Chronic exposure to cafeteria-style diet in rats alters sweet taste preference and reduces motivation for, but not 'liking' of sucrose

Justine Fam[1], Kelly J. Clemens[1], R. Fred Westbrook[1], Margaret J. Morris[2], & Michael D. Kendig[2]*

1. School of Psychology, UNSW Sydney, NSW, 2052, Australia.

2. School of Medical Sciences, UNSW Sydney, NSW, 2052, Australia.

*corresponding author: m.kendig@unsw.edu.au

Declarations of interest: none

Funding: work supported by a UNSW Sydney School of Medical Sciences Accelerator grant to MDK, a National Health & Medical Research Council Project grant to MJM and RFW (APP1126929) and an Australian Research Council Distinguished Early Career Research award to JF (DE200100856).

Data availability statement: data are available on request from the corresponding author (m.kendig@unsw.edu.au)

Acknowledgements: the authors thank Ms. Lily Peng, Ms. Lucy Koh, Ms. Katarina Kikas and Mr. Nick Kennedy for assistance with feeding during the diet intervention.

Abstract

Obesity is associated with changes to taste perception and brain reward circuitry. It is important to understand how these effects alter the preference for palatable foods and drinks, given that these are widely consumed, and leading risk factors for obesity. This study examined the effects of diet-induced obesity on sweet taste preference by analysing the microstructure of licking for sugar solutions and assessing pERK expression in the nucleus accumbens shell and insula. Adult male Sprague-Dawley rats were fed standard chow (Control; n=16) or a varied, palatable cafeteria diet (Caf; n=16) for 12 weeks. Two-choice preference tests between 2%, 8% and 32% sucrose solutions were conducted at baseline and in weeks 11-12 of the diet. Rats in the Caf group trebled energy intake and doubled weight gain relative to controls. In tests held under water restriction after 11 weeks of diet, the Control group reliably preferred higher sucrose concentrations (i.e., 32% > 8% > 2%). Relative to controls, the Caf group showed a stronger preference for 32% vs. 2% sucrose, lower preference for 32% vs. 8% sucrose, and were indifferent to 8% vs. 2% sucrose. Testing without water restriction increased preference for higher sucrose concentrations in both groups. Chronic Caf diet increased the latency to lick, decreased total licks and reduced alternations between spouts, but did not alter lick cluster size, a measure of hedonic appraisal, on any test. Following a final exposure to a novel sucrose concentration, neuronal activity (pERK) in the insula and nucleus accumbens shell was significantly reduced in the Caf group. Results indicate that differences in 'liking' do not underlie obesity-induced changes to sweet taste preference.

269 words

1. Introduction

The widespread availability and consumption of palatable processed foods rich in fat and sugar contributes to high rates of obesity and metabolic disease around the world (Morris et al., 2015). Obesity in humans is associated with cognitive and neurological changes (O'Brien et al., 2017; Raji et al., 2010), such as impairments in forms of short-term memory and executive function (Prickett, Brennan & Stolwyk, 2015), decreased hippocampal volume (Yau et al., 2012; Jagust et al., 2005), increased brain inflammation (Thaler et al., 2012) and reduced availability of dopamine D2 (Wang et al., 2001) and mu-opioid receptors (Karlsson et al., 2015). However, evidence for effects of obesity on behaviour towards food-associated stimuli is mixed. On the one hand, recent meta-analyses of human data have failed to detect any association between obesity and attentional biases towards food cues measured in the dot-probe, Stroop, and eye-tracking tasks (19 studies; Hagan et al., 2020) or with neural responses to pictures of food (13 studies; Morys, Garcia-Garcia & Dagher, 2020). On the other hand, neuroimaging studies suggest that striatal activation and other neural responses to the sight and/or taste of palatable food predict prospective weight gain (Stice et al. 2010; Stice & Burger, 2019). More research is needed to understand how diet-induced obesity influences reward seeking and the neural correlates of these changes.

The effects of obesity on the rewarding properties of food can be studied by analysing sweet taste perception and preference. Understanding changes in sweet taste is important given that consumption of sugar-sweetened beverages is associated with an increased risk of obesity and metabolic and cognitive impairment in people and rodents (Hu, 2013; Maersk et al., 2012; Kendig, 2014). Notably, a stronger preference for sweeter solutions has been associated with prospective weight gain in populations genetically prone to developing obesity (Salbe et al., 2004). Evidence for an association between sweetness preference and obesity is also mixed: some studies have found increased sweet preference in people with obesity (Berthoud & Zheng, 2012; Bartoshuk et al., 2006; Nishihara et al., 2019), but others have found no differences (e.g., Drewnowski, Kurth, & Rahaim,

1991; Wooley, Wooley, & Dunham, 1972; Bobowski & Mennella, 2017). One reason for such conflicting evidence is the use of rating scales which may obscure differences in taste sensitivity due to between-subject variability in how sweetness is perceived (Bartoshuk et al., 2006). It is therefore key to develop alternative methods of taste measurement that can be linked to neural changes.

Animal models permit changes in sweet taste preference to be linked to neural substrates. The microstructure of rodents' licking behaviour provides information about the motivational value and palatability of the solution ingested. Rodents ingest fluids in clusters of licks, with increasing cluster size taken to reflect palatability of the solution (Davis, 1973; Davis & Smith, 1992; Dwyer, 2012). Lick cluster size increases monotonically for more concentrated sucrose solutions (Davis & Smith, 1992) and decreases with stronger concentrations of quinine (Hsiao & Fan, 1993). Higher sucrose concentrations also produce monotonic increases in orofacial responses indicative of liking (Grill & Norgren, 1978; Wilmouth & Spear, 2009), activity of the taste-sensitive chorda tympani nerve (Hagstrom & Pfaffmann, 1959) and the number of licks in brief (10-30s) exposure tests (Davis, 1973; Shin et al., 2011; Spector, Klumpp & Kaplan, 1998). Together, these data suggest that cluster size is closely related to the construct of 'liking' (Dwyer, 2012). Total intake of sucrose, however, is highest at 8-12% (w/v) concentrations (Richter & Campbell, 1940; Spector & Smith, 1983; Collier & Bolles, 1968) because satiety signals inhibit intake at higher concentrations. Removing such signals by implanting a gastric fistula results in higher intake of more concentrated solutions (Mook, 1963; Nissenbaum & Sclafani, 1987). Thus, the parameters of sucrose intake in rodents have been well characterised, making this a useful measure with which to examine the influence of diet and obesity on the perception of sweetness.

Obesogenic diets have produced mixed effects on sweet taste preference in studies employing rodent models. Shin et al. (2011) found that 16 weeks access to a purified high-fat, high-sugar diet increased 'liking' (measured by taste reactivity tests and the number of licks per 10s) for sucrose concentrations above 0.06M, and decreased liking for low sucrose concentrations (0.01M – 0.03M) in

rats, but increased the time taken to traverse a runway for a sweet reward (Froot Loops cereal). By contrast, 12 weeks access to a purified high-fat diet reduced total intake, burst size and burst number in mice exposed to 5, 10 or 20% sucrose solutions (Johnson, 2012). Ducrocq et al. (2019) also found that relative to chow-fed controls, mice fed a purified high-fat diet for 13 weeks exhibited fewer licks, fewer lick bursts, and longer latency to lick when drinking 10% sweetened condensed milk. However, no differences in burst size were observed. Obese-prone and obese-resistant rat strains fed a purified high-fat, high-sugar diet exhibited lower preference for a range of sucrose concentrations (0.0342 – 34.2%) versus water in preference tests conducted in the home-cage (Duca et al., 2014). Finally, Treesukosol et al. (2014) reported decreased sham-feeding (where ingested food bypasses the stomach) of 34.2% sucrose in rats given 7 weeks exposure to a purified high-fat, high-sugar diet supplemented with liquid *Ensure* relative to groups fed purified high-fat or control diets. Thus, past work indicates that diets high in fat and/or sugar reliably decrease consumption of sucrose solutions but have been variously shown to increase, decrease or have no effect on hedonic measures.

Few studies have explored the neural correlates of diet-induced changes to sweet taste preference. Sub-regions of the nucleus accumbens mediate both the incentive motivational properties ('wanting') and the hedonic properties ('liking') of rewards (Berridge, Robinson & Aldridge, 2009) and diets high in fat and sugar reduce evoked dopamine release (Geiger et al., 2009; Ducrocq et al., 2019), dopamine turnover (Davis et al., 2008) and expression of dopamine D1 (Alsiö et al., 2010) and D2 receptors (Johnson & Kenny, 2010) in these regions. Naneix et al. (2016) reported that young adult rats chronically exposed to a 5% sucrose solution in early adolescence (postnatal day 30-46) drank less sucrose or saccharin and exhibited fewer orofacial 'liking' reactions than control rats. This study also reported that these consummatory effects of chronic sucrose consumption were accompanied by reduced c-fos expression in the nucleus accumbens shell and core, but not in brain regions involved in taste processing, such as the insula.

Previous studies examining dietary effects on sweet taste preference have used homogeneous diets high in fat (Johnson, 2012; Ducrocq et al., 2017), fat and sugar (Duca et al., 2014; Treesukosol et al., 2015; Shin et al., 2011) or sugar alone (Naneix et al., 2016), which have yielded mixed effects on body weight gain. Here we examined sweet taste preference and neuronal activity in rats given chronic exposure to a cafeteria-style (Caf) diet containing chow and a range of commercially available, processed foods rich in fat and sugar. Two-choice preference tests between low, moderate and highly concentrated sucrose solutions at baseline and in weeks 11-12 of Caf diet exposure. We first tested rats under mild water restriction and then repeated the tests without restriction to examine whether any effect of the diet manipulation would be specific to deprivation state. Further, we tested under both restricted and unrestricted conditions in light of recent evidence in people that a liquid meal and water satiation had a greater effect on reducing 'wanting' for sugar-sweetened beverages than reducing 'liking' for these beverages (Pender, Stevenson, Francis & Oaten, 2019). Finally, we used phosphorylated extracellular-regulated kinase (pERK) as a marker of neuronal activity in the insula, a region involved in taste processing (Small, 2010) and the nucleus accumbens shell, a key hedonic 'hotspot' that regulates palatable food intake (Peciña & Berridge, 2005).

