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Individual differences in behavioral responses to palatable food or to CCK predict subsequent diet-induced obesity

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Abstract

Objective: This study investigated whether individual differences in behavioral responses to palatable food and to the satiation signal cholecystokinin (CCK) in outbred chow-maintained Sprague Dawley rats enabled prediction of individual differences in weight gained after subsequent high-fat/high-sugar diet (HFHSD) maintenance.

Methods: Meal size, meal number and early dark cycle intake during initial HFHSD exposure were measured as was early dark cycle sucrose solution and chow intake, chow meal size and meal number, the intake suppressive effect of 0.5μg/kg CCK injection, and CCK-induced c-Fos activation in the nucleus tractus solitarius (NTS). Subsequently, rats were maintained on HFHSD for five weeks and weight gain determined.

Results: Rats that took larger and less frequent meals on the first day of HFHSD exposure, whose early dark cycle intake (HFHSD and sucrose) was larger during initial HFHSD exposure, gained more weight after HFHSD maintenance. Rats with lesser sucrose intake suppression in response to CCK gained more weight after HFHSD maintenance and also displayed reduced CCK-induced c-Fos activation in the NTS.

Conclusions: Together, these data identify individual differences in behavioral responses to palatable food and to CCK as novel predictors of diet-induced obesity.

Keywords

Obesity; meal size; NTS; c-Fos; cholecystokinin

Introduction

In humans and rats, long-term consumption of energy-dense diets leads to variability in magnitude of weight gain. For example, outbred male Sprague Dawley (SD) rats show individual differences in body weight (BW) gain after multi-week palatable, energy-dense diet maintenance[1,2]. Selective breeding of high and low weight gainers over multiple generations amplified these traits in a fashion reflecting a polygenic pattern of inheritance[2,3].

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For the rat gaining weight after HFHSD maintenance, multiple effects of weight gain itself could contribute to further weight gain. For example, rats on energy-dense diet maintenance are less responsive to the intake suppressive effects of exogenously administered gastrointestinal (GI) peptides, e.g., CCK, glucagon-like peptide-1, and bombesin[4–6]. For this reason, the current experiments sought to determine whether individual differences in behavioral and physiological features of food intake control exist in chow-maintained SD rats and whether, if identified, such differences could predict magnitude of HFHSD-induced BW gain.

Food intake and its underlying parameters including meal size and meal number are controlled by actions of various energy-availability signals on central nervous system cells and circuits. One such signal, the GI peptide CCK, is of particular interest. CCK is released from intestinal I cells in response to ingestion. It acts on CCK-1 receptors (CCK-1Rs) expressed on vagal afferent neurons innervating the GI tract that project centrally to caudal NTS neurons, and activating both inhibits food intake [7,8]. Palatable food taste provides a hedonic signal, arising from oral sensors innervated by cranial nerve afferents that synapse on rostral NTS neurons to stimulate food intake[9–11]. While CCK action and responsiveness to hedonic properties of food influence consumption, it is unknown whether individual differences in either predict diet-induced obesity (DIO).

Here, we utilized a rat model of DIO to investigate the hypotheses that individual differences in sensitivity to the effects of (a) CCK-induced satiation and (b) hedonic properties of palatable food on intake and meal parameters, exist in chow-maintained rats and predict the magnitude of HFHSD-induced BW gain. Overall, our data support the hypotheses and identify individual differences in behavioral responses to palatable food and to CCK-induced suppression of a palatable diet as novel predictors of HFHSD-induced BW gain.

Methods

Subjects

Adult male SD rats (250–265g on arrival, Charles River Laboratories, Wilmington, MA) were selected for documented BW heterogeneity during HFHSD maintenance[1,2]. Upon arrival, rats were individually housed in wire-bottomed hanging cages under 12-h light/12-h dark cycle with *ad libitum* access to pelleted chow (Purina 5001, St. Louis, MO) and water for at least one week. All procedures conformed to the institutional standards of the University of Pennsylvania IACUC and were consistent with the NIH Guide for the Care and Use of Laboratory Animals.

General Experimental Procedures

CCK sensitivity procedures were conducted using within-subject, counterbalanced designs, with at least 48h intervening between experimental conditions. All correlation analyses were between-subject comparisons. CCK (Bachem Americas Inc., H-2080) was dissolved in sterile physiological 0.9% saline (Fisher Scientific) and kept on ice. Drugs were delivered through intraperitoneal (IP) injection (1ml/kg).

