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TASTE UNCOUPLED FROM NUTRITION FAILS TO SUSTAIN THE REINFORCING PROPERTIES OF FOOD

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Abstract

Recent findings suggest the reward system encodes metabolic value independent of taste, provoking speculation that the hedonic value of taste could be derived from nutritional value as a secondary appetitive property. We therefore dissociated and compared the impact of nutrition and taste on appetitive behavior in several paradigms. Though taste alone induces preference and increased consumption, in the absence of nutritional value its reinforcing properties are greatly diminished and it does not, like sucrose, induce increased responding over time. In agreement with behavioral data, saccharin- but not sucrose- evoked dopamine release is greatly attenuated following pre-exposure, suggesting that nutritional value is critical for dopamine mediated reward and reinforcement. Further supporting the primacy of nutrition over taste, genetically increased dopaminergic tone enhances incentive associated with nutritional value with minimal impact on taste-based, hedonic incentive. Overall, we suggest that the sensory-hedonic incentive value associated with taste functions as a conditioned stimulus that requires nutritional value to sustainably organize appetitive behavior.

Keywords

dopamine; reinforcement; feeding; obesity; palatability; reward; cyclic voltammetry

INTRODUCTION

Ingesting food provides two primary forms of reward value: nutritional and hedonic. Nutritional reward arises from metabolic and homeostatic signaling and hedonic reward arises from the sensory properties perceived as pleasurable. Historically, palatability has been believed to arise as a consequence of both of these values. Recently, there has been an increased focus on motivation arising from hedonic aspects of food independent of need state and nutritional value, so-called ‘hedonic hunger’ (Lowe & Butryn, 2007). Though highly palatable foods will motivate consumption even in a calorically replete state, the independence of this hedonic motivation from homeostatic mechanisms has been challenged. A study by de Araujo et al (de Araujo *et al.*, 2008) used *trpm5* knock-out (*trpm5* KO) mice that cannot transduce sweet taste to demonstrate that nutritional value alone can induce conditioning and preference. Though not elaborated upon, they also show that sweet taste alone, though it does induce preference, does not induce conditioning, even in wild-type mice that transduce sweet taste. Glendinning et al (Glendinning *et al.*, 2010) recently

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demonstrated that nutritional content rather than taste responsiveness determines daily intake of sweet solutions in mice. Recently, Haase and colleagues (Haase *et al.*, 2009) demonstrated that activation of various brain regions in response to pure gustatory stimuli (ie, hedonic value) varies according to motivational state (see also Fontanini & Katz, 2009). In short, in the regulation of appetitive and consummatory behavior, the relationship between sensory and hedonic signaling on one hand and metabolic and homeostatic on the other remains an unresolved but critical question.

The above controversy is also reflected in the role of dopamine in reward and motivation (Schultz *et al.*, 1997; Salamone *et al.*, 1997; Salamone *et al.*, 2005; Wise, 2008; Berridge, 2009) and in the pathogenesis of overeating and obesity (Volkow & Wise, 2005; Kenny, 2010; Volkow *et al.*, 2010). On one hand, accumulating data emphasize a close linkage between homeostatic systems and dopamine (Figlewicz & Sipols, 2010; Berthoud *et al.*, 2011) with one group proposing that dopamine acts as a metabolic sensor (de Araujo *et al.*, 2010). On the other hand, studies of dopamine activity in response to sensory signals suggest that the sensory-hedonic properties of food can induce a dopamine response independent of nutritional value. For example, both artificial sweeteners (Wheeler *et al.*, 2011) and sucrose sham-feeding induce a dopamine response (Hajnal *et al.*, 2004). This suggests that sweet taste is rewarding independent of nutritional value. The apparent independence of sensory, hedonic activation of dopamine challenges the notion of tight coupling between dopamine and homeostatic systems. Can sensory-hedonic value override this coupling? The question is at the heart of current theories implicating dopamine in obesity. To our knowledge, whether dopamine responds to calorie-free sweet taste is maintained *after* the animal learns to discriminate the artificial sweetener from real sugar (ie., given the opportunity to learn that saccharin does not have any caloric value) has not been tested.

