. 3A). Notably, this was not explained by employment of the alternative strategy of checking the food cup (Fig. 3B), and is consistent with previous studies reporting deficits in reward processing in rodents chronically exposed to poor quality and junk food diets, as indicated by increased brain self-stimulation thresholds [6], decreased conditioned place preference for amphetamine [71], decreased ethanol consumption [40], and decreased motivation for reward on a progressive ratio task [72]. Interestingly, decreased motivation for food on progressive ratio [46,72] or incentive runway [73] tasks is seen in several conditions associated with poor quality diets and obesity, such as after junk food exposure, with [46,72] or without [73] weight gain, and even in obesity-prone rats in the absence of obesity or junk food exposure [73]. The lack of a statistically significant main effect of weight gain on cue-induced food seeking, or an interaction of weight gain with diet on this measure in our study argues against a conclusion that the dietary effects we observed on cue-induced lever-pressing were secondary to metabolic effects of weight gain alone, but rather supports a more direct effect of diet on the motivational influence of cues. However, since the ad libitum junk food-fed rats gained more body fat (statistically significant following 6 weeks of exposure) than control or intermittent junk food-fed rats, secondary metabolic effects remain a possible cause of the apparent motivational deficit in the Ad Libitum group.

We found no evidence that ad libitum junk food-fed rats found SCM significantly less palatable than their chow-fed counterparts (Fig. 4). Indeed, low weight-gainers in this group 'liked' SCM *more* than their chow-fed counterparts. It is noteworthy, however, that high weight-gaining rats in this group trended towards lower palatability responses than their high weight-gaining chow-fed counterparts (Fig. 4). Further, unlike chow-fed and intermittent junk food-fed animals, high weight-gaining rats among those exposed to an ad libitum junk food diet did not show evidence of elevated palatability responses relative to low weight-gainers fed the same diet (Fig. 4). Thus, while this diet tended to produce the highest weight gains (Fig. 1), this is likely not due to increased palatability of sweet/fatty food or to increased cue-precipitated incentive motivation. Collectively, these observations may be considered somewhat in agreement with studies elsewhere suggesting that increases in palatability may not explain the development of obesity: that is, obese humans experience reduced sweetness [74], and obese [73] and obesity-prone [45] rats "like" low concentrations of sucrose and fat less than lean or obesity-resistant rats, respectively, an effect that can be normalized with weight loss (but not with acute food deprivation) [41].

Growing evidence points to disruption of the dopamine system as a likely neuroadaptation mediating the reduction in incentive motivation observed in our ad libitum junk food-fed rats: chronic consumption of poor quality, junk food diets produces lower basal and evoked dopamine in the rat NAc [71,75], and downregulated D2 receptors (D2R) [6,76], similar to the decreased D2 receptors reported in pathologically obese humans [77–79]. Diet-induced downregulation of the mesolimbic dopamine system may function as a satiety-signal by

reducing the motivational impact of food-paired cues when sufficient food has already been consumed: striatal dopamine signaling is required to maintain feeding behavior [80] and to attribute salience to environmental cues associated with reward [81]. Conversely, it has also been reported that diet-induced D2R downregulation is also associated with compulsive-like feeding and increased reward seeking [6], possibly in an attempt to restore homeostasis to an underactive reward system, similar to the allostatic model of drug addiction [82].