Experiment 1: Assessing whether individual differences in feeding responses during initial HFHSD exposure predict HFHSD-induced BW gain

Naïve chow-maintained rats (n=15) were individually housed in a custom, automated food intake monitoring system consisting of hanging, wire-bottom cages modified with an access hole to a food cup resting on an electronic scale. Scale output connected to software (LabView) that calculated food intake from changes in cup weight every 10s. The method has been well validated[12,13]. Following 5-day apparatus habituation with access to powdered Purina 5001 chow [58% carbohydrates – 3% sucrose - 13% fat], rats were switched to powdered HFHSD [51.4% kcal from carbohydrate, 26% kcal from sucrose, 32% kcal from fat,; D12266B Research Diets, Inc.]. Food intake, meal size and meal number were measured for the first two days of HFHSD access. Three rats left excessive spillage, impairing accuracy of food intake measurements, thus final analyses include n=12 of 15 rats. Measurement of HFHSD intake ended after exposure day two. Subsequently, rats were shifted to 5-week HFHSD maintenance with BW measured weekly.

Experiments 2a and b: Assessing whether [1] individual differences in CCK-induced intake suppression and [2] baseline intake of sucrose and chow predict HFHSD- induced BW gain

CCK Behavioral Sensitivity Procedures—Two experiments in separate groups of rats were conducted to assess CCK's effect on intake suppression. For one, CCK's intake inhibitory effect was measured in rats consuming 15% sucrose solution as described previously[14,15], and for the other, CCK's intake inhibitory effect was measured in rats consuming chow.

Sucrose. Naïve rats (n=20) were habituated to home cage access to 15% sucrose solution (referred to as sucrose going forward) for up to one week to reach intake stability within 15% of average responding for three consecutive days. Rats were also habituated every other day to IP 0.9% saline. Rats were then subjected to a 4-condition counterbalanced experiment where (0, 0.1, 0.5, or 2.0 μg/kg) CCK was administered immediately before dark onset and sucrose intake was measured every 15min for 1h post dark onset. Chow maintenance diet was withheld 2h prior to dark onset to limit gastric content variability. *Chow.* Another group of naïve rats (n=20) was habituated to IP 0.9% saline and on test days food was withheld for 2h prior to dark onset. Rats were injected with (0, 0.5, or 2μg/kg) CCK 2h after dark onset to ensure high baseline rates of short-term intake, and intake was measured at 30 and 60min.

CCK sensitivity was calculated as percentage intake suppression from baseline intake. Greater positive values (greater intake suppression) reflect greater CCK sensitivity and negative values (lesser intake suppression) reflect lesser CCK sensitivity.

Following both CCK sensitivity procedures, groups were shifted to 5-week HFHSD maintenance with BW measured weekly. To determine whether CCK sensitivity and sucrose or chow consumption predicted HFHSD-induced BW gain, behavioral CCK sensitivity and 60-min sucrose or chow intake (from vehicle condition) were correlated with 5-week BW gain. In the sucrose cohort, one rat was water deprived during 5-week BW measurement. Correlation analyses were conducted for n=19 of 20 rats. In the chow cohort, one rat was ill

and another did not eat under any treatment. Correlation analyses were conducted for n=18 of 20 rats.

Experiment 3: Evaluating whether individual differences in chow meal size and meal number predict HFHSD-induced BW gain

Naive rats (n=15) were individually housed in a custom, automated food intake monitoring system and were habituated to the apparatus with access to powdered Purina 5001 chow (described in Experiment 1). Once habituated, meal size and meal number were measured for four days. One rat left excessive spillage, impairing accuracy of intake measurements. Final meal analyses include n=14 of 15 rats. After meal pattern measurement, rats were shifted to 5-week HFHSD maintenance with BW measured weekly.

Experiment 4: Assessing whether individual differences in CCK behavioral sensitivity correlate with CCK-induced NTS neuronal activation x

A group of naïve rats (n=25) was used to determine whether CCK behavioral sensitivity correlated with CCK-induced NTS neuronal activation using c-Fos immunoreactivity (IR). Several days after evaluating CCK sensitivity to sucrose (Experiment 2a), rats were administered 0.5μg/kg CCK (n=20) or 0.9% saline (n=5) as this was the lowest effective dose when individual differences were observed. Rats were deeply anesthetized and transcardially perfused with 4% paraformaldehyde 90min after injection, a time-point producing maximal c-Fos IR[16]. Coronal sections (30μm) were cut using a cryostat and processed for fluorescence microscopy in the NTS at the rostrocaudal level of the AP as described previously[17,18]. Briefly, sections were washed with 1% sodium borohydride, blocked in 0.1M PBS with 5% donkey serum (Jackson Immuno Research Lab, Inc.) and incubated in goat primary antibodies for c-Fos (1:1000: sc-53–2-G: Santa Cruz Biotechnology, Inc.). Following incubation in fluorescent secondary antibodies (594 Alexa Fluor Donkey Anti Rabbit IgG: Jackson Immuno Research Lab Inc.), sections were mounted on slides and coverslipped with Fluorogel (Electron Microscopy Sciences; Hatfield, PA). Neurons expressing c-Fos were visualized and quantified (Nikon 80i; NIS-Elements AR 3.0) at ×10 and ×20 magnification, from coronal sections of the caudal brainstem at −14.2, −14 and −13.8mm from bregma (3 sections/rostral-caudal coordinate), according to the stereotaxic atlas of Paxinos and Watson (2005). One animal had insufficient numbers of sections for quantification so analyses included 24 of 25 rats.