In the studies reported here, we dissociate taste and nutrition using *trpm5* KO mice that cannot transduce sweet taste (Damak *et al.*, 2006; de Araujo *et al.*, 2008) to isolate nutritional signaling and calorie-free artificial sweeteners to isolate hedonic signaling. We use several behavioral paradigms, each providing a window onto different aspects of appetitive behavior. This methodological strategy is critical to avoid drawing incorrect inferences based on data from a single behavioral paradigm only. To examine the role of dopamine, we used both dopamine transporter knock-down mice (DATkd) with elevated tonic dopamine (Zhuang *et al.*, 2001) as well as DAT knock-down and *trpm5* KO double mutants. Overall, a pattern of results emerge from our data suggesting that nutritional value is critical to reward and in its absence, the reinforcing properties of hedonic value are greatly attenuated. To reconcile this with established literature demonstrating that sensory signaling alone can elicit a robust dopamine response—which should be reinforcing—we used cyclic voltammetry to compare the dopamine response evoked by both sucrose and saccharin in rats given prior exposure to both, allowing them to learn about each and stabilize their behavior before recording. Under these conditions, we observe a dramatic attenuation of evoked dopamine in response to saccharin compared to sucrose. Together, these data suggest that the incentive motivation underlying ‘hedonic hunger’ or non-homeostatic feeding arises primarily from nutritional, not hedonic, reinforcement.

MATERIALS AND METHODS

Subjects

Mice were grouped housed in standard conditions on a 6:00 to 18:00 light cycle except during the two-bottle preference tests and the homecage operant tests where they were singly housed. Standard chow and water were provided *ad libitum* unless otherwise noted. Experiments were carried out during the light cycle, except during the two-bottle preference

tests and homecage operant tests which were conducted across a period of 30 and 7 days, respectively. Male and female mice between 8 and 16 weeks were used, age and sex matched across groups within each experiment. No mice were used in multiple experiments or conditions (ie., all studies were between not within subjects) unless otherwise noted in the results. To precisely control for genetic background, littermate controls were used for all experiments with genetically altered lines. There were no significant body weight differences between groups except as noted in the text. All Animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Chicago. In the voltammetry experiments, male Sprague-Dawley rats (postnatal day ~75 at start of experiment; Charles River) were individually housed in plastic cages (26.5 × 50 × 20 cm) in a temperature (22°C) and humidity (30%) controlled environment on a 12:12 h light/dark cycle. Voltammetry experiments were conducted in the Behavioral Neuroscience Division at University of Illinois at Chicago and approved by the Institutional Animal Care and Use Committee at the University of Illinois at Chicago.

The DATkd mice have been extensively described and characterized elsewhere (Zhuang *et al.*, 2001; Pecina *et al.*, 2003; Cagniard, Balsam, Brunner, & Zhuang, 2006; Cagniard, Beeler, Britt, McGehee, Marinelli, & Zhuang, 2006; Yin *et al.*, 2006; Tilley *et al.*, 2007). They exhibit a 85% reduction in dopamine transporter expression, elevated extracellular dopamine concentrations, slowed striatal dopamine reuptake kinetics and increased frequency of tonic dopamine cell firing. In contrast to the DAT knockouts (Bosse *et al.*, 1997) they do not exhibit any developmental abnormalities or learning deficits (Cagniard, Beeler, Britt, McGehee, Marinelli, & Zhuang, 2006; Yin *et al.*, 2006). Trpm5 KO mice were originally derived by Damak *et al.* (Damak *et al.*, 2006) and heterozygote breeding pairs used to generate both homozygotes and wild-type controls. The trpm5 KO homozygote mice were crossed with DATkd homozygotes to generate DATkd(het):trpm5(het) animals which were in turn bred to obtain DATkd(hom):trpm5(hom) and DATkd(wild-type)- trpm5(hom) to generate hyperdopaminergic trpm5 KO animals and littermate controls. All genotypes were verified by PCR.