2. Materials and Methods

2.1 Subjects

Thirty-two experimentally naïve, adult, male Sprague Dawley rats were obtained from Animal Resources Centre (Perth, Western Australia) and were housed in groups of four in plastic boxes (67 cm depth x 40 cm width x 22 cm height) in an air-conditioned colony room maintained on a 12-hour reverse light cycle (lights on 7pm – 7am) at 22°C and 40-60% humidity. Rats were acclimated for 7 days, handled and weighed daily for a further 7 days, when mean body weight was 405g (SEM: 4.0, range 365-471g). Experimental procedures were conducted in accordance with the Australian code for

the care and use of animals for scientific purposes 8th edition (2013) and were approved by the Animal Care and Ethics Committee at UNSW Sydney (approval #19/114B).

2.2 Apparatus

Drinking tests were conducted in four identical Perspex chambers (30 x 30 x 30 cm) with floors of stainless-steel rods (1.5 mm diameter, spaced 10 mm apart). Two stainless steel drinking spouts were located on the front wall of the chamber (50 mm apart, 20 mm from the floor). Each spout was connected via silicon tubing to its own peristaltic pump which delivered sucrose from a beaker when activated. An electrical sensor on each spout detected licking to the nearest 1ms and activated fluid delivery. Two plastic shields were mounted to the left and right of each spout to prevent rats grasping the spout and triggering the electrical sensor. Chambers were located in separate compartments of a sound-attenuating cabinet. Data were recorded using custom-written programs in MATLAB (The MathWorks, Inc.).

2.3 Procedures

Drinking tests were 15 minutes in duration and were held once daily between 0900-1300h. Water restriction was implemented to induce a mild deprivation state to ensure appreciable amounts of licking in the drinking chambers. Food access was never restricted. Rats were adapted to 2h per day of water access in the home cages for 5 days and maintained on this fluid schedule across testing unless otherwise specified. Access to water occurred approximately 2 hours after each test. During the 11-week diet exposure between pre- and post-diet testing, water was available ad-libitum.

2.3.1 Spout training (4 sessions): Prior to baseline preference tests, rats were acclimated to the apparatus and sucrose solutions in 4 consecutive daily sessions where spouts delivered water (day 1), 2% sucrose (day 2), 8% sucrose (day 3) and 32% sucrose (day 4). Sucrose solutions consisted in sugar dissolved in tap water (w/v). We chose to test 2%, 8% and 32% sucrose solutions because these concentrations are linearly related by a factor of 4 (i.e., 2% x 4 = 8%, 8% x 4 = 32%) and have been

used extensively to study sweet taste preference in rats through one- and two-bottle preference tests, sham-feeding experiments, instrumental response tests and runway mazes (see Sclafani, Hertwig, Vigorito & Feigen, 1987; Sclafani, 1987, for review).

2.3.2 Baseline preference testing (3 sessions): Rats received two 2-choice preference tests between 8% and 32% sucrose on consecutive days under water restriction, where the location of 8% and 32% was swapped from day 1 to day 2. To assess whether preference was affected by water-restriction, rats were provided with ad-libitum access to water in their home cages and tested again on day 3.

2.3.3 Group allocation: Rats were matched on baseline body weight, licks during spout training, and baseline preference for 8 vs. 32% sucrose solution, then allocated to groups fed chow (Control, n = 16 rats) or Cafeteria diet (Caf, n = 16 rats). Matching was successful as there were no significant group differences in baseline measures of sucrose preference or intake (all $t_{(30)} < 1$, all $p > 0.40$).

2.3 Cafeteria diet (12 weeks): Both groups received ad-libitum access to chow (Specialty Feeds, 14 kJ/g) and water, except during water-restricted tests. Caf diet comprised chow plus 4-5 commercially available savoury and sweet foods each day, including meat pies, dim sims, chips, cakes, and cookies. Caf diet was replenished daily, with no food presented on more than 2 consecutive days (see Leigh, Kendig & Morris, 2019). Food intake was measured once per week by weighing individual food items (to the nearest 0.1g) for each cage; 24-h later, all remnants were carefully collected from the bedding, sorted, and re-weighed. Total energy intake was estimated by calculating the difference in weights for each food and converting to kJ using manufacturers' information.

2.3.5 Post-diet water re-training (2 sessions; week 11): Post-diet testing began after 10 weeks of dietary exposure. To re-acclimate rats to the drinking chambers, rats were placed on the water

restriction schedule for 3 days prior to 2 re-training sessions where water was available from both spouts.

2.3.6 Post-diet preference testing (6 sessions; week 11-12): Three sucrose preference tests were conducted under water restriction comparing 2% vs. 8%, 8% vs. 32%, and 2% vs. 32% sucrose concentrations. Test order was counterbalanced within each group, and the spout containing the higher concentration alternated across tests. Results were not significantly altered by test order. Home-cage water access was then provided *ad libitum*, and the three preference tests were repeated in the manner described.

2.3.7 Final consumption test and transcardial perfusion: At the time of the final test, total intake of the 2%, 8% and 32% sucrose solutions differed widely between rats, and using one of these would have complicated interpretation of the pERK data. Therefore, we presented a novel 20% sucrose solution at the midpoint of the two most-preferred concentrations (8% and 32% sucrose). Immediately after this test, rats were anesthetized via intraperitoneal injection of sodium pentobarbital (Lethabarb) and transcardially perfused with ice-cold 4% paraformaldehyde in 0.1M sodium phosphate buffer (PFA). Perfusions began 5-8 min after the test to coincide with peak induction of pERK (Gao & Ji, 2009). Brains were extracted, post-fixed overnight in PFA solution at 4°C and placed in 30% sucrose solution for 48-72 hours. Brains were then embedded in OCT compound, frozen, and sectioned coronally at 40 µm using a cryostat (Leica Microsystems CM1950). Sections were stored in cryoprotective solution (30% ethylene glycol, 30% glycerol, 0.1M sodium phosphate buffer) at -20°C until processing.

2.3.8 Immunofluorescence: Sections were rinsed in Tris-buffered saline (TBS) three times (10 min each) and incubated for 2 h at room temperature in TBS with 0.2% Triton X-100 for membrane permeabilization. Sections were then rinsed 3 times in TBS (10 min) and then incubated in monoclonal rabbit anti-phospho-p44/42MAPK(Erk1/2) primary antibody (1:300, #4370 Cell Signaling

Technology, diluted in TBS) for 24 h at 4°C on a rocking platform. Next, sections were rinsed three times with TBS (10 min) and incubated in donkey anti-rabbit Alexa Fluor-488 secondary antibody (1:400, #A21206, Invitrogen, diluted in TBS) for 1 h at room temperature. Finally, sections were rinsed three times in TBS (10 min), mounted onto Superfrost Plus-coated slides (Thermo Fisher Scientific), dried and cover-slipped with Vectashield Hardset mounting medium (Vector Laboratories).

2.3.9 Cellular quantification: Fluorescent imaging was acquired with a confocal microscope (Olympus FV1200) using a 10x objective. For each rat, 4 sections were selected to encompass the insular cortex and rostral nucleus accumbens shell along the anterior-posterior axis according to boundaries defined by Paxinos and Watson (2006). Open Source ImageJ software (MacBiophotonics upgrade v. 1.43u, Wayne Rasband, National Institutes of Health, Bethesda, MD) was used to determine cell counts, which were performed by a trained observer who was blind to group allocation. Regions of interest (ROI) were first manually selected corresponding to the brain region of interest, and then the number of pERK-immunoreactive cells within the ROI were quantified. Cell counts were taken from a single confocal plane and were expressed relative to the size of the ROI (mm^2).

2.4 Data analysis

Licks during preference tests were expressed as a proportion, calculated as the number of licks on the spout delivering the higher sucrose concentration divided by the sum of licks on both spouts. The inter-lick interval criteria used to define lick cluster size was determined by examining the frequency distributions of inter-lick intervals during pre-diet testing. A criterion of 0.5s was chosen, as this captured > 92% of inter-lick intervals (Supplementary Table 1). Varying this criterion did not alter any results (Supplementary Figure S3). We also explored whether the distribution of inter-lick intervals varied between groups. As shown in Supplementary Figure S4, the distributions of inter-lick intervals were comparable for Chow and Caf groups for every test, suggesting that the diet did not

alter oromotor behaviour related to fluid intake. Finally, the pattern of differences in the number of lick clusters was closely related to the pattern of differences in total licks, as shown in Figure S5.

Data were analysed using SPSS (IBM, v26). Body weight, energy intake and preference test data were analysed in mixed-model analyses of variance (ANOVA) with group (chow or Caf) as the between-subjects factor and 'test', 'spout' or 'time' as the repeated measures factor, as appropriate. One rat in the Caf group did not lick on two of the unrestricted preference tests, thus degrees of freedom differ slightly for measures collected on these tests. Mann-Whitney U-tests were used for latency data, which violated assumptions of normality. The cage was the unit of analysis for energy intake data across the experiment. Post-hoc pairwise comparisons controlled with the Bonferroni correction were used to determine the source(s) of significant interactions. The criterion for rejection of the null hypothesis was set at .05 (two-tailed). All data are shown as mean ± SEM.

3. Results

3.1 Sucrose exposure and baseline preferences

As shown in Supplementary Figure S1, total licks increased significantly across sessions with 2%, 8% and 32% sucrose ($F_{(2, 60)} = 31.22$, $p < .001$). Relative to 2%, rats licked significantly more for 8% ($F_{(1, 30)} = 63.75$, $p < .001$) and 32% ($F_{(1, 30)} = 43.96$, $p < .001$) with no difference between total licks for 8% and 32% ($F < 1$). Lick cluster size differed significantly between concentrations ($F_{(2, 60)} = 5.90$, $p = .005$), with larger clusters for 32% than 8% sucrose ($F_{(1, 30)} = 4.34$, $p = .046$) or 2% ($F_{(1, 30)} = 7.41$, $p = .011$) and a trend towards larger clusters for 8% than 2% sucrose ($F_{(1, 30)} = 4.03$, $p = .054$). These effects did not differ between rats subsequently allocated to chow and Caf groups ($F < 1$ for all group x test interactions).