Statistical Analyses

All analyses were conducted with Prism 8 (GraphPad Inc.). Sucrose and chow intake were analyzed by two-way repeated measures ANOVA for main effects and Dunnett's multiple comparisons test for interaction effects. Changes in plasma CCK were analyzed by paired t-tests. Unpaired T-tests were used to compare c-Fos activated neurons under 0 or 0.5μg/kg CCK. Pearson's correlations were used for all correlation analyses and the Benjamani, Hochberg and Yekutieli method was used to control false discovery rate. Alpha levels were set to 0.05 for all analyses.

Results

Experiment 1:Individual differences in meal size, meal number, and intake in the first hour of dark phase during initial HFHSD exposure predict HFHSD-induced BW gain.

Displayed in Fig 1A, individual differences in average meal size for all meals on day 1 (24 h) of HFHSD exposure correlated positively with 5-week HFHSD-induced BW gain[R(10)=0.8, p=0.002, r^2=0.64]. Additionally, individual differences in average meal number for day 1 of HFHSD exposure (Fig 1B) correlated negatively with BW gain [R(10)= −0.73, p=0.007, r^2=0.54]. Rats who took fewer meals on day 1 also took larger-sized meals [R(10)= 0.61, p=0.003, r^2=0.61] and, as noted, also gained more weight during HFHSD maintenance. In addition, individual differences in HFHSD intake in the first 60min after dark onset for Days 1 and 2 of HFHSD exposure (average of those two days) positively correlated with individual differences in 5-week HFHSD-induced BW gain [R(10)=0.68, p=0.02, r^2=0.46] (Fig1C). There was a trend toward positive correlation between HFHSD intake in the first 60min after dark onset on Day 1 of exposure and 5-week BW gain but this was not statistically significant [R(10)=0.56,p=0.06, r^2=0.31] (Figure 1D). Controlling false discovery rate (FDR), results in figure 1A, 1B and 1C were considered discoveries. BW gain and individual differences in BW gain were observed (Average %BW gain = 39; SD %BW gain = 7).

Experiment 2a: Individual differences in sensitivity to CCK's intake inhibitory effects on sucrose intake and in baseline sucrose intake predict HFHSD-induced BW gain.

Peripheral CCK injection reduced sucrose intake dose-dependently (Figure 2A). Two-way repeated measures ANOVA revealed a significant main effect of dose [F(3)=13.92, p<0.001], a significant main effect of time [F(3)=53.19, p<0.001], and a significant dose by time interaction [F(3)=3.43, p=0.01]. Dunnett's multiple comparisons test revealed that at 15min post-injection, sucrose intake under vehicle treatment (M= 12.06 SD=2.57) was significantly different than under 0.5 (M=9.14 SD=4.57) and 2μg/kg CCK (M= 5.50 SD=4.57). Additionally, at 30 and 45min post-injection, sucrose intake under vehicle (30: M=13.14 SD=3.09, 45: M= 13.55 SD=2.73) was significantly different than intake under 0.5 (30: M=10.75 SD=3.90, 45: M=11.78 SD=3.74) and 2μg/kg CCK (30: M=8.84 SD=5.35, 45: M= 9.58 SD=5.12). 60min post-injection, sucrose intake under vehicle (M=14.35 SD=3.05) was significantly different than under 2μg/kg CCK (M=11.50 SD=5.01). Treatment with 0.1μg/kg CCK did not significantly reduce sucrose intake at any time point compared with vehicle. Pearson's correlations revealed that individual differences in CCK-induced intake suppression (at 30min post 0.5μg/kg CCK injection) negatively correlated with percentage 5-week HFHSD-induced BW gain(Fig. 2B) [R(17)=0.49, p=0.03, r^2=0.24]. Additionally, individual differences in CCK-induced intake suppression at 15min post 2μg/kg CCK injection negatively correlated with percentage BW gained [R(17)= −0.49,p=0.03, r^2=0.25] (Fig. 2C). Consistent with results in Fig. 1C, this experiment revealed that individual differences in 60min sucrose intake under vehicle positively correlated with BW gain (Fig. 2D) [R(17)=0.59, p=0.01, r^2=0.35]. Controlling FDR, all correlations reported were considered discoveries. Individual differences in 15min intake suppression to 0.5μg/kg CCK did not significantly correlate with %BW gain and was not

deemed a discovery after controlling FDR. BW gain and individual differences in BW gain were observed (Average %BW gain = 23.4g; SD %BW gain = 4).

Experiment 2b: Individual differences in sensitivity to CCK's suppressive effects of chow intake, and baseline chow intake did not predict HFHSD induced BW gain.