Matching sweet intensity of sweeteners

Often, equivalency between sweeteners is established by finding the concentrations of two sweeteners that elicit comparable behavioral responses. When comparing nutritive and non-nutritive sweeteners, this method is inappropriate. The reward value of sucrose derives from two sources, taste and nutrition while the value derived from calorie-free sweeteners arises only from taste. To achieve commensurate responding, the concentration of the calorie-free sweetener has to be adjusted such that sweet intensity alone is as rewarding as *both* sweetness and nutrition in sucrose. In essence, you have to increase the sweetness of calorie-free reward to compensate for the absence of nutrition. Young and Madsen (1963) report that the most preferred saccharin solution, 0.4%, is isopreferred with 3.5% sucrose. Preference for sucrose clearly increases at concentrations above 3.5% (eg., our preference data) while further increases in saccharin do not induce greater preference. We suggest this is because sweet taste alone can only match sweet plus nutrition at concentrations where the nutritional component is negligible. That is, at 3.5% sucrose, which does not elicit a maximal response to sucrose, sweet intensity of artificial sweeteners can compensate for a lack of nutritional value but as sucrose concentration increases, this becomes impossible.

In these studies, we selected sweetener concentrations that were iso-sweet based on the concentration-dependent characterization of each sweetener in comparison to water in a comprehensive behavioral study of sweeteners in mice (Bachmanov *et al.*, 2001). In this study, maximal preference and consumption EC₅₀ for sucrose, sucralose and saccharin solutions were at ~100, 1 and 2 mM, respectively. From this we used a 1:100 ratio for sucrose and sucralose solutions, which yielded iso-preference at low concentrations (ie., Fig

1 small but equivalent increase at .2/.002% solutions). Sucralose and saccharine pellets were at 10 and 6 mM, respectively, reflecting maximal consumption in the Bachmanov et al. data.

Behavioral Procedures

Two-Bottle Preference—Mice were singly housed in cages that included four identical water bottles (15ml round bottom polypropylene tubes with rubber stopper on the mouth and sipper tube with ball bearing) and ad libitum food. Two bottles contained water and two contained a tastant solution, either sucrose or sucralose (Sigma Aldrich, St. Louis, MO), at varying concentrations in the course of the experiment (sucrose: 0.0, 5.5, 145, 290, and 435 mM; sucralose: 0.0 0.055, 1.45, 2.90, 4.35 mM). Each concentration of tastant was provided for 6 days. Bottles containing tastant and water were placed on opposite sides of the cage. The position of bottles was rotated daily to counterbalance potential position preferences during the DATkd experiment. During the trpm5 KO and DATkd:trpm5 experiments, the position of bottles were fixed in order to provide a cue for the animals, but the position of tastant bottles were counterbalanced between cages. A cage without mice was maintained and weighed daily to track spillage, which remained minimal and is not reported.

Quinine adulteration preference test—One bottle contained water and one contained a tastant solution at a constant sweetener concentration (15 and .15% for sucrose and sucralose, respectively) adulterated with increasing quinine hydrochloride (Sigma-Aldrich, St. Louis, MO) concentrations (0, 0.03, 0.06, 0.1 and 0.5mM). Each concentration was provided for 4 days. In the last part of the experiment, quinine concentration was held constant (0.5mM), while sucrose/sucralose concentrations were decreased (10/.10, 5/.05 and 0/0%, respectively). The position of bottles was rotated daily to counter potential position bias.

Two bottle conditioning tests—Mice were singly housed and put on a 22 hour food and water restriction. Animals were conditioned for 6 days with a 30-min session every day of free access to water or tastant (sucrose 0.8M or 30mM sucralose, replicating (de Araujo *et al.*, 2008) on alternate days, eg. days 1, 3, and 5: water; days 2, 4 and 6: tastant. Water and tastant were presented in the same middle position during training in identical water bottles (15ml round bottom polypropylene tubes with rubber stopper on the mouth and sipper tube with ball bearing) with sipper tubes colored either white or black to serve as cues (counter-balanced between tastant and water). After conditioning, animals were tested for conditioned preference in a 10 minute two-bottle access test where both bottles contained water but with different black/white sipper tubes as conditioned cues. Amount of water consumed from each bottle reflects an animal's preference for the conditioned cue previously associated with tastant or water.