Interestingly, previous work has shown that cafeteria diet-induced obese rats displayed no increase in extracellular dopamine in response to standard lab chow (in contrast to controls), and only showed such increases in response to a cafeteria-diet “challenge” [75]. Such a finding may be pertinent to why our Ad Libitum group displayed little instrumental responding for a 50% SCM solution – a food reward that supported cue-evoked food-seeking in the other diet groups, but that may hold little value for rats accustomed to a richer, more varied diet. The decreased incentive motivation seen in ad libitum-exposed rats may also reflect the emergence of a depression-like phenotype, as obesity is associated with increased risk of mood disorders, including depression [83], which is partly characterized by decreased interest or pleasure and changes in appetite [84]. The mesolimbic dopamine system has been implicated in the etiology of mood disorders [85], in addition to mediating appetitive behaviors such as food liking, craving, and seeking [80,86]. A high-fat diet can induce a depression-like phenotype in mice, indicated by increased behavioral despair [87]. Although the latter study employed a longer period of exposure (12 weeks) than used here, poor quality diets (i.e., high sucrose, high fat, junk foods, etc.) have been shown to effect changes in behavior within the timeframe of our study [37,88–91], including anxiety [92], which is highly comorbid with depression [93]. The brief withdrawal from the junk-food diet used in our experimental design may also have contributed to the expression of such a phenotype.

### **Intermittent junk food access produces indiscriminate cue responsivity and increased reward palatability under sated conditions**

Emerging evidence suggests a strong role for the pattern of diet consumption (i.e., binge eating versus constant “grazing”) in the susceptibility to maladaptive eating, where restricted and binge eating are associated with addiction-like behaviors [43,44]. Like the relationship between drug-paired cues and drug relapse, food-paired cues can potentiate non-homeostatic eating in intermittent-fed rats [26]. Here, we modeled restricted eating by using intermittent (2h/day) junk food exposure. We found that, unlike their ad libitum junk food access counterparts, these rats displayed significantly increased lever pressing in response to reward-paired cues, when sated. However, this invigoration was no greater than that observed in chow-fed animals. Rather, the distinguishing characteristic of the Intermittent group was their equal lever invigoration response to a neutral cue. While they did not discriminate between the two cues in terms of their lever pressing, there was a noticeable trend towards such a discrimination with respect to food cup entries during cue presentation, suggesting an intact ability to discern the two cues. It also suggests that they remained susceptible to the conditioned effects of the reward-paired cue even if this does not induce them to expend significantly more effort in an attempt to procure the reward. Their indiscriminate lever pressing suggests an increased susceptibility to the excitatory effects of

environmental cues when sated, including generalizing to those that are similar to, but distinct from, those previously paired with reward.

As alluded to above, sensitization of mesolimbic dopamine transmission is strongly implicated in the invigoration of reward seeking precipitated by reward-paired cues [64,81,94]. Intermittent sucrose access has been shown to repeatedly release dopamine in the NAc shell [95,96], alter the expression [97] and availability [76] of dopamine receptors, and facilitate locomotor sensitization to a dopamine agonist [98,44,99] suggesting that such dietary interventions may impact the dopaminergic systems involved in learning about and responding to reward-paired cues [100–103]. Interestingly, dopamine neurons will fire in response to familiar stimuli non-predictive of reward, but to a lesser degree than firing in response to cues predicting reward, suggesting dopamine neurons may support stimulus generalization [104,105]. This is notable because our intermittent-fed rats appeared to overgeneralize the excitatory response-invigorating effects of the CS<sup>+</sup> to the seemingly neutral CS<sup>0</sup> stimulus. Although the CS<sup>0</sup> was never directly paired with food reward, it may have acquired (or been attributed) latent motivational properties due to its perceptual similarity to the CS<sup>+</sup>, or through its second-order relationship with reward, in that it was presented in a context strongly associated with food reward. Regardless, it is not uncommon for “neutral” or ambiguous cues to acquire incentive motivational properties. For instance, previous studies have shown that cues that are presented in a random fashion with respect to food reward can still acquire the ability to stimulate food-seeking behavior [106]. Similarly, cues that signal the cancelation of food access acquire the ability to potentiate feeding [107], even though such a relationship might be expected to support inhibitory rather than excitatory learning. Although the CS<sup>0</sup> stimulus used in the current experiment did not elicit an overt motivational influence over reward seeking in the control (chow) condition, intermittent junk food exposure appeared to instigate or uncover this underlying motivational influence, either through over-attribution of incentive salience to the CS<sup>0</sup>, or through a nonspecific reduction in the motivational threshold for the elicitation of reward-seeking behavior. Interestingly, it has been shown that intermittent exposure to cocaine [63,81] or amphetamine [64] can also potentiate cue-triggered food-seeking behavior. Further research will be needed to determine how such effects relate to the motivational effects of junk food exposure, including whether they depend on a common set of neuroadaptations. Indeed, this hypergeneralization and hypersensitivity to reward-paired contexts and cues is a hallmark of both drug addiction and binge eating disorder [10,108], and growing evidence suggests remarkable parallels between drug addiction and food binging [10]. For instance, rats provided intermittent access to a sweet solution (thus enabling food binging) show similarities to rodents in drug-abuse paradigms, exhibiting escalating intake [76], increased motivation to obtain sucrose [109], naloxone- and food-deprivation-induced signs of withdrawal [110], and accelerated development of habitual behavior [37]. Notably, sugar-binging rats show cross-sensitization with amphetamine, while rats with ad libitum sugar access do not [44].