Peripheral CCK injection reduced chow intake dose-dependently (Figure 3). Two-way repeated measures ANOVA revealed a significant main effect of dose [$F(2)=4.03$, $p=0.039$], a significant main effect of time [$F(1)=35.57$, $p<0.001$] and a significant dose by time interaction [$F(2)=6.28$, $p=0.01$]. Dunnett's multiple comparisons test revealed that at 30min post injection, chow intake under vehicle (M= 5.27 SD=1.48) was significantly different than under 0.5 (M=3.76 SD=2.25) and 2μg/kg CCK (M=3.48 SD=1.79). There was no significant effect of either dose on intake inhibition at 60min. In contrast to results above, Pearson's correlations showed that individual differences in CCK's effect on chow intake inhibition (0.5μg/kg at 30min chosen *a priori* based on Experiment 2a) did not correlate with subsequent HFHSD-induced BW gain [$R(16)=0.36$, $r^2=0.15$, $p=0.13$]. There was no significant correlation between 60min chow intake under vehicle treatment and BW gain [$R(16)=0.30$, $r^2=0.09$, $p=0.22$]. After controlling FDR, correlations reported were not considered discoveries. BW gain and individual differences in BW gain were observed (Average %BW gain = 25.6g; SD %BW gain = 5.8).

Experiment 3: Individual differences in chow meal size and meal number do not predict HFHSD-induced BW gain.

Shown in Table 1, individual differences in average meal size and meal number on day 1 of chow measurements did not significantly predict BW gain [$R(12)=0.09$, $r^2= 0.01$, $p=0.77$ and $R(12)=-0.02$, $r^2=0.0003$, $p=0.95$, respectively]. Controlling FDR, correlations were not considered discoveries. BW gain and individual differences in BW gain were observed (Average %BW gain =29.17 SD %BW gain = 4.38).

Experiment 4: Individual differences in sensitivity to CCK's suppressive effects of sucrose intake correlate with c-Fos IR in the NTS.

Peripheral CCK injection dose-dependently reduced sucrose intake. Two-way repeated measures ANOVA revealed a significant main effect of dose [$F(3)=7.40$, $p=0.001$], a significant main effect of time [$F(3)=67.94$, $p<0.001$], but not a significant dose by time interaction[$F(3)=1.391$, $p=0.25$]. Dunnett's multiple comparisons test was used to determine dose effects, revealing that sucrose intake under vehicle (M=12.10 SD=3.44) was significantly different than both 0.5 (M=10.89 SD=3.21) and 2μg/kg CCK (M=9.92 SD=2.92) (Figure 4A). There was no significant difference between sucrose intake under vehicle treatment and 0.1μg/kg CCK. Based on Experiment 2a, CCK sensitivity at 30min post 0.5μg/kg tretment, was chosen *a priori* for correlations with c-Fos IR. Treatment with 0.5μg/kg CCK significantly increased c-Fos IR in the NTS compared to saline [$t(22)=-2.331$, $p=0.029$]. In addition, individual differences in CCK-induced intake suppression positively correlated with individual differences in CCK induced c-Fos IR in the NTS [$R(17)=-0.51$, $p=0.02$, $r^2=0.26$] (Fig. 4B and 4C), revealing that rats with lesser CCK sensitivity had lower CCK induced c-Fos IR in the NTS while rats with greater CCK sensitivity showed greater CCK-induced c-Fos IR in the NTS.

Discussion

Experiments sought to determine whether individual differences in behavioral and physiological features of food intake control could predict individual differences in HFHSD-induced BW gain. Results identify individual differences in behavioral and physiological responses to exogenous diet/satiation signal challenges measured in outbred SD rats that predicted susceptibility to DIO. Chow-fed rats that were less sensitive to the sucrose intake inhibitory effects of treatment with CCK gained more weight during subsequent multi-week HFHSD maintenance than rats with greater CCK sensitivity. In addition, rats who consumed larger-sized and fewer numbers of meals during their initial day of HFHSD exposure, gained more weight during subsequent multiweek HFHSD maintenance than rats with opposite characteristics. Another predictor of larger weight gain was greater consumption of palatable diet (sucrose or HFHSD) during the first hour of the dark cycle. Together, these experiments identified individual differences in responsiveness to GI signals and palatable food that predicted DIO.

Differential sensitivity to CCK's suppressive effects on sucrose intake that predicted weight gain, was separately correlated with differential CCK treatment-driven activation of caudal NTS neurons. Rats with lesser behavioral CCK sensitivity also displayed lesser CCK-driven c-Fos IR in NTS neurons, with the opposite observed for rats showing greater sensitivity. CCK-induced intake inhibition results from CCK-1R mediated, vagal afferent excitation and glutamate release that activates caudomedial NTS neurons[19], whose axons project to local caudal brainstem neurons[20,21] and to basal forebrain neurons and circuits whose actions reduce meal size and cumulative food intake[20,22–28]. This result suggests that the biological basis of lesser CCK sensitivity in obesity prone rats might result from lesser CCK-1R expression on vagal afferents, as rats lacking CCK-1Rs show reduced CCK treatment driven c-Fos in the dorsal vagal complex[4].