Supplementary Figure S2 shows results from pre-diet preference tests between 8% and 32% sucrose solution. On average, there was no preference for 32% over 8% sucrose in tests under water

restriction (mean preference for 32% sucrose = 50.1 ± 3.3%; $t_{(31)} < 1$, $p = .99$), but there was significant preference for 32% over 8% sucrose when rats were tested with *ad libitum* home-cage water access (mean preference = 82.0 ± 2.6%; $t_{(31)} = 12.54$, $p < .01$). This difference was statistically significant ($F_{(1, 30)} = 100.43$, $p < .001$) and did not differ between rats allocated to chow and Caf groups (preference x group interaction, $F < 1$).

3.3 Diet intervention

Energy intake and body weight during the diet intervention are shown in Figure 1. Energy intake was significantly higher in the Caf group ($F_{(1,6)} = 214.87$, $p < .001$) with no significant changes over time ($F_{(11, 66)} = 1.35$, $p = .22$) and no group x time interaction ($F_{(11, 66)} = 1.28$, $p = .26$). Body weight significantly increased across time (linear trend: $F_{(1, 30)} = 357.01$, $p < .001$) with a significantly greater increase in the Caf than chow group (group x time linear interaction trend: $F_{(1, 30)} = 248.65$, $p < .001$). Mean body weight was approximately 30% greater in the Caf than the chow group at endpoint (Caf: 810.2 ± 30.2g; Chow: 621.2 ± 15.8g; $F_{(1, 30)} = 30.80$, $p < .001$).

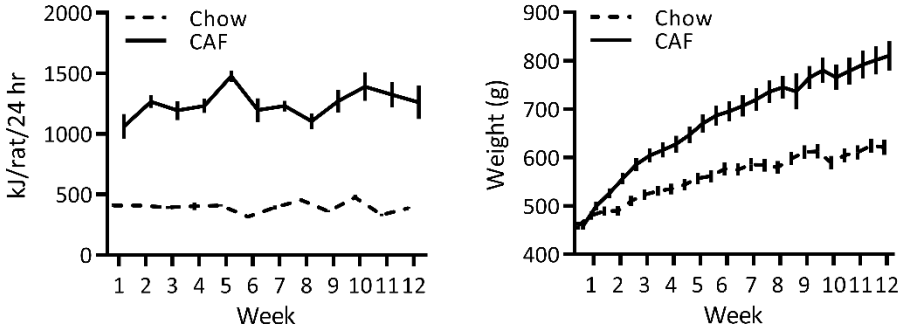


Figure 1. Energy intake and body weight across the diet intervention. Energy intake (left) was doubled by Cafeteria diet exposure and remained stable across the 12-week diet intervention, with a corresponding increase in body weight gain (right panel). $n = 16$/group. Data show mean ± SEM.

3.4 Post-diet preference tests

Sweet taste preference was assessed in weeks 11 and 12 of the diet intervention with daily tests of 2% vs. 8%, 8% vs. 32%, and 2% vs. 32% sucrose solutions, held first under water restriction and then repeated with *ad libitum* access to water in the home cage. The order of tests and the left/right position of the solutions was counterbalanced within each group.

3.4.1 Restricted preference tests

Figure 2 shows data from the three preference tests held under water restriction. The primary measure was the proportion of total licks of the higher concentration of sucrose in each test, shown in Figure 2A. Preference for the higher concentration of sucrose differed significantly between the three restricted tests ($F_{(2, 56)} = 10.85$, $p < .001$), with trends towards a test x group interaction ($F_{(2, 58)} = 2.47$, $p = .09$) and a group main effect ($F_{(1, 29)} = 3.28$, $p = .08$). Pairwise comparisons applying the Bonferroni correction indicated that relative to controls, the Caf group exhibited higher preference for 32% over 2% sucrose ($F_{(1, 29)} = 4.89$, $p = .035$) and lower preference for 32% over 8% sucrose ($F_{(1, 29)} = 4.93$, $p = .034$). Preference for 8% versus 2% sucrose did not differ between groups ($F_{(1, 29)} = 1.39$, $p = .25$). One-sample *t*-tests were then run to evaluate whether the preference for the higher sucrose concentration was statistically significant within each group (i.e., vs. no preference or 0.5). These showed that the Caf group showed no preference in the 8% vs. 2% sucrose test ($t_{(14)} = 0.57$, $p = .58$), but that both groups significantly preferred the higher concentration in the 32% vs. 8% and 32% vs. 2% tests (all $t_{(15)} > 2.35$, $p < .05$).

In all three tests, the Caf group exhibited longer latencies to first lick (Fig. 2B, Mann-Whitney U-tests; all $p < .01$), alternated less frequently between spouts (Fig. 2C, all $F_{(1, 30)} > 6.96$, $p < .013$) and made fewer licks (Fig. 2D, all $F_{(1, 29)} > 6.22$, $p < .019$) than the chow group. Lick cluster size in each test is shown in Figure 2E and was analysed in a mixed ANOVA (concentration x group). In the 8% vs. 2% test, cluster size did not differ significantly between concentrations, groups, or by the group x concentration interaction (all $F < 1$). Cluster size was significantly larger for 32% in the test against

8% sucrose ($F_{(1, 28)}$ = 30.06, p < .001) and in the test against 2% sucrose ($F_{(1, 28)}$ = 26.81, p < .001) but did not differ between groups on either test (all F < 1).

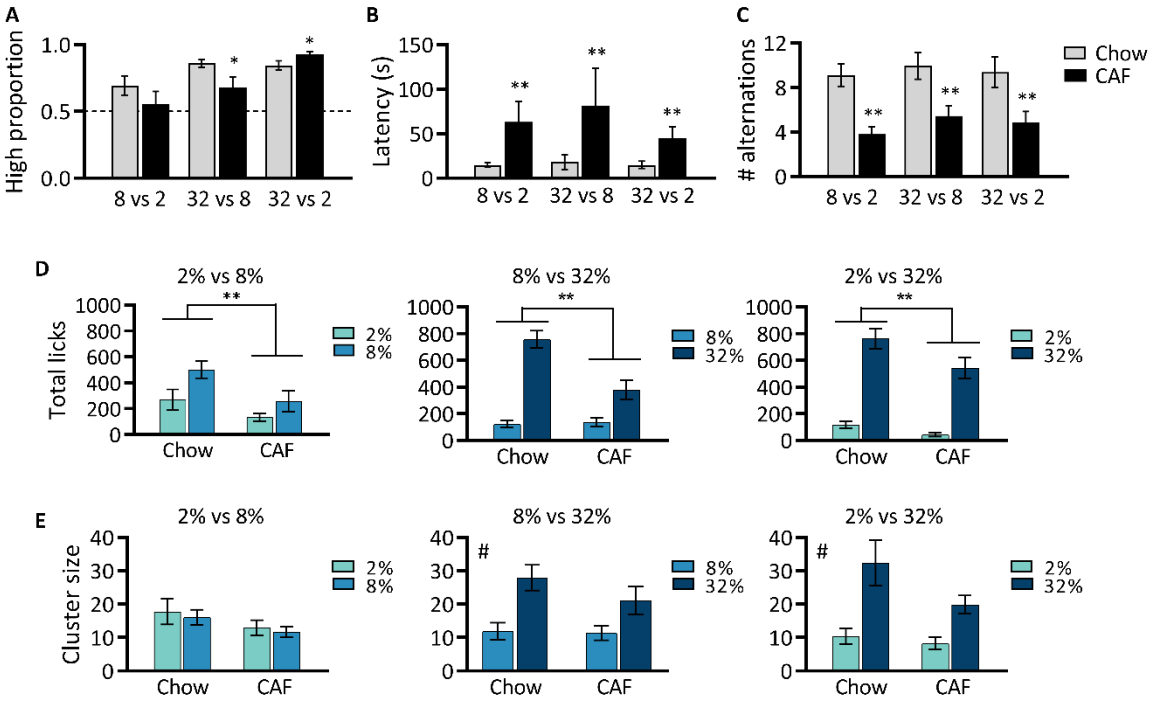


Figure 2. Sucrose preference and liking during three preference tests held under water restriction. Panel A shows the proportion of total licks made on the spout delivering the higher sucrose concentration. Caf diet decreased the preference for 32% vs. 8% and increased preference for 32% vs. 2% sucrose. Caf diet also increased the latency to engage in licking (B), reduced alternations between spouts (C) and decreased total licks (D) relative to group Chow. Lick cluster size did not vary between groups on any test but was higher for 32% vs. both 8% and 2% sucrose (E). *p < .05, **p < .01 vs. Chow group; #p < .01, main effect of sucrose concentration. Figures show mean ± SEM.

3.4.2 Unrestricted preference tests

Licking for sucrose in the three unrestricted preference tests is shown in Figure 3. Sucrose preference differed between the three tests (Fig. 3A; $F_{(2, 56)}$ = 3.82, p = .028) with a significant group x test interaction: $F_{(2, 58)}$ = 3.48, p = .038) but no group main effect ($F_{(1, 29)}$ = 2.81, p = .11). The significant interaction was driven by significantly lower preference for 8% over 2% sucrose in the Caf group ($F_{(1, 29)}$ = 4.25, p = .049). Preference for 32% vs. 8% and for 32% vs. 2% sucrose did not differ

between groups (both $F < 1$). One-sample *t*-tests confirmed that both chow and Caf groups showed significant preferences for the higher concentration in all three tests (smallest $t_{(13)} = 4.97$, $p < .01$). As in the restricted tests, Caf diet increased the latency to lick in each test (Fig. 3B; Mann-Whitney U-tests; all $p < .01$) and reduced alternations between spouts on the 8% vs. 2% test (Fig. 3C; $F_{(1, 30)} = 9.26$, $p < .01$), 8% vs. 32% test ($F_{(1, 30)} = 4.44$, $p = .044$) but not on the 2% vs. 32% test ($F_{(1, 30)} = 1.18$, $p = .29$). Total licks (Fig. 3D) were significantly reduced in the Caf group in tests between 2% vs. 8% ($F_{(1, 28)} = 19.36$, $p < .01$), 8% vs. 32% ($F_{(1, 28)} = 5.94$, $p = .021$) and 2% vs. 32% sucrose ($F_{(1, 28)} = 6.58$, $p = .016$). Cluster size (Fig. 3E) was significantly larger for the high sucrose concentration in every test (8% > 2%: $F_{(1, 28)} = 28.06$, $p < .001$; 32% > 8%: $F_{(1, 28)} = 56.02$, $p < .001$); 32% > 2%: $F_{(1, 28)} = 45.49$, $p < .001$), but there were no differences between chow and Caf groups (all $F < 1$).