Progressive Ratio—Tests were conducted in operant conditioning chambers with two retractable levers (Med Associates, St. Albans, VT). Mice were food restricted receiving two hours of ad lib feeding after each session. Animals were trained to lever press at FR1 ratio overnight for two nights. They were then trained on an FR1 in daily 30-minute sessions until reaching criteria (earning 18 pellets in a 30-minute session for 3 consecutive days). Animals were then trained with 2-hour sessions for two days on a progressive ratio schedule where after each pellet earned the required number of presses for the next reward increases by 3 (PR3). Following this training, mice were tested on a PR7 schedule (cost of each subsequent pellet increments by 7) with 2-hour sessions for 5 consecutive days. 20 mg grain pellets (Bio-Serv, Frenchtown, NJ) were used as reinforcers during FR1 and PR3 sessions and replaced with 20 mg sucrose, sucralose or saccharin pellets (Bio-Serv) during PR7 test sessions.

Homecage Progressive Ratio and Concurrent Choice—Homecage operant boxes are modified standard cages equipped with two levers placed on one side of the cage approximately 15 cm apart with a food hopper between the levers. Animals were singly housed and water and standard chow was available *ad libitum*. Upon initial placement in the homecages operant boxes, the first 50 rewards (20 mg sucrose, sucralose or saccharin pellets) are delivered on an FR1 schedule. Subsequent rewards were delivered on a PR2 schedule where the required number of presses increments by two after each reward delivery. The progressive ratio would reset to 2 (ie., start the progression at the beginning) after 30 minutes of inactivity. Experiments lasted 7 days during which freely available chow consumption was measured daily.

Fast-scan cyclic voltammetry

Prior to training and during recovery from surgery rats had *ad libitum* access to both standard lab chow and water. During training rats were food-restricted to approximately 90% of their free-feeding weight. Training sessions took place between 08:00 and 12:00. Rats were trained with either banana-flavored sucrose pellets (45 mg; F06645; Bio-Serv) or grape-flavored saccharin pellets (1.1%; 45 mg; F06646; Bio-Serv) on alternate days (e.g. days 1, 3, 5, 7, and 9 with sucrose, days 2, 4, 6, 8, 10 with saccharin or vice versa). Rats were placed in a behavioral chamber (19 cm [l] × 11 cm [w] × 14 cm [h]; UIC Machine shop) containing a houselight and white noise generator (Med Associates, St. Albans, VT). The houselight and white noise were on throughout all training and testing sessions. Pellets were delivered into the food receptacle at random intervals (60 ± 30 s). The number of pellets eaten was recorded. After 10 sessions (5 sessions with each pellet), rats were fed *ad libitum* for 3-10 days before surgery for voltammetric recordings was performed. Surgery has been described in detail elsewhere (Ebner *et al.*, 2010). Briefly, rats were anesthetized with ketamine (100 mg/kg, intraperitoneal) and xylazine (10 mg/kg, intraperitoneal). A guide cannula was implanted dorsal to the nucleus accumbens core (from Bregma: 1.3 mm anterior, 1.5 mm lateral) and a Ag/AgCl reference electrode was placed in the contralateral forebrain. Dental cement and screws were used to secure the guide cannula and reference electrode to the skull. A micromanipulator was used to lower a carbon-fiber electrode into the nucleus accumbens. A bipolar stimulating electrode was positioned dorsal to the VTA and lowered in 0.2 mm increments until electrically-evoked dopamine release was detected with the carbon fiber electrode. Dopamine was measured using fast-scan cyclic voltammetry (FSCV). In FSCV, a triangular voltage waveform (-0.4 V to +1.3V to -0.4 V; 400 V/s; 10 Hz) is applied to a carbon fiber electrode, which results in oxidation and reduction of electroactive species at the electrode surface. The amount of current produced by this process is directly proportional to the oxidation/reduction taking place and thus electroactive species can be quantified. Background subtraction was used to remove the contribution of stable oxidation/reduction taking place at the electrode surface. Dopamine was identified by a change in current at its oxidation/reduction values ($\sim +0.6$ V/ ~ -0.2 V). After optimizing electrically-evoked release, the stimulating electrode was cemented into position and the carbon fiber electrode was removed and replaced with an obturator.