Recently de Git et al (2018) identified that differential sensitivity to the intake suppressive effects of leptin predicted DIO, paralleling the relationship between differential sensitivity to CCK's effect on sucrose intake and DIO described here[29]. Chow-maintained Wistar rats showing lesser sensitivity to leptin's food intake inhibitory effect gained more BW when later maintained on an energy-dense diet, a finding consistent with data published by Ruffin et al. (2004) in Wistar rats and by Levin and Dunn-Meynall (2002) in SD rats using similar paradigms[30,31]. It is possible that individual differences in behavioral response to leptin and to CCK, that each predict DIO, are interrelated. Indeed, many publications have determined that the behavioral actions of leptin depend on its modulation of within-meal satiation signals like CCK[32–35]. De Git et al. attributed reduced leptin responsivity to reduced CNS leptin receptor signaling in the dorsomedial and ventrolateral hypothalamic nuclei, but did not examine contributions of extrahypothalamic sites[29]. While hypothalamic leptin receptor signaling has received attention for its possible role in mediating leptin-driven feeding suppression, a more compelling case has been made for hindbrain leptin receptor signaling (NTS and AP) in both leptin and CCK-induced feeding suppression. Forty percent of caudomedial NTS neurons activated by GI satiation signals express leptin receptors[35]. Hayes et al. (2010) showed that rats with a 40% knockdown in leptin receptor expression in NTS and AP, overate, became obese, and were unresponsive to CCK[14]. These data show that

reduction in NTS and AP leptin receptor signaling can explain reduced CCK sensitivity and thus an anatomical link between differences in leptin receptor signaling and differences in CCK treatment effects on food intake.

Two individual difference factors relating to consumption of palatable, energy-dense foods, predicted DIO. Rats who consumed greater amounts of palatable diet (HFHSD during initial exposure or sucrose) during the first hour of the dark cycle gained more weight after chronic HFHSD maintenance than rats consuming lesser amounts of HFHSD. Relatedly, chow-maintained rats who consumed larger-sized and fewer numbers of meals, during their initial day of exposure to HFHSD, gained more weight during subsequent HFHSD maintenance, further suggesting that energy-dense, palatable food intake (measured as cumulative intake or meal parameters) is a predictor of DIO. This is consistent with prior work showing that rats consuming larger meals over nine-day high-energy diet exposure exhibited larger changes in adiposity over this time[36]. To determine whether these differential responses were specific to palatable food, we tested whether cumulative chow intake during the first hour of the dark cycle predicted subsequent HFHSD-induced BW gain, but did not find such relationship. Additionally, 24h chow meal size and meal number did not correlate with BW gain. Such evidence suggests that greater responsiveness to palatable, energy-dense, food is a pre-existing factor that predicts obesity. This is consistent with prior work showing rats displaying greater cue-triggered approaches to a sucrose cue and to a food cup[37] and rats that work harder to obtain sucrose pellets[38] both gain more weight after HFHSD maintenance.

It is important to note that CCK sensitivity, a predictor of BW gain, was measured as percent suppression of sucrose intake, yet, short-term sucrose intake itself predicted DIO. For this reason, we separately evaluated whether individual differences in CCK-induced intake suppression of chow also predicted BW gain, but did not find a significant correlation. The non-significant correlation between CCK sensitivity on chow and BW gain cannot be explained by an absence of individual differences in either. In fact, there was greater variability in CCK-induced intake suppression of chow than of sucrose [SD= 36.7 and 22, respectively] suggesting that individual differences in CCK sensitivity is present regardless of diet type. Additionally, average %BW gain and variability in %BW gain were similar for both chow [M= 25.6; SD=5.8] and sucrose [M= 23.4; SD=4.5] groups, suggesting differences between the two groups in BW gained did not contribute to differential predictive relationships. Together, this suggests that diet type is the only meaningful difference between chow and sucrose groups, indicating that CCK sensitivity and responsivity to palatable diet interact and might not be independent obesity predictors. Future studies are needed to understand the mechanisms contributing to this interaction.

This study identified individual differences in responsivity to sensory cues associated with palatable foods affecting intake and meal size, and sensitivity to CCK-inducd intake inhibition predicted DIO. Rats that were less sensitive to CCK and who consumed more palatable food gained more BW after subsequent 5-week HFHSD maintenance. Humans consuming high-energy diet also display individual differences in BW gain, and recent evidence suggests that focusing on individual differences among patients with obesity, can inform treatment decisions to maximize weight loss. More specifically, Smith and

colleagues showed that humans with obesity with higher sweet preference lose significantly more BW after Roux-en-Y gastric bypass than others treated with vertical sleeve gastrectomy (K. Smith et al., unpublished). Additionally, Acosta et al. (2015) identified that patients with obesity with reduced sensitivity to satiety lose more weight in response to phentermine-topiramate treatment[39]. Thus, higher sweet taste responsivity and lesser responsivity to satiety, which we have also identified as predictors of BW outcome in our animal model, can be used to select obesity treatment to maximize weight loss in human populations, making our findings of clinical relevance.