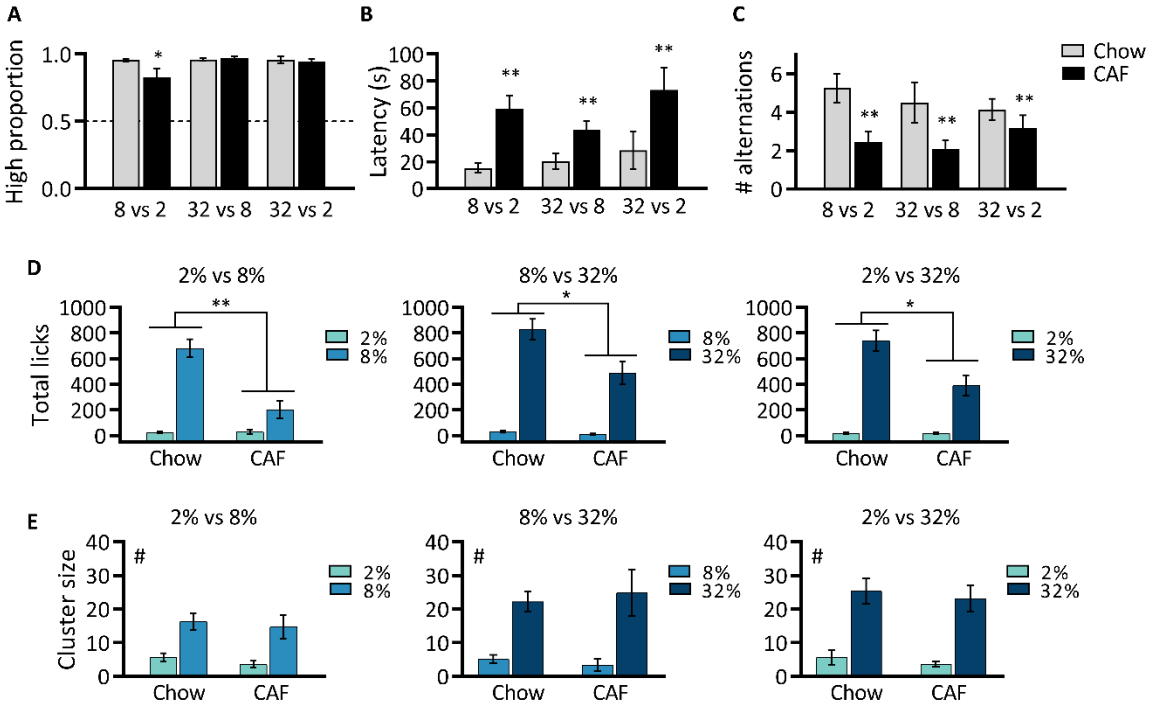


Figure 3. Licking during the three unrestricted preference tests. Caf diet significantly reduced the preference for 8% over 2% sucrose (A). Latency to lick (B) and alternations between spouts (C) were significantly reduced and increased, respectively, in the chow relative to Caf group. Cluster size was significantly greater for the higher concentration in each test (E) and did not differ between groups. *$p < .05$, **$p < .01$ vs. Chow group; #$p < .01$, main effect of sucrose concentration. All bars indicate mean ± SEM.

3.4.3 Contrast effects in sucrose 'liking'

The response to a reward can be influenced by the available alternatives, so-called simultaneous incentive contrast (Flaherty, 1982). Here, each sucrose concentration was tested twice; for example, 8% sucrose was presented with 2% and, separately, 32% sucrose. Thus, we examined whether cluster size for each solution differed according to the alternative and whether this was modulated by diet history. Figure 4 shows cluster sizes for 2%, 8% and 32% sucrose averaged across restricted and unrestricted tests, with cluster size for each concentration analysed in a 2 x (2) ANOVA (group x [alternative]). Cluster size for 2% sucrose tended to be higher when 8% sucrose was the alternative, relative to 32% sucrose ('test' main effect; $F_{(1, 29)} = 3.37$, $p = .076$. Cluster size for 8% sucrose was significantly higher in the test against 2% sucrose than in the test against 32% sucrose ($F_{(1, 29)} = 14.68$, $p = .001$), while cluster size for 32% sucrose did not differ between tests where 2% and 8% sucrose were the alternatives ($F < 1$). There were no significant group main effects or group x test interactions for any concentration (largest $F_{(1, 29)} = 3.38$, $p = .08$).

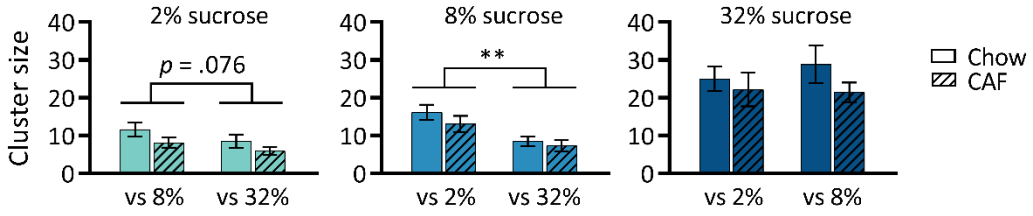


Figure 4. Comparing lick cluster size to evaluate incentive contrast. Rats licked in larger clusters for 8% sucrose when presented with a weaker alternative (2% sucrose) than a stronger alternative (32% sucrose) (middle, $^{**}p < .01$, test main effect). The alternative solution did not significantly change cluster size for the weakest (2%; left) and strongest concentration (32%; right) and there were no significant group differences in sensitivity to contrast. All bars indicate mean ± SEM.

3.4.4 Final test

Figure 5 shows data from the final 15-min consumption test, where a novel 20% sucrose solution was presented immediately prior to euthanasia. The Caf group exhibited a similar pattern of licking as in preference tests, with fewer total licks (Fig. 5A, $F_{(1, 30)} = 20.93$, $p < .01$), higher latency to lick (Fig. 5B, Mann-Whitney U: $p = .014$), and no group difference in cluster size relative to controls (Fig. 5C, $F < 1$). Notably, cluster size appeared significantly higher for this novel 20% sucrose solution than for the 32% solution presented in previous tests (Fig. 5C, cf. Fig. 2 & 3). Neuronal activation in the insula and nucleus accumbens shell, as indexed by immunofluorescent staining for pERK, is shown in Figure 5D, with representative images shown in Figure 5E. Cell counts (# positive cells/mm^2) in both regions were compared between groups using a 2 x (2) ANOVA (group by [region]), with total licks entered as a covariate to control for the group difference in this variable. pERK expression was significantly lower in the Caf than chow group ($F_{(1, 29)} = 5.15$, $p = .03$) with no effect of total licks ($F < 1$). The region main effect, region x total licks and region x group interactions were not significant (largest $F_{(1, 29)} = 2.94$, $p = .10$). Subsequent analyses confirmed that Caf diet significantly reduced pERK expression in both the insula ($F(1, 30) = 5.20$, $p = .03$; Figure 5D) and accumbens shell ($F(1, 30) = 12.35$, $p = .001$; Figure 5D). Finally, we examined partial correlations between insula and shell pERK, cluster size, latency to first lick, and terminal body weight, controlling for total licks. Expression in the insula and nucleus accumbens shell correlated positively (Spearman's rho (ρ) = 0.54. $p = .002$) but neither was associated with cluster size, latency, or body weight (largest $\rho = -0.31$, $p = .10$).

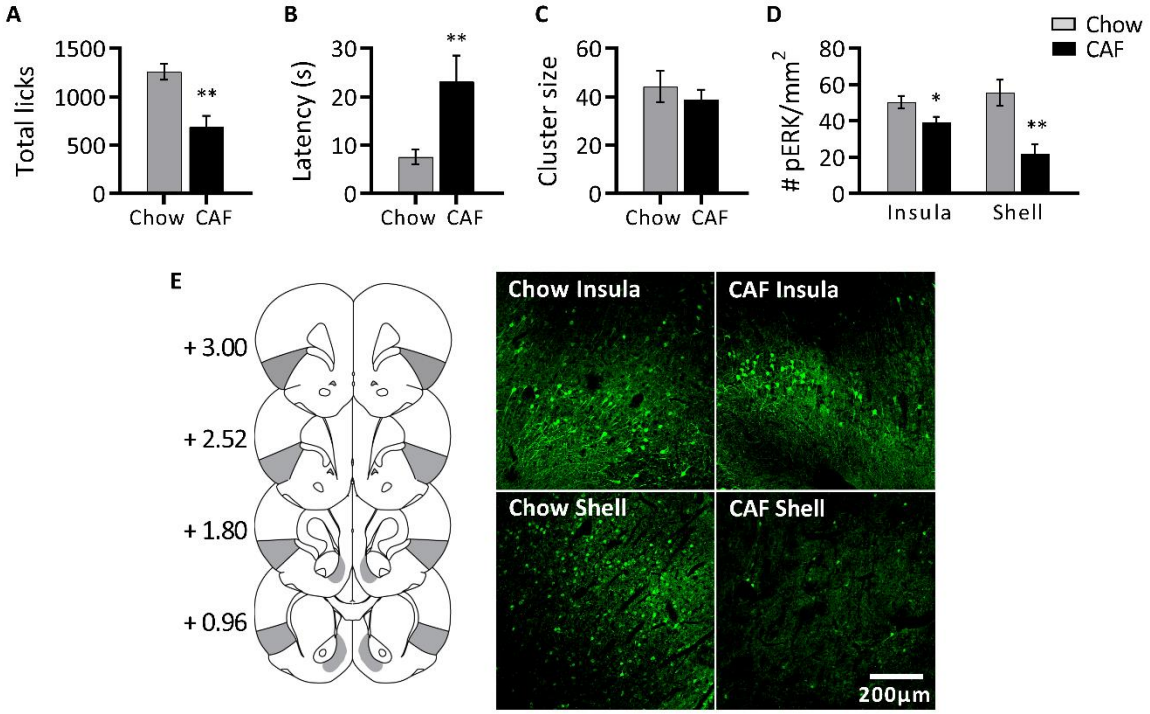


Figure 5. Final test data. Rats were perfused immediately following exposure to a novel 20% sucrose solution for pERK immunofluorescent quantification in the insula and nucleus accumbens shell. Total licks were halved in the Caf group (A) and latency to lick was almost trebled (B), with no difference in cluster size (C). Neuronal activity was significantly reduced by Caf in the insula and accumbens shell (D). Grey regions indicate areas where pERK was quantified (E). *$p < .05$, **$p < .01$ vs. Chow group. All bars indicate mean ± SEM.