After 5-7 days of recovery rats were food restricted again and trained with a single session for each pellet (sucrose, saccharin) to acclimatize them to the headstage before testing. On test day rats were placed in the behavioral chamber and a carbon fiber electrode was lowered into the nucleus accumbens core (6.6-7.2 mm from brain surface) using a micromanipulator. Voltammetric recordings were made while pellets were delivered in blocks of 20 (sucrose-saccharin-sucrose). When these sessions ended, dopamine was evoked by electrical stimulation of the midbrain. A range of stimulation parameters were used to generate dopamine release events of different magnitudes (30/60 Hz; 5-24 pulses). As well as dopamine release, electrical stimulation results in a shift in pH, due to neuronal activity.

Representative current by voltage plots (cyclic voltammograms) were obtained for dopamine and pH. These cyclic voltammograms were used to construct a training set to allow principal component analysis to extract dopamine concentration traces from behavioral sessions (Heien *et al.*, 2004; Day *et al.*, 2007). Application of voltage changes to the electrode as well as the sampling of electrochemical data and dopamine extraction was performed using computer software written in LabVIEW (National Instruments, Austin, TX, USA; (Heien *et al.*, 2004).

Data Analysis

All statistical analyses were performed using R statistical software (R version 2.12.1 (2010-12-16); The R Foundation for Statistical Computing, <http://www.r-project.org>).

RESULTS

Preference Tests

The two-bottle preference experiments, in which mice self-regulate their consumption of two alternatives without experimenter imposed constraints (ie., no food/water restriction, temporal limitations or imposition of costs), allows the measurement of consummatory choice behavior. From these studies, we can discern not simply preference but the degree to which that preference alters consumption when alternatives are freely and continuously available.

Both nutritional and hedonic value induces preference and increase consumption

Both sucrose and sucralose induce preference with wild-type mice drinking significantly more of either than water (Fig 1A; bottle main effect sucrose, $F_{(1,11)} = 372.98$, $p < .0001$; sucralose, $F_{(1,11)} = 62.05$, $p < .0001$) in a concentration dependent manner (both tastants, $F_{(1,22)} = 178.2$, $p < .0001$). However, sucrose induces an approximately two-fold greater consumption of tastant (Fig 1A; tastant main effect, $F_{(1,22)} = 42.16$, $p < .0001$) and exhibits greater concentration sensitivity (concentration $\times$ tastant, $F_{(1,22)} = 56.9$, $p < .0001$). At the lowest concentrations tested, both tastants induce a small but equivalent increase in consumption, suggesting that at a sucrose concentration where the nutritional value may be less significant (see discussion in Methods), the concentration ratio between the sweeteners (1:100) is iso-sweet and, at this concentration, iso-preferred.

To test the contribution of nutritional value independent of taste, we utilized the *trpm5* knock-out mice (*trpm5* KO) that do not transduce sweet taste (Damak *et al.*, 2006; de Araujo *et al.*, 2008). Consistent with previous reports (de Araujo *et al.*, 2008), the *trpm5* KOs exhibit a preference for sucrose (Fig 1B; bottle main effect, $F_{(1,5)} = 50.34$, $p < .001$). However, compared to wild-type littermates, the *trpm5* KO mice show diminished preference at lower concentrations. From these data we cannot determine whether this is due to decreased sensitivity to lower sucrose concentrations or reflects slower learning. In the absence of nutritional value, the *trpm5* KOs show no difference between water and sucralose consumption (Fig 1C; bottle main effect, $F_{(1,5)} = .128$, $p = .7384$), consistent with their inability to distinguish the two based on taste.

These data suggest that both hedonic and nutritional value can induce preference and increase consumption. The greatest effect arises when taste and nutritional values are combined, suggesting an additive or synergistic relationship between the two forms of reward value, consistent with previous observations (Ramirez, 1994; 1997; Myers & Scalfani, 2003; Scalfani & Glendinning, 2003). Alternatively, the two types of signals may play different roles in incentive motivation, which will be examined in subsequent experiments.