The intermittent-fed rats’ indiscriminant lever pressing may also be due to decreased response inhibition or increased impulsivity, both of which are strongly associated with binge eating [111–113] and addiction [114–116] disorders. Recent reports indicate that rodents exhibit increased impulsivity after high-fat, high-sugar, and palatable diets [117], an

effect that can be passed on to offspring as a result of an “unfavorable intrauterine nutritional environment” [118]. While trait impulsivity has been thought to play a *causative* role in these disorders [114,119], drug use is thought to also exacerbate impulsivity and disrupt response inhibition [120], creating a vicious cycle of impulsive drug seeking [116]. Given the behavioral and neurochemical similarities between drug addiction and binge eating disorder [121], it is possible that an impulsive phenotype may be both a product of intermittent palatable feeding, and a driving factor in humans with binge eating disorder.

The second notable characteristic of the intermittent junk food access rats is the elevated palatability measure (lick bout length) (Fig. 4). In particular, rats that gained the most weight on the intermittent diet access appeared to ‘like’ the SCM more than respective control-fed or ad libitum-fed rats. (While the difference between High Weight Gainer Controls and High Weight Gainer Intermittent rats failed to reach significance with this measure (bouts:  $p = 0.096$ ), the total licks comparison was significant.) Our results are consistent with previously reported evidence of increased reward ‘liking’ after 5 weeks of palatable-food binging [122]. Limited-access diets are known to potentiate not only dopamine [95,96] but also opioid activity [76], neurochemical systems known to positively regulate motivation and reward-learning [101], and palatability/hedonia [86], respectively. Given overwhelming evidence that palatable food consumption can be induced and abolished by facilitation and impairment of opioid signaling, respectively [65,123,124], it is possible that intermittent junk food access upregulates opioid systems, potentiating reward ‘liking’. In fact, this effect is consistent with reports that binge eating in humans is associated with a “gain-of-function” mutation in the mu-opioid receptor gene, which is also associated with increased self-reported food liking [125].

## Summary

Access to a junk food diet produced profound alterations in cue-induced food seeking and food “liking” under sated conditions that varied with the pattern of access provided to the junk food. Rats provided ad libitum access were generally unresponsive to reward-paired cues when sated despite apparently ‘liking’ the SCM to a similar degree or, in the case of low weight-gainers, significantly *more* than chow-fed animals. The deficit in these animals was therefore primarily motivational rather than hedonic. Unsurprisingly, these animals tended to gain more weight than the other groups but neither motivational nor palatability differences could account for within-group variability in weight gain. On the other hand, restricted junk food access induced development of a *cue generalization* phenotype in sated animals. In these animals, as in ad libitum chow-fed controls, within-group differences in weight gain could potentially be accounted for by the degree to which the SCM reward was “liked” upon consumption. The data underline the importance of the *pattern* of consumption as a factor impacting diet-behavior interactions and are particularly interesting in the context of research highlighting different subtypes of overeating and obesity. For some individuals, overeating is a steady, perhaps habitual action characterized by frequent snacking, large portion sizes, and poor quality foods [126]. For others, it can be compulsive and *driven*, characterized by food binges and marked distress about overeating, as in the case of binge eating disorder [127]. Our data suggest that such intermittent junk food “binges” may cause cues that are only loosely associated with eating to take on motivational significance when

sated, and may also increase the hedonic impact of palatable food, which may be of particular relevance to binge eating.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