4. Discussion

4.1. Cafeteria diet shifted preference and reduced motivation for sucrose

Obesity-associated changes to brain reward circuitry are thought to foster increased consumption of palatable foods rich in sugar and fat, but evidence for this hypothesis is mixed. Most studies in rats and mice have used homogeneous purified diets that do not encapsulate the variety of foods characteristic of the modern human diet. To our knowledge this is the first study to characterise changes in sweet taste preference and neuronal activity with a diet-induced, rodent obesity model involving a range of highly palatable, processed foods eaten by people. Chronic exposure to this Caf diet increased preference for higher sucrose concentrations over weak alternatives (i.e., 32% vs. 2%) and reduced preference against moderately concentrated alternatives (i.e., 32% vs. 8%) relative to

chow-fed controls. In tests between the two lower concentrations (8% vs. 2%), Caf-fed rats showed no preference under water restriction and a weaker preference than controls when tested without water restriction. Our results are analogous to those reported by Shin et al. (2011), who observed higher lick rates for concentrated sucrose solutions and lower lick rates for weak sucrose solutions in diet-induced obese rats, relative to controls. If confirmed in people, this shift in preference towards highly concentrated sucrose solutions could manifest as the selection of unhealthy food options in day-to-day life.

In addition to the effects of diet, our preference test data showed that removing water restriction increased preference for the higher sucrose concentration available. This result is analogous to prior work showing that non-deprived rats exhibit a preference for sucrose solution over water at lower concentrations than water-restricted rats (0.43% vs. 1.28% sucrose solution; Beck et al., 1965). Overall, a preference for higher sucrose concentrations was still evident under water restriction, as shown previously in two-choice preference tests (Beck & Nash, 1969) and two-choice discrimination tasks (Cohen & Hachey, 1980).

Our use of two-choice preference tests, where rats could freely switch between spouts, enabled the temporal components of licking behaviour to be analysed alongside overall preference and lick microstructure. This approach revealed that Caf diet-induced changes to sweet taste preference occurred against a backdrop of substantial changes in the motivation to seek out and consume sucrose. Latency to lick was tripled and total licks were halved in the Caf group in both restricted and unrestricted tests. The use of two-choice preference tests also permitted analysis of how frequently rats alternated between spouts, a measure which, like total licks, was approximately halved in rats fed Caf diet in all tests. Thus, we believe that in this case, fewer alternations in Caf rats was a function of their fewer licks overall, reflecting similar underlying motivational changes. However, differences in alternation when groups are matched on total licks could be interpreted in other ways (e.g. the tendency to explore vs. exploit reward locations). Our results are consistent with previous studies of sweet taste

preference in obese rats and mice (Ducrocq et al., 2019; Duca et al., 2014; Johnson, 2012) and agrees with past work showing decreased motivation following diet-induced obesity, such as reduced progressive ratio breakpoints (Tracy et al., 2015; Tantot et al., 2017), increased runway transit time (Shin et al., 2011) and reduced conditioned place preference (Lesser et al., 2017) for food reward. The effects of obesogenic diets on progressive ratio tests of motivation appear to be biphasic, with breakpoints for food reward increased after short diet durations (3-5 weeks; la Fleur et al., 2007; Figlewicz et al., 2013) and decreased after longer diet durations (~12 weeks; Davis et al., 2008; Finger et al., 2012). These results are in line with the blunted motivation for sucrose observed in the present study, which extends prior work by demonstrating that these effects hold when a cafeteria diet is used (vs. purified diets) and in tests where rewards are freely available.

Here we observed pronounced reductions in motivation to consume sucrose in Caf-fed rats despite pre-diet exposure to 2%, 8% and 32% sucrose. This is noteworthy in light of prior work showing that pre-exposing rats to reward pellets prior to 10 weeks' access to a purified high-fat diet prevented the diet-induced reduction in progressive ratio breakpoints for these pellets (Tracy et al, 2015). Our data may imply that this pre-exposure effect applies only in instrumental tasks where effort must be expended to obtain rewards. Differences in diet palatability may also explain the absence of a pre-exposure effect in the present study: while Tracy et al. (2015) used a purified high-fat diet that may have been less palatable than the sucrose-sweetened reward pellets used in tests, the Caf diet used here likely matched or exceeded the palatability of the sucrose solutions tested.

4.2. Caf diet reduces neuronal activity in response to novel sucrose concentrations

Chronic Caf diet reduced neuronal activity in the nucleus accumbens shell and insula following consumption of a novel 20% sucrose solution. Few previous studies have directly compared neuronal activity in these regions, which are key components of brain 'wanting' and 'liking' circuitries (Berridge et al., 2009). Fry et al. (2020) found that cluster size for a flavour previously paired with

456 sucrose correlated with activity in the insula, but not in the nucleus accumbens. The insula is thought
457 to encode the sensory properties of flavours (Fry et al., 2020; Yamamoto et al., 1989) and is activated
458 by sucrose and other taste modalities, as indexed by *in vivo* optical imaging (Accolla, Bathellier,
459 Petersen & Carleton, 2007). Naneix et al. (2016) showed that adolescent exposure to sucrose reduced
460 c-fos activation in response to sucrose in the nucleus accumbens shell and core, but not insular cortex
461 (Naneix et al., 2016). The longer diet exposure and robust obesity produced by Caf diet exposure may
462 explain the reduced expression in the insula observed here. Clarifying the time-course of dietary effects
463 on neuronal activity is an important future direction. While our focus here was on acute neural response
464 to sweet taste, long-term exposure to high-fat diets has also been shown to downregulate dopamine D1
465 and cannabinoid CB1 receptor expression in the nucleus accumbens of rats (Arcego et al., 2020).

466 In this study access to Caf diet continued throughout preference tests and the final exposure to
467 a novel sucrose concentration. Given that acutely switching from an obesogenic diet to regular chow
468 can normalise measures of sucrose preference in obese rats and mice (Johnson, 2012; Shin et al., 2011),
469 future work should evaluate whether the substantial reduction in neuronal activity observed under the
470 present conditions is maintained after withdrawal of the Caf diet.

471 *4.3. Caf diet did not alter lick cluster size for sucrose*

472 In contrast to the marked effects observed on sucrose consumption and preference, chronic Caf
473 diet did not alter lick cluster size, which reflects palatability of the tastant, and has been used to infer
474 changes in 'liking' (Dwyer, 2012). Rats licked in larger clusters for more concentrated sucrose
475 solutions, consistent with past work (Davis, 1973, Smith & Davis, 1992) but diet history did not
476 modulate cluster size for any concentration in either the restricted or unrestricted tests. Thus, long-
477 term exposure to palatable foods high in fat and sugar did not alter the palatability of sucrose, implying
478 that this variable is unrelated to the changes in sweet taste preference and consumption produced by
479 Caf diet. Cluster size was unaffected by deprivation state, except for the 8% vs. 2% test, where testing

under restricted water access revealed no difference between concentrations but testing unrestricted conditions did reveal a clear increase in the size of the preference for 8% over 2%. Importantly, our results were robust to manipulating the criterion for defining cluster size (Supplementary Figure S3). While our data imply that control and Caf rats 'liked' sucrose solutions comparably, this hypothesis should be assessed by examining taste reactivity measures.

Therefore, our results do not support the notion that diet-induced obesity is accompanied by greater hedonic value of sweetness. This is consistent with prior work in rodents showing obesogenic diet-induced changes in motivational but not hedonic measures. A recent study in rats found that 10 weeks access to high-fat diet beginning at weaning did not alter hedonic orofacial responses to percentage 0.1M or 1.0M sucrose, but reduced motivation to work for sweet food rewards (Arcego et al., 2020). Similarly, Lesser et al. (2017) showed that chronic exposure to a high-fat, high-sugar 'junk-food' diet (a purified mix of cookies, potato chips, peanut butter, chocolate powder and rat chow) suppressed approach behaviour to a food-paired cue but did not alter hedonic orofacial responses to 1, 3 and 9% sucrose in Sprague-Dawley rats. An intriguing strain difference was observed, with junk-food reducing both 'wanting' and 'liking' in Long-Evans, relative to Sprague-Dawley rats. Ducrocq et al. (2019) found reduced instrumental response rates but no difference in burst size in high-fat diet-fed rats licking for dilute sweetened condensed milk. Finally, rats fed isocaloric high-fat, high-sugar or control diets exhibited comparable 'liking' of fat and sugar, despite high-fat and high-sugar groups preferring their respective nutrient in choice preference tests between fat and sugar (Steele et al., 2019). These data and the present results are consistent with a recent study in humans showing that liking and wanting of a palatable milkshake was unrelated to participants' adiposity (Wall et al., 2020) and direct future research to interrogate other parameters related to food-seeking and consumption in obesity.