Sucralose intake is more sensitive to adulterating bitterness

It is possible that mice drink less sucralose simply because they do not like the taste as much as sucrose (ie., it is less hedonically rewarding rather than because of a lack of caloric value). Artificial sweeteners do not taste identical to sugar and often have an associated bitter taste, which may reduce preference. Sclafani et al (Sclafani *et al.*, 2010) suggest that sucralose has a bitter taste that is masked by its sweet taste in B6 WT mice at concentrations < 3 mM. As only the final concentration tested here exceeds 3 mM (ie., .15% = 3.7 mM), a bitter taste is unlikely to have significantly impacted preference and consumption. In taste reactivity tests, rodents show enhanced positive hedonic reactions to saccharin compared to water (Kozlov *et al.*, 2008; Neath *et al.*, 2010), but to our knowledge sucrose and sucralose have not been directly compared in taste reactivity.

To address potential effects of adulterating sweet taste with bitter, we conducted an experiment in which wild-type mice were provided a choice between water and a solution sweetened with either sucrose or sucralose. However, as the experiment progressed, the tastant solutions were increasingly adulterated with quinine, introducing a bitter taste. After the maximal quinine concentration used, the quinine was then held constant and the sweetener concentrations progressively reduced. Adulteration of sucrose solution with bitterness had no effect on consumption except at the combination of highest quinine and lowest sucrose (Fig 2). Sucralose consumption, in contrast, showed greater reduction in response to quinine adulteration (Fig 2A, concentration $\times$ tastant, $F_{(1,477)} = 5.15$, $p = .023$). The greater impact of adulterating bitterness on sucralose consumption can be seen in a shift in the quinine/sweetener dose-response curve where preference declines at lower ratios of quinine in the sucralose than the sucrose group (Fig 2B, concentration $\times$ tastant, $F_{(1,477)} = 21.08$, $p < .001$).

This result complements a taste reactivity study in which the addition of quinine to a sucrose solution did not diminish positive hedonic reactions to an adulterated sucrose solution (Badia-Elder *et al.*, 1996). These data demonstrate that the introduction of mild bitterness has little impact on consumption of *sucrose*, indicating that preference for sweet taste, at least in the presence of caloric value, is not significantly diminished by adulterating bitterness. Consumption and preference for calorie-free sweetener, however, is more sensitive to adulteration. These data, together with Sclafani's observation (Sclafani *et al.*, 2010) that the bitter taste of sucralose at concentrations > 3 mM is masked by the sweet taste in B6 WT mice, suggest the primary difference underlying reduced consumption of sucralose observed in the preference tests (Fig 1) is not a reduced hedonic value due to an associated bitterness—bitterness does not reduce the value of sucrose— but its lack of nutritional value.

Elevated dopamine has limited impact on preference and consumption

Dopamine, associated with enhanced willingness to work for a preferred food (Salamone, 2009) and enhanced incentive value (Berridge, 2009), has recently been implicated in obesogenic behaviors (Volkow & Wise, 2005; Kenny, 2010; Volkow *et al.*, 2010). To assess the impact of elevated dopamine on consumption of a preferred food, we used mice in which the dopamine transporter has been genetically knocked down in both a wild-type and a trpm5 KO background (DATkd and trpm5 KO:DATkd, respectively) resulting in elevated tonic dopamine (Zhuang *et al.*, 2001; Cagniard, Beeler, Britt, McGehee, Marinelli, & Zhuang, 2006).

Elevated dopamine (ie., DATkd on both backgrounds) does not enhance consumption of either sucrose (Fig 3A, wild-type, $F_{(1,11)} = 34.72$, $p = .5874$; trpm5, $F_{(1,9)} = 33.4$, $p = .46$) or

sucralose (Fig 3B, $F_{(1,11)} = 2.27$, $p = .16$). These data suggests that under conditions of free access, elevated dopamine has little effect on choice and consumption.

Two-bottle conditioning test

The preference tests do not distinguish between ‘reward,’ a positively evaluated experience, and ‘reinforcement,’ the degree to which that reward will organize and motivate future behavior. One measure of the reinforcing properties of a stimulus is the degree to which other, neutral stimuli can acquire incentive properties through association. The two-bottle conditioning test examines the reinforcing properties of sucrose and sucralose by comparing their efficacy in conferring incentive value on neutral cues.