**Funding:** This work was supported by the National Institutes of Health/National Institutes of Diabetes and Digestive and Kidney Disorders (grant number #DK098709 to NT Maidment, NP Murphy and SB Ostlund).

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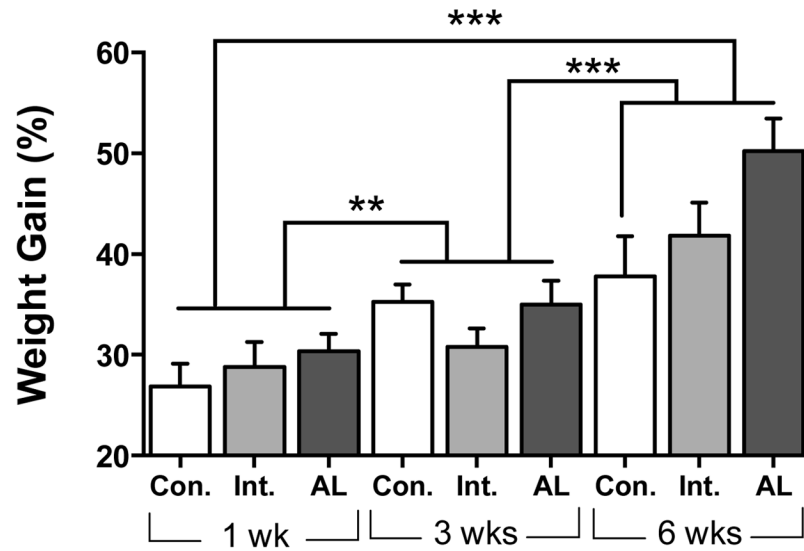
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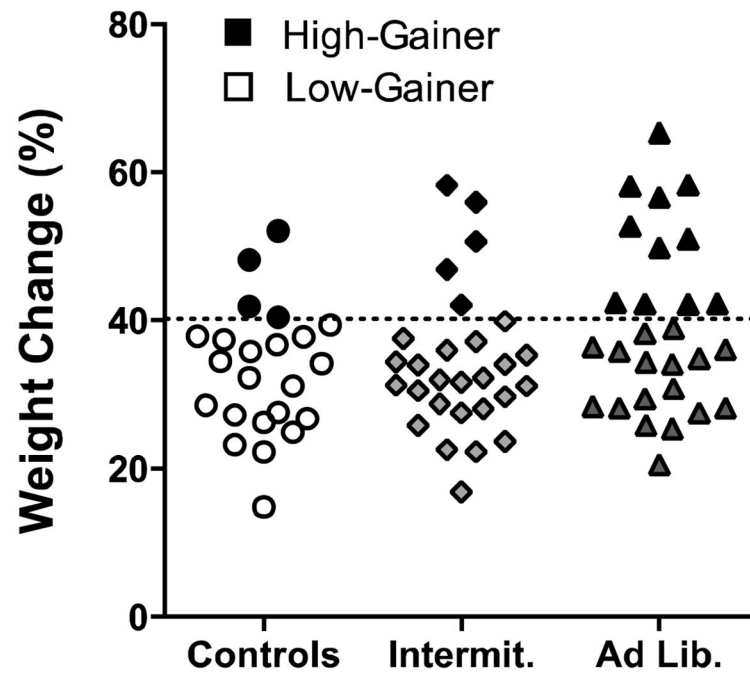
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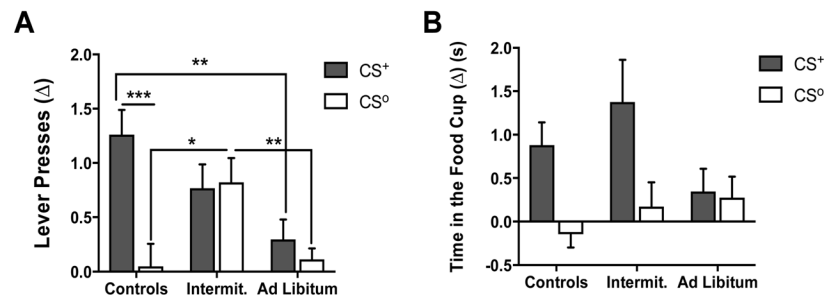
**Figure 1. Changes in Body Weight**