Although lick cluster size was unaffected by diet history, analyses indicated that the alternative provided in each test modulated the palatability of each concentration. Lick cluster size for 8% sucrose was significantly higher in the test against 2% sucrose than in the test against 32% sucrose. A non-

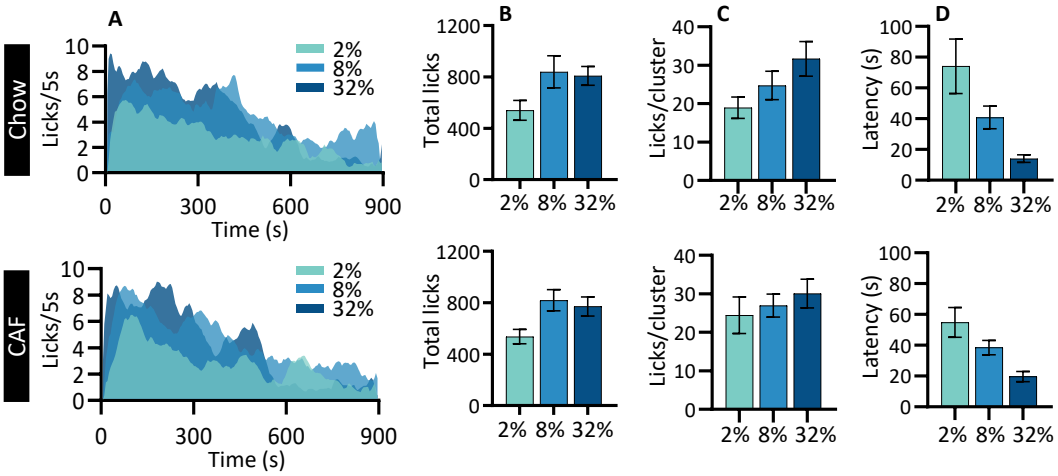
significant effect was observed for 2% sucrose, with a trend toward lower cluster size when pitted against the strongly-preferred 32% sucrose than the moderately-preferred 8% sucrose solution. Past work in mice has shown analogous contrast effects using successive contrast procedures (Austen, Strickland & Sanderson, 2016). These results demonstrate the ability of the surrounding environment to modulate the appraisal of rewards. Under these conditions, however, sensitivity to contrast was not altered by Caf diet exposure.

4.4. Future directions & conclusions

Exposure to Caf diet increased body weight by ~30% relative to chow-fed controls, and our past work has shown that Caf diet induces metabolic impairments that include increased adiposity, glucose intolerance, and elevated plasma insulin, leptin and triglycerides (e.g., Hossain et al., 2020). Future work should clarify whether diet-induced changes to sweet taste preference are driven by excess weight gain or to direct effects of the diet *per se*. Previous work has found dissociations between weight gain and the hedonic responses to palatable food. For example, in rats given restricted (2.5h/day) or continuous access to a high-fat, high-sugar solution, initial lick rate and burst size increased in the restricted but not continuous group, while body weight was significantly greater in the continuous group (Lardeux, Kim & Nicola, 2013). Finally, given recent evidence that long-term consumption of diets high in fat and sugar induces pronounced changes to the mouse tongue proteome (Dutt et al., 2021), future work should identify the molecular changes associated with the behavioural changes in sucrose preference observed here.

In summary, our results show that chronic consumption of a cafeteria-style diet alters sweet taste preference and exerts pronounced motivational effects in the absence of changes to sweet taste processing. The effects of our highly palatable and varied Caf diet on behavioural indices of consumption and measures of neuronal activity extend prior work using purified diets high in fat and/or

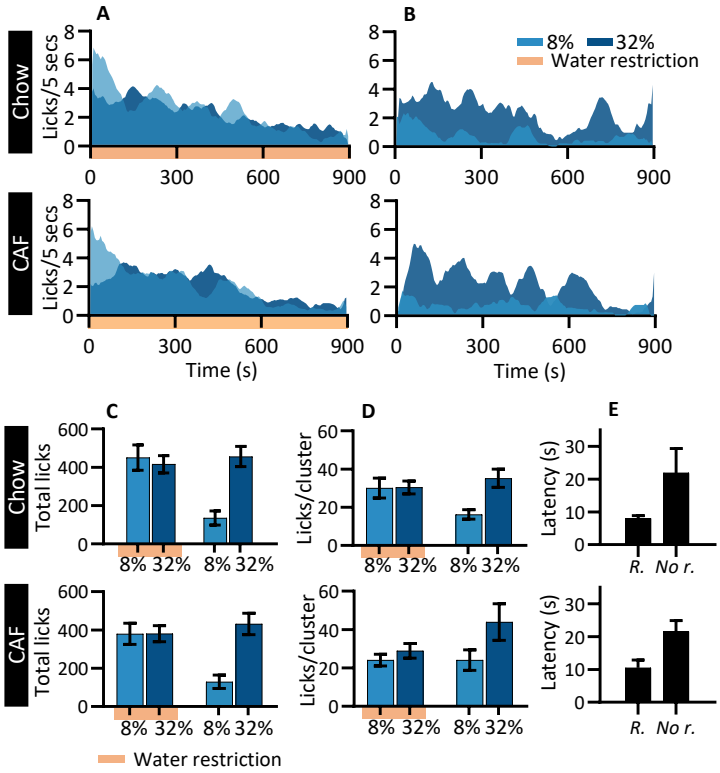
sugar. The effects of chronic exposure seen here call for further work to characterise the onset of these changes across diet exposure and the contribution of diet-induced weight gain *per se*.



532

533 Supplementary Figure S1. Pre-exposure to 2%, 8% and 32% sucrose solution. Chow (top row) and
534 Caf (bottom row) groups were matched on all parameters, including licks across the 15 min sessions
535 (Panel A) and total licks (Panel B), mean licks per cluster (Panel C) and latency to first lick (Panel
536 D). All bars indicate mean ± SEM

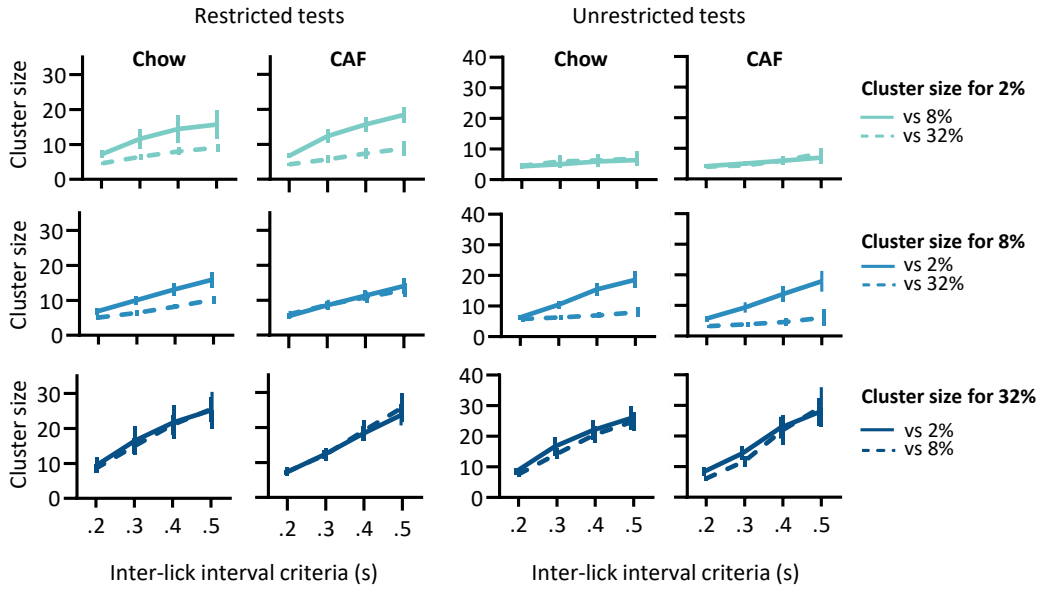
537



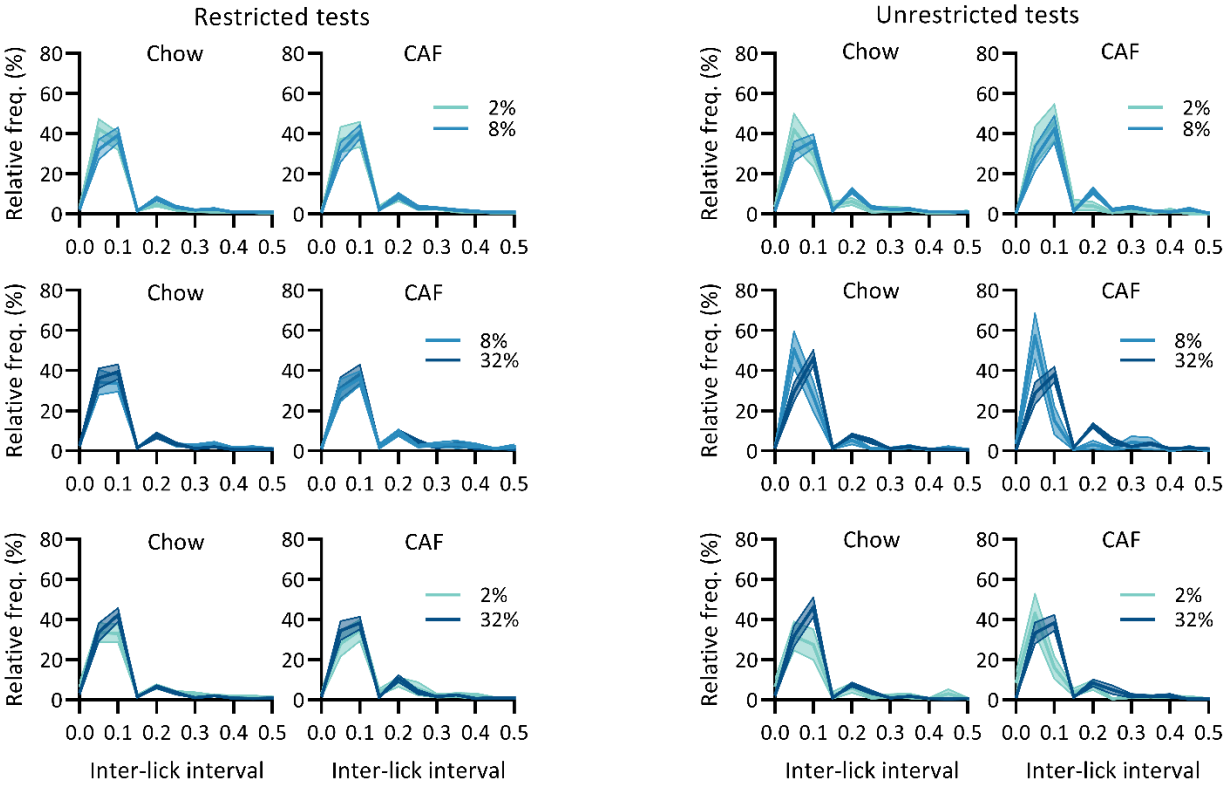
538

539 Supplementary Figure S2. Baseline preference tests between 32% and 8% sucrose with and without
540 water restriction. Licks over time are shown in Panels A and B. Chow and Caf groups were matched
541 on total licks (C), licks per cluster (D) and latency to first lick (E). All bars indicate mean ± SEM.