Hedonic value alone does not induce conditioning

It was recently reported that nutritional value alone can induce conditioning. Though not highlighted, that report also showed that hedonic value alone does *not* induce conditioning (de Araujo *et al.*, 2008), Fig 1H). To confirm this result, we conducted a two-bottle conditioning test with wild-type mice that received six days of training presented on alternating days with a bottle containing either water or tastant (two groups: sucrose or sucralose) for one hour sessions. During training, only one bottle was available and always placed in the center position. The sipper tubes for tastant and water were enameled either white or black providing a cue to associate with the tastants (counter-balanced in assignment to water or tastant). On testing, mice were presented two bottles with white and black sipper tubes, both filled with water, and allowed 10 minutes to drink. As both tubes contain water and only the sipper tube color distinguishes them, the degree to which a subject drank from one over the other tubes during the test reflects the degree to which the cue (ie., sipper tube color) has acquired incentive value through association with reward.

Consistent with the preference data, both sucrose and sucralose induced greater consumption than water (Fig 4 A). The tastants induced similar preference, which increased across the training days (ie., 3 pairs of water/tastant days, Fig 4B). In contrast to the preference data, sucralose was as effective in increasing consumption as sucrose. We attribute this to a ceiling effect in that mice had only limited time (60 minutes) to consume the tastants. In the preference test, they can continue to return to the tastants without limit. As expected, sucrose induces conditioning (Fig 4C left bar). That is, sucrose conferred incentive properties to its associated cue inducing mice to preferentially approach and sample that tube even when it contained water. Despite identical experience with both sucrose and sucralose during the training sessions, sucralose did not facilitate conditioning and mice showed no preference during the test sessions (Fig 4C right bar, sucrose vs. sucralose conditioning, $t = -2.41$, $p = .0365$). Experience with sucralose did not organize behavior during the test session in the absence of sucralose itself, while prior experience with sucrose did.

Progressive ratio

Another way to dissociate reward and reinforcement is to measure how much effort an animal is willing to expend to obtain the reward, essentially assessing the importance of the reward in organizing behavioral choice and effort. The classic measurement of reinforcer efficacy is the progressive ratio operant paradigm (Hodos, 1961) in which the cost (typically in lever presses) of each reward increases throughout a session measuring how much the animal is willing to pay for this particular reward.

Nutritional but not hedonic value supports increased appetitive responding over time

In these studies, the cost (lever presses) of a pellet increments by 7 (PR7) after each reward is earned, yielding a breakpoint: the cost at which the animal fails to earn a pellet. We

compared three reinforcers, sucrose and two calorie-free sweeteners, sucralose and saccharine, using wild-type and *trpm5* KO mice (sucrose only) with and without dopamine transporter knock-down, as before.

We initially trained mice using grain pellets and then switched them to either sucrose or calorie-free pellets for 5 days of PR7. Subsequently, the sucrose and calorie-free pellets were reversed and PR7 was continued for another 5 days. The wild-type and DATkd with sucrose, sucralose and saccharin were littermate controls and comprise a single experiment. The *trpm5* KO and *trpm5* KO:DATkd littermates were a separate experiment and analyzed separately. Though initial body weight between genotypes is not significantly different (wild-type vs. DATkd, 23.01 and 21.1 g, respectively, $t = .88$, $p = .38$), all analyses include body weight as a main effect, i.e., a covariant independent variable.

Type of reinforcer (sucrose, sucralose and saccharine) was a significant predictor of breakpoint (Fig 5A, $F_{(1,213)} = 62.9$, $p < .001$). Pairwise comparison shows a significant difference between sucrose and both calorie-free reinforcers (sucralose, $F_{(1,105)} = 26.04$, $p < .001$; saccharine, $F_{(1,105)} = 39.8$, $p < .001$) but no significant difference between sucralose and saccharin ($F_{(1,19)} = 1.94$, $p < .17$). In the remainder of the analysis, the sucralose and saccharin data are pooled as calorie-free reward.