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Study Importance Questions

- Outbred Sprague-Dawley rats, like humans, display individual differences in weight gain on high energy diet maintenance with some displaying and others resisting obesity.
- When maintained on energy dense diets, obesity prone rats are behaviorally and physiologically distinguishable from obesity resistant rats, but such differences could be consequences of the diet-induced obesity itself.
- We examined whether individual differences are present in chow-maintained Sprague-Dawley rats, prior to the differential obesity that would result from high energy diet maintenance and also asked whether observed difference predicted subsequent weight gain
- We found individual differences in responsivity to sensory properties of palatable food and sensitivity to the feeding inhibitory effect of CCK exist that predicted subsequent weight gain on energy dense diet maintenance
- In probing the neural basis for this predictive relationship, we found that rats with lesser sensitivity to the feeding inhibitory effect of CCK displayed reduced CCK-induced c-Fos activation in the NTS

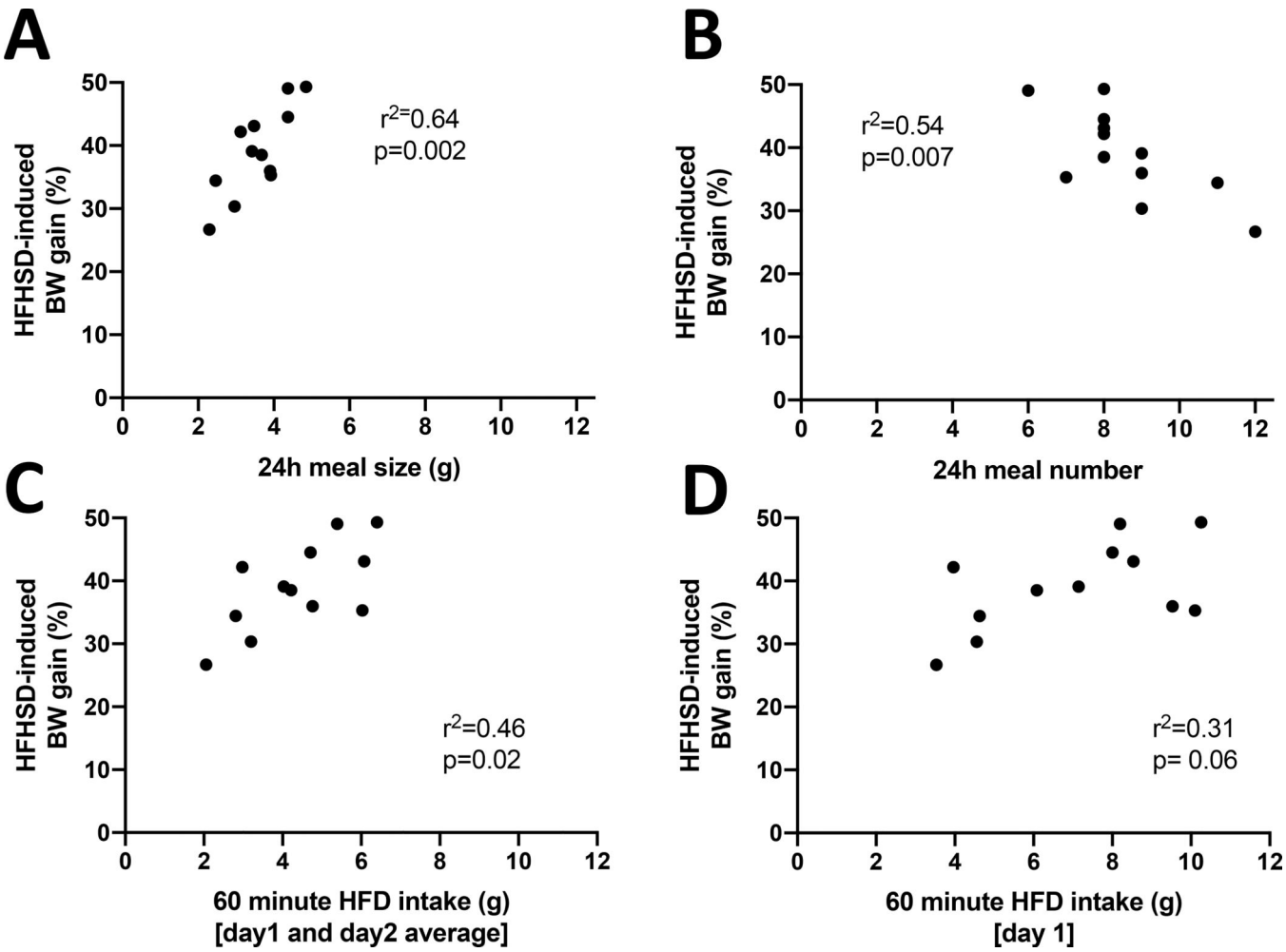

Figure 1:

Individual differences in HFHSD meal size, meal number, and early dark cycle cumulative intake predict HFHSD-induced BW gain. [A] 24h meal size (on the first day of HFHSD exposure) positively correlates with HFHSD-induced BW gain [B] 24h meal number (on the first day of HFHSD exposure) negatively correlates with HFHSD-induced BW gain [C] 60 minute HFHSD intake (an average of the first two days of HFHSD exposure) positively correlates with HFHSD-induced BW gain [D] 60 minute HFHSD intake (on the first day of HFHSD exposure) had a trend toward a positive correlation with HFHSD-induced BW gain. HFHSD-induced BW gain is measured as percentage BW gained after 5 weeks of HFHSD maintenance [32%kcal from fat].

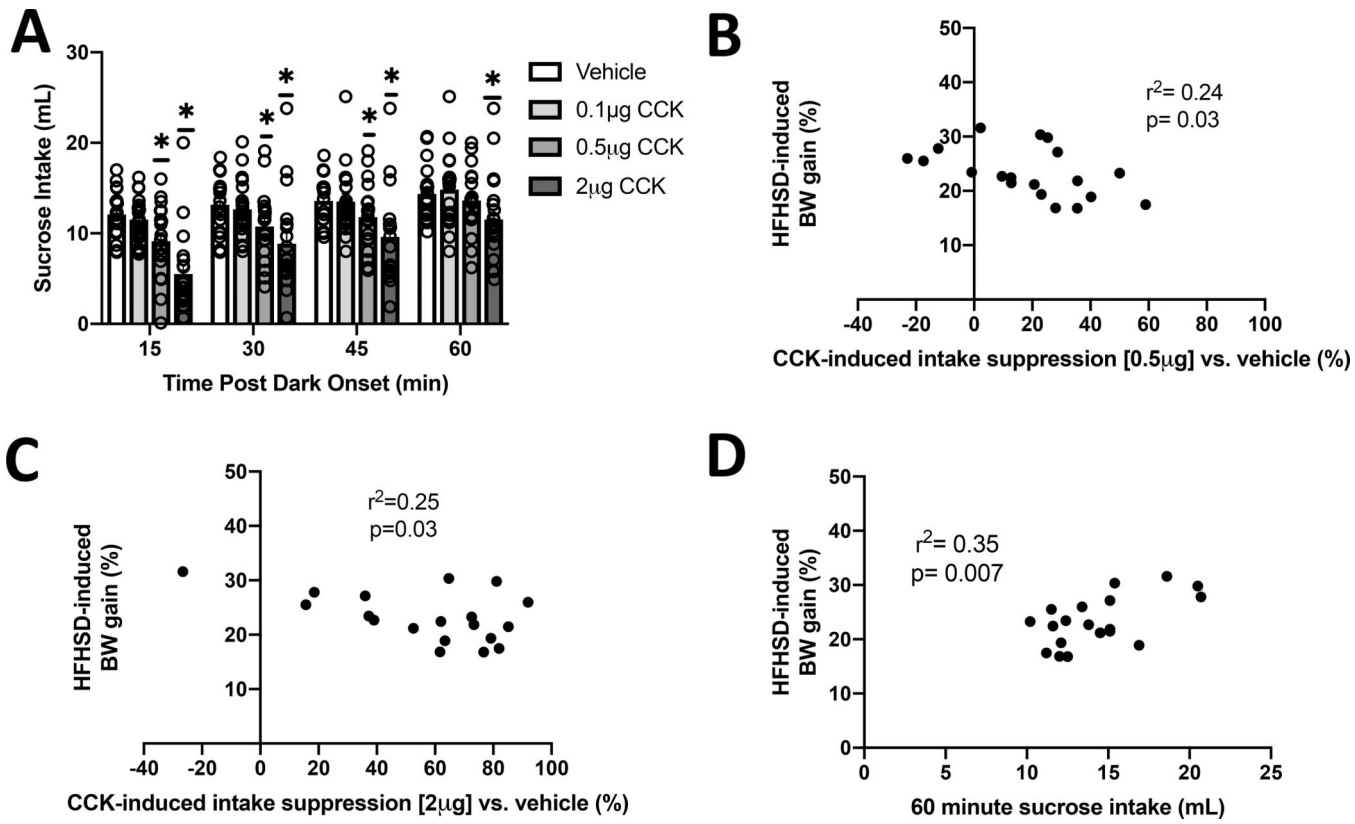

Figure 2:

Individual differences in CCK-Induced intake suppression of sucrose and sucrose intake itself predict HFHSD-induced BW gain. [A] CCK dose dependently reduces sucrose intake [B] Individual differences in sensitivity to the intake suppressive effects of 0.5μg/kg CCK at 30min post injection, and [C] individual differences in sensitivity to the intake suppressive effects of 2μg/kg CCK at 15min post injection negatively correlate with HFHSD-induced BW gain. [D] 60 minute intake of 15% sucrose solution, positively correlates with HFHSD-induced BW gain. CCK-induced intake suppression is measured as percent suppression of intake after injection of 0.5 or 2μg/kg CCK compared to vehicle treatment. Positive values are greater intake suppression and negative values are lesser intake suppression. BW Gain is measured as the percent BW gained after 5-weeks HFHSD maintenance [32% kcal from fat]. * indicates significant differences from vehicle treatment.