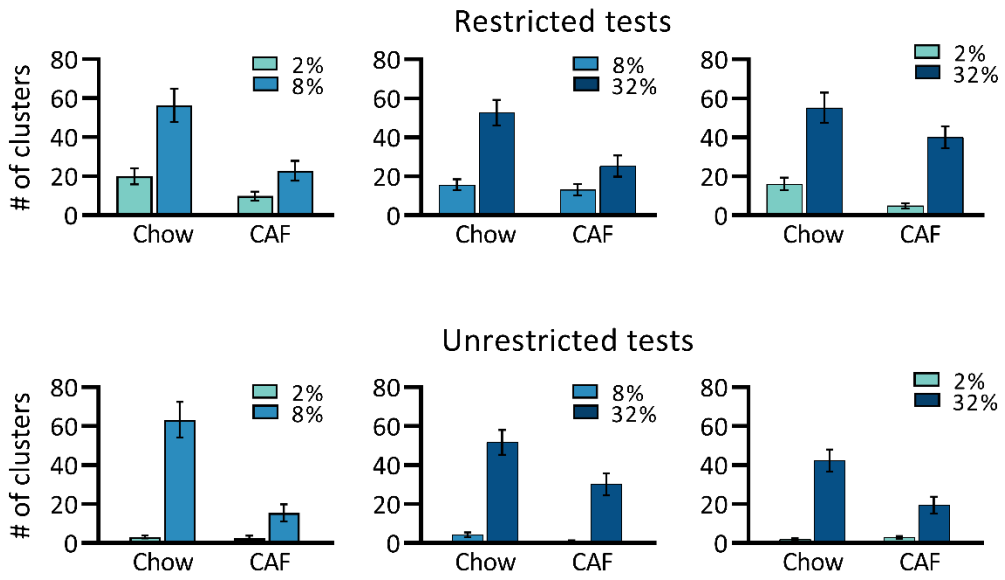
542



Supplementary Figure S3. Variations in the inter-lick interval criterion used to determine a lick cluster. A criterion of 0.5s was used for analyses. Group differences, and the relative differences in cluster size between 2%, 8% and 32% sucrose, were consistent at lower criteria.



Supplementary Figure S4. Distributions of inter-lick intervals (ILI) in sucrose preference tests. Chow and Caf groups exhibited similar distributions, with most ILIs less than 0.1s. ILIs were grouped into bins of 0.05s and plotted to the lick cluster criterion of 0.5s. Approximately 85% of all inter-lick intervals fell within this criterion; this proportion did not vary significantly between chow and Caf groups.



Supplementary Figure S5. The number of lick clusters in sucrose preference tests matched the pattern of total licks (cf. Figures 2-3), with more lick clusters for more concentrated sucrose solutions, and fewer lick clusters in Caf than chow rats.

Supplementary Table S1. Frequency distribution of inter-lick intervals during pre-exposures to 2%, 8% and 32% sucrose solution. Over 90% of inter-lick intervals were less than 0.5s.

	2% pre-diet		**8% pre-diet**		**32% pre-diet**	
Inter-lick interval (s)	**Chow**	**CAF**	**Chow**	**CAF**	**Chow**	**CAF**
0-.1	56.31	54.47	48.38	52.59	54.03	57.77
.1-.2	81.76	78.53	80.61	79.49	86.01	83.93
.2-.3	88.53	86.35	88.56	87.96	92.14	91.43
.3-.4	91.67	89.72	91.59	91.57	94.43	94.05
.4-.5	93.35	92.05	93.18	93.63	95.49	95.40

References

Accolla R, Bathellier B, Petersen CC, Carleton A. Differential spatial representation of taste modalities in the rat gustatory cortex. Journal of Neuroscience. 2007 Feb 7;27(6):1396-404.

Alsiö J, Olszewski PK, Norbäck AH, Gunnarsson ZE, Levine AS, Pickering C, Schiöth HB. Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. Neuroscience. 2010 Dec 15;171(3):779-87.

Arcego DM, Krolow R, Lampert C, Toniazzo AP, dos Santos Garcia E, Lazzaretti C, Costa G, Scorza C, Dalmaz C. Chronic high-fat diet affects food-motivated behavior and hedonic systems in the nucleus accumbens of male rats. Appetite. 2020 Oct 1;153:104739.

Bartoshuk LM, Duffy VB, Hayes JE, Moskowitz HR, Snyder DJ. Psychophysics of sweet and fat perception in obesity: problems, solutions and new perspectives. Philosophical Transactions of the Royal Society B: Biological Sciences. 2006 Jul 29;361(1471):1137-48.

Beck RC, Nash R. Thirsty rats do prefer sucrose solutions. Psychonomic Science. 1969 Jul;15(1):19-20.

Beck RC, Self JL, Carter DJ. Sucrose preference thresholds for satiated and water-deprived rats. Psychological reports. 1965 Jun;16(3):901-5.

Berthoud HR, Zheng H. Modulation of taste responsiveness and food preference by obesity and weight loss. Physiology & behavior. 2012 Nov 5;107(4):527-32.

Bobowski N, Mennella JA. Personal variation in preference for sweetness: effects of age and obesity. Childhood obesity. 2017 Oct 1;13(5):369.

Cohen JS, Hachey GJ. Development of sucrose preferences under two levels of water deprivation. Psychological reports. 1980 Jun;46(3):820-2.

Collier G, Bolles R. Some determinants of intake of sucrose solutions. Journal of Comparative and Physiological Psychology. 1968 Jun;65(3p1):379.

Davis JD. The effectiveness of some sugars in stimulating licking behavior in the rat. Physiology & behavior. 1973 Jul 1;11(1):39-45.

Davis JD, Smith GP. Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. Behavioral neuroscience. 1992 Feb;106(1):217.

Davis JF, Tracy AL, Schurdak JD, Tschöp MH, Lipton JW, Clegg DJ, Benoit SC. Exposure to elevated levels of dietary fat attenuates psychostimulant reward and mesolimbic dopamine turnover in the rat. Behavioral neuroscience. 2008 Dec;122(6):1257.

Drewnowski A, Kurth CL, Rahaim JE. Taste preferences in human obesity: environmental and familial factors. The American journal of clinical nutrition. 1991 Oct 1;54(4):635-41.

Duca FA, Swartz TD, Covasa M. Effect of diet on preference and intake of sucrose in obese prone and resistant rats. PloS one. 2014 Oct 20;9(10):e111232.

Ducrocq F, Hyde A, Fanet H, Oummadi A, Walle R, De Smedt-Peyrusse V, Layé S, Ferreira G, Trifilieff P, Vancassel S. Decrease in operant responding under obesogenic diet exposure is not related to deficits in incentive or hedonic processes. Obesity. 2019 Feb;27(2):255-63.

Dutt M, Ng YK, Molendijk J, Karimkhanloo H, Liao L, Blazev R, Montgomery MK, Watt MJ, Parker BL. Western Diet Induced Remodelling of the Tongue Proteome. Proteomes. 2021 Jun;9(2):22.

Dwyer DM. Licking and liking: The assessment of hedonic responses in rodents. Quarterly journal of experimental psychology. 2012 Mar;65(3):371-94.

Figlewicz DP, Jay JL, Acheson MA, Magrisso IJ, West CH, Zavosh A, Benoit SC, Davis JF. Moderate high fat diet increases sucrose self-administration in young rats. Appetite. 2013 Feb 1;61:19-29.

Finger BC, Dinan TG, Cryan JF. Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task. Psychopharmacology. 2012 Mar;220(1):173-81.

Flaherty CF. Incentive contrast: A review of behavioral changes following shifts in reward. Animal Learning & Behavior. 1982 Dec;10(4):409-40.

Fry BR, Russell N, Gifford R, Robles CF, Manning CE, Sawa A, Niwa M, Johnson AW. Assessing reality testing in mice through dopamine-dependent associatively evoked processing of absent gustatory stimuli. Schizophrenia bulletin. 2020 Jan 4;46(1):54-67.

Gao YJ, Ji RR. c-Fos and pERK, which is a better marker for neuronal activation and central sensitization after noxious stimulation and tissue injury?. The open pain journal. 2009 Jan 1;2:11.

Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience. 2009 Apr 10;159(4):1193-9.

Green E, Jacobson A, Haase L, Murphy C. Reduced nucleus accumbens and caudate nucleus activation to a pleasant taste is associated with obesity in older adults. Brain research. 2011 Apr 22;1386:109-17.

Grill HJ, Norgren R. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain research. 1978 Mar 24;143(2):263-79.

Hagan KE, Alasmar A, Exum A, Chinn B, Forbush KT. A systematic review and meta-analysis of attentional bias toward food in individuals with overweight and obesity. Appetite. 2020 Apr 13:104710.

Hagstrom EC, Pfaffmann C. The relative taste effectiveness of different sugars for the rat. Journal of Comparative and Physiological Psychology. 1959 Jun;52(3):259.

Harris JA, Thein T. Interactions between conditioned and unconditioned flavor preferences. Journal of Experimental Psychology: Animal Behavior Processes. 2005 Oct;31(4):407.