Increases in body weight, expressed as a percentage of individual starting weight. *Con.* = Control group; *Int.* = Intermittent group; *AL* = Ad Libitum group.



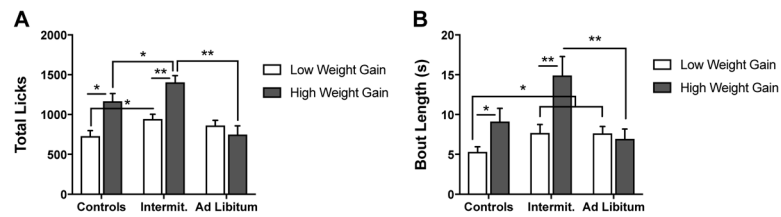
**Figure 2. Individual Differences in Weight Gain**

Rats were split into low vs. high weight-gainers using 2 group k-means clustering.



**Figure 3. Pavlovian-to-Instrumental Transfer Test**

(A) Increase in lever pressing from pre-cue (baseline) responding per 30 sec, averaged across 4 30-s CS presentations. (B) Increase in time (in sec) spent in the food cup from baseline per 30 sec, averaged across 4 30-s CS presentations.  $CS^+$  = reward-paired cue;  $CS^0$  = neutral cue.



**Figure 4. Licking Microstructure Analysis**

(A) Total number of licks and (B) bout length (in seconds) during a 5-min sated lick test for sweetened condensed milk immediately after the PIT test.

**Table 1**

Training and Testing Timeline.

| Phase                      | Duration       | Procedure                                                                 |
|----------------------------|----------------|---------------------------------------------------------------------------|
| Magazine Training          | 1 d            | Noncontingent reward                                                      |
| Instrumental Training      | 10 d           | Lever-press → Reward                                                      |
| Pavlovian Conditioning     | 10 d           | CS <sup>+</sup> → Reward                                                  |
| Diet Exposure              | 7, 21, or 42 d | Control, Intermittent or 24 h Ad Libitum exposure                         |
| Return to Food Restriction | 3 d            | 2 h chow per day                                                          |
| Instrumental Retraining    | 3 d            | Lever-press → Reward                                                      |
| Pavlovian Re-Conditioning  | 1 d            | A.M.: CS <sup>0</sup> → No reward<br>P.M.: CS <sup>+</sup> → Reward       |
| Instrumental Extinction    | 1 d            | Press → No reward                                                         |
| PIT & Lick Test 1          | 1 d            | Lever extended with CS <sup>+</sup> and CS <sup>0</sup> (both unrewarded) |
| Instrumental Retraining    | 3 d            | Lever-press → Reward                                                      |
| Pavlovian Re-Conditioning  | 1 d            | A.M.: CS <sup>0</sup> → No reward<br>P.M.: CS <sup>+</sup> → Reward       |
| Instrumental Extinction    | 1 d            | Press → No reward                                                         |
| PIT & Lick Test 2          | 1 d            | Lever extended with CS <sup>+</sup> and CS <sup>0</sup> (both unrewarded) |