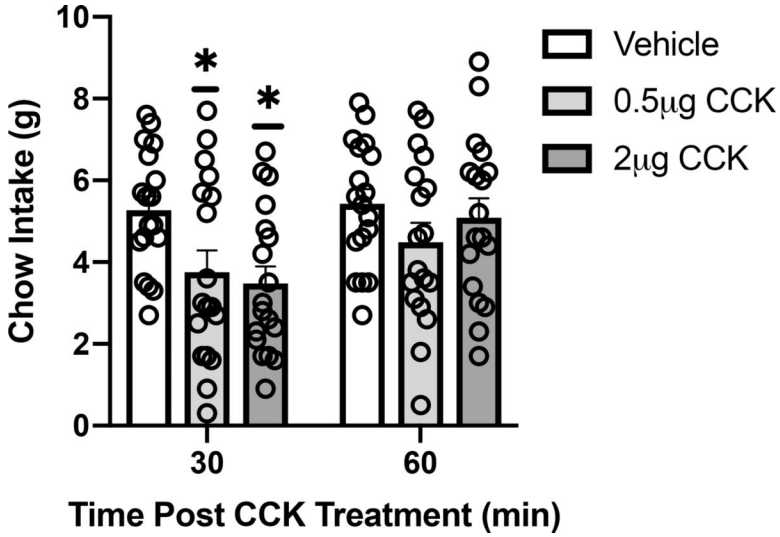

Figure 3:

CCK dose dependently reduces chow intake.

* indicates significant differences from vehicle treatment.

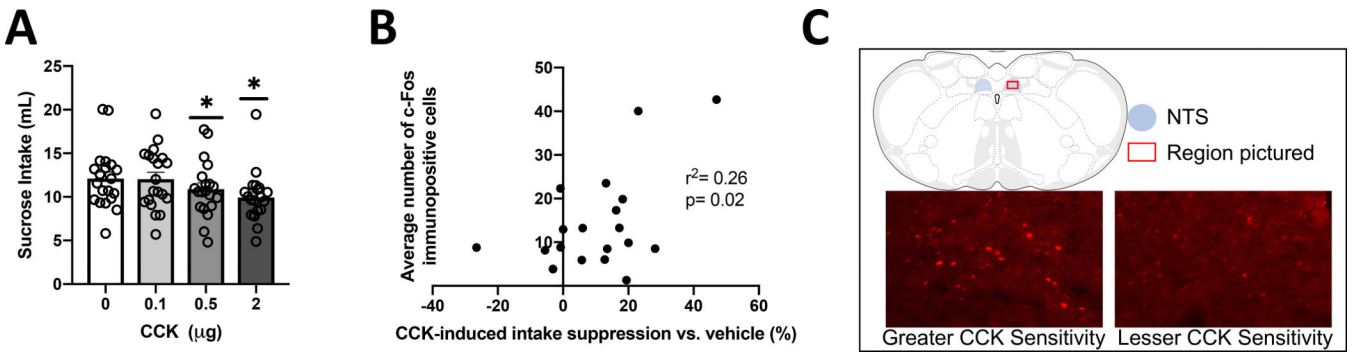

Figure 4:

Individual differences in CCK-induced intake suppression of sucrose solution correlate with individual differences in CCK-induced c-Fos activation in the NTS. [A] CCK dose dependently reduces sucrose intake [B] Individual differences in behavioral CCK sensitivity positively correlates with CCK-induced NTS neuronal activation. [C] Representative images of c-Fos immunoreactivity in rats with greater and lesser CCK sensitivity. CCK-induced intake suppression is measured as percent suppression of intake after injection of 0.5μg/kg CCK compared to vehicle treatment. Positive values are greater intake suppression and negative values are lesser intake suppression. Neuronal activation is measured as the total average number of c-Fos immunopositive cells in the NTS at the level of the area postrema. * indicates significant differences from vehicle treatment.

Table 1:

Individual differences in CCK-induced intake suppression of chow, 60 minute chow intake, and 24h average chow meal size and meal number do not predict HFHSD-induced BW gain.

	HFHSD- Induced BW gain (%)		
Chow Intake measurement	**R**	**r^2**	**p**
0.5μg CCK 30min	0.36	0.13	0.15
Vehicle 60min	0.30	0.09	0.22
Meal size 24h	0.09	0.01	0.77
Meal number 24h	−0.02	0.00	0.95