Hossain MJ, Kendig MD, Wild BM, Issar T, Krishnan AV, Morris MJ, Arnold R. Evidence of Altered Peripheral Nerve Function in a Rodent Model of Diet-Induced Prediabetes. Biomedicines. 2020 Sep;8(9):313.

Hsiao S, Fan RJ. Additivity of taste-specific effects of sucrose and quinine: microstructural analysis of ingestive behavior in rats. Behavioral neuroscience. 1993 Apr;107(2):317.

Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. Obesity reviews. 2013 Aug;14(8):606-19.

Jagust W, Harvey D, Mungas D, Haan M. Central obesity and the aging brain. Archives of neurology. 2005 Oct 1;62(10):1545-8.

654 Johnson AW. Dietary manipulations influence sucrose acceptance in diet induced obese mice.
655 Appetite. 2012 Feb 1;58(1):215-21.

656 Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and
657 compulsive eating in obese rats. Nature neuroscience. 2010 May;13(5):635.

658 Karlsson HK, Tuominen L, Tuulari JJ, Hirvonen J, Parkkola R, Helin S, Salminen P, Nuutila P,
659 Nummenmaa L. Obesity is associated with decreased μ-opioid but unaltered dopamine D2
660 receptor availability in the brain. Journal of Neuroscience. 2015 Mar 4;35(9):3959-65.

661 Kendig MD. Cognitive and behavioural effects of sugar consumption in rodents. A review. Appetite.
662 2014 Sep 1;80:41-54.

663 Kendig MD, Rooney KB, Corbit LH, Boakes RA. Persisting adiposity following chronic
664 consumption of 10% sucrose solution: Strain differences and behavioural effects. Physiology &
665 behavior. 2014 May 10;130:54-65.

666 La Fleur SE, Vanderschuren LJ, Luijendijk MC, Kloeze BM, Tiesjema B, Adan RA. A reciprocal
667 interaction between food-motivated behavior and diet-induced obesity. International journal of
668 obesity. 2007 Aug;31(8):1286-94.

669 Lardeux S, Kim JJ, Nicola SM. Intermittent access to sweet high-fat liquid induces increased
670 palatability and motivation to consume in a rat model of binge consumption. Physiology &
671 behavior. 2013 Apr 10;114:21-31.

672 Leigh SJ, Kendig MD, Morris MJ. Palatable Western-style Cafeteria Diet as a Reliable Method for
673 Modeling Diet-induced Obesity in Rodents. Journal of visualized experiments: JoVE. 2019 Nov
674 1(153).

675 Lesser EN, Arroyo-Ramirez A, Mi SJ, Robinson MJ. The impact of a junk-food diet during
676 development on 'wanting' and 'liking'. Behavioural brain research. 2017 Jan 15;317:163-78.

677 Maersk M, Belza A, Stødkilde-Jørgensen H, Ringgaard S, Chabanova E, Thomsen H, Pedersen SB,
678 Astrup A, Richelsen B. Sucrose-sweetened beverages increase fat storage in the liver, muscle, and
679 visceral fat depot: a 6-mo randomized intervention study. The American journal of clinical
680 nutrition. 2012 Feb 1;95(2):283-9.

681 Morris MJ, Beilharz JE, Maniam J, Reichelt AC, Westbrook RF. Why is obesity such a problem in
682 the 21st century? The intersection of palatable food, cues and reward pathways, stress, and
683 cognition. Neuroscience & Biobehavioral Reviews. 2015 Nov 1;58:36-45.

684 Morys F, García-García I, Dagher A. Is obesity related to enhanced neural reactivity to visual food
685 cues? A review and meta-analysis. Social Cognitive and Affective Neuroscience. 2020 Aug 12.

686 Mook DG. Oral and postingestional determinants of the intake of various solutions in rats with
687 esophageal fistulas. Journal of Comparative and Physiological Psychology. 1963 Aug;56(4):645.

688 Naneix F, Darlot F, Coutureau E, Cador M. Long-lasting deficits in hedonic and nucleus accumbens
689 reactivity to sweet rewards by sugar overconsumption during adolescence. European Journal of
690 Neuroscience. 2016 Mar;43(5):671-80.

691 Nishihara T, Nozaki T, Sawamoto R, Komaki G, Miyata N, Hosoi M, Sudo N. Effects of weight loss
692 on sweet taste preference and palatability following cognitive behavioral therapy for women with
693 obesity. Obesity facts. 2019;12(5):529-42.

694 Nissenbaum JW, Sclafani A. Sham-feeding response of rats to Polycose and sucrose. Neuroscience
695 & Biobehavioral Reviews. 1987 Jun 1;11(2):215-22.

696 O'Brien PD, Hinder LM, Callaghan BC, Feldman EL. Neurological consequences of obesity. The
697 Lancet Neurology. 2017 Jun 1;16(6):465-77.

698 Paxinos G, Watson C. The rat brain in stereotaxic coordinates: hard cover edition. Elsevier; 2006
699 Nov 2.

700 Peciña S, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do μ-opioids cause
701 increased hedonic impact of sweetness? Journal of neuroscience. 2005 Dec 14;25(50):11777-86.

702 Pender S, Stevenson RJ, Francis HM, Oaten MJ. Wanting and liking for sugar sweetened beverages
703 and snacks differ following depletion and repletion with energy and fluids. Appetite. 2019 Jun
704 1;137:81-9.

705 Prickett C, Brennan L, Stolwyk R. Examining the relationship between obesity and cognitive
706 function: a systematic literature review. Obesity research & clinical practice. 2015 Mar 1;9(2):93-
707 113.

708 Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW,
709 Thompson PM. Brain structure and obesity. Human brain mapping. 2010 Mar;31(3):353-64.

710 Richter CP, Campbell KH. Taste threshold and taste preferences of rats for five common sugars.
711 Journal of Nutrition. 1940;20:31-46.

712 Salbe AD, DelParigi A, Pratley RE, Drewnowski A, Tataranni PA. Taste preferences and body
713 weight changes in an obesity-prone population. The American journal of clinical nutrition. 2004
714 Mar 1;79(3):372-8.

715 Sclafani A. Carbohydrate taste, appetite, and obesity: an overview. Neuroscience & Biobehavioral
716 Reviews. 1987 Jun 1;11(2):131-53.

717 Sclafani A, Hertwig H, Vigorito M, Feigin MB. Sex differences in polysaccharide and sugar
718 preferences in rats. Neuroscience & Biobehavioral Reviews. 1987 Jun 1;11(2):241-51.

719 Shin AC, Townsend RL, Patterson LM, Berthoud HR. "Liking" and "wanting" of sweet and oily
720 food stimuli as affected by high-fat diet-induced obesity, weight loss, leptin, and genetic
721 predisposition. American Journal of Physiology-Regulatory, Integrative and Comparative
722 Physiology. 2011 Nov;301(5):R1267-80.

723 Small DM. Taste representation in the human insula. Brain Structure and Function. 2010 Jun
724 1;214(5-6):551-61.

725 Spector AC, Klumpp PA, Kaplan JM. Analytical issues in the evaluation of food deprivation and
726 sucrose concentration effects on the microstructure of licking behavior in the rat. Behavioral
727 neuroscience. 1998 Jun;112(3):678.

728 Spector AC, Smith JC. A detailed analysis of sucrose drinking in the rat. Physiology & behavior.
729 1984 Jul 1;33(1):127-36.

730 Steele CC, Pirkle JR, Davis IR, Kirkpatrick K. Dietary effects on the determinants of food choice:
731 Impulsive choice, discrimination, incentive motivation, preference, and liking in male rats.
732 Appetite. 2019 May 1:136:160-172.

733 Stice E, Yokum S, Blum K, Bohon C. Weight gain is associated with reduced striatal response to
734 palatable food. Journal of Neuroscience. 2010 Sep 29;30(39):13105-9.

Tantot F, Parkes SL, Marchand AR, Boitard C, Naneix F, Layé S, Trifilieff P, Coutureau E, Ferreira G. The effect of high-fat diet consumption on appetitive instrumental behavior in rats. Appetite. 2017 Jan 1;108:203-11.

Thaler JP, Yi CX, Schur EA, Guyenet SJ, Hwang BH, Dietrich MO, Zhao X, Sarruf DA, Izgur V, Maravilla KR, Nguyen HT. Obesity is associated with hypothalamic injury in rodents and humans. The Journal of clinical investigation. 2012 Jan 3;122(1):153-62.

Tracy AL, Wee CJ, Hazeltine GE, Carter RA. Characterization of attenuated food motivation in high-fat diet-induced obesity: Critical roles for time on diet and reinforcer familiarity. Physiology & behavior. 2015 Mar 15;141:69-77.

Treesukosol Y, Liang NC, Moran TH. Alterations in sucrose sham-feeding intake as a function of diet-exposure in rats maintained on calorically dense diets. Appetite. 2015 Sep 1;92:278-86.

Wall KM, Farruggia MC, Perszyk EE, Kanyamibwa A, Fromm S, Davis XS, Dalenberg JR, DiFeliceantonio AG, Small DM. No evidence for an association between obesity and milkshake liking. International Journal of Obesity. 2020 Aug;44(8):1668-77.

Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusll N, Fowler JS. Brain dopamine and obesity. The Lancet. 2001 Feb 3;357(9253):354-7.

Wilmouth CE, Spear LP. Hedonic sensitivity in adolescent and adult rats: taste reactivity and voluntary sucrose consumption. Pharmacology Biochemistry and Behavior. 2009 Jun 1;92(4):566-73.

Wooley OW, Wooley SC, Dunham RB. Calories and sweet taste: effects on sucrose preference in the obese and nonobese. Physiology & behavior. 1972 Nov 1;9(5):765-8.

Yamamoto T. Role of the cortical gustatory area in taste discrimination. Neural Mechanisms in Taste. 1989 Aug 31(494):197.

Yau PL, Castro MG, Tagani A, Tsui WH, Convit A. Obesity and metabolic syndrome and functional and structural brain impairments in adolescence. Pediatrics. 2012 Oct 1;130(4):e856-64.