During the initial five days with either sucrose or calorie-free sweeteners, sucrose induced greater responding that increased across the 5 sessions, while the lower rate of responding with calorie-free reward remained flat (Fig. 5B, left; sucrose vs. calorie-free reward, $F_{(1,209)} = 141.8$, $p < .001$; reinforcer $\times$ session, $F = 31.99_{(1,209)}$, $p < .001$). Switching from sucrose to calorie-free reward resulted in a decline in responding (Fig 5B, right). In contrast, switching to the sucrose from calorie-free pellets resulted in increased responding (Fig 5B, right). There was no significant main effect of genotype ($F_{(1,19)} = 1.9$, $p = .17$) but a significant genotype and reinforcer interaction ($F_{(1,209)} = 20.12$, $p < .001$) suggesting that elevated dopamine enhanced responding for sucrose but not artificial sweeteners. In the *trpm5* KO mice, elevated dopamine increased responding for sucrose (Fig 5A/B, breakpoint, genotype main effect, $F_{(1,9)} = 5.42$, $p = .044$; active lever presses, $F_{(1,9)} = 11.6$, $p < .01$), with the *trpm5* KO:DATkd mice exhibiting the same progressive increase across sessions (genotype $\times$ session, $F_{(1,41)} = 6.80$, $p = .012$) observed with sucrose in mice that can taste sweet (Fig 5B, left). There were no significant genotype differences or interactions in responding on the inactive lever (data not shown). Taken as a whole, these data suggest that (a) sucrose is more efficacious than taste alone as a reinforcer and induces *increasing* effort over time, (b) taste alone induces less responding that does not increase over time (c) dopamine preferentially increases responding associated with nutritional but not hedonic value. With nutrition alone, elevated dopamine dramatically increased responding, consistent with dopamine preferentially enhancing nutritional value. Similarly, in the *trpm5*:DATkd, responding increased over time, as observed with sucrose and wild-type mice, but did not in *trpm5* KO without DATkd.

These data indicate the importance of looking at behavior *over time*. For example, Reilly et al (Reilly & Trifunovic, 1999) have reported that saccharin and sucrose induce equal responding in a PR test. However, they tested only a single session (notably after multiple training sessions using sucrose for all groups), precluding observation of a decline in calorie-free and increase in sucrose responding as seen here (and also observed in Sclafani *et al.*, 2010).

In order to make iso-sweet sucrose and calorie-free pellets, the sucralose and saccharin pellets require the addition of fiber to achieve equivalent pellet mass. It is possible that this fiber has aversive qualities that may contribute to the present findings. However, this is

unlikely. Calorie-free consumption is approximately equal to sucrose consumption on the first day (Fig 5B, left) and remains constant while sucrose consumption increases, suggesting it is the enhanced reward of sucrose differentiating it from the calorie-free sweeteners, not the aversive quality of fiber (or adulterating bitterness, as discussed above). Further, in the voltammetry study reported below, both saccharin and sucrose pellets are consumed equally, again suggesting the fiber used in calorie-free pellets does not induce a significant aversive response.

Homecage progressive ratio concurrent choice (HCPGCC)

The above data suggest that sustained behavioral reinforcement that increases responding over time requires caloric value. However, the standard operant test examines behavior under food restriction in time limited sessions. Such a paradigm does not directly examine self-organized behavior in the context of energy balance. The homecage progressive ratio concurrent choice paradigm is designed to examine how energy balance is maintained or affected by food-seeking behavior. Moreover, it captures the advantages of the preference studies, where mice self-regulate with minimal experimenter interference, and the advantages of operant tests, which allow sophisticated measurement of the reinforcing properties of different rewards. In these studies, mice are singly housed in operant equipped homecages and have a continuous choice of consuming a freely available standard chow or working for a reinforcer (either sucrose, sucralose or saccharin) on a progressive ratio schedule (PR2). These studies examine how the reinforcing properties of hedonic or nutritional reward modulate self-regulated appetitive motivation and behavioral choices in a semi-naturalistic environment. Thirty minutes of inactivity reset the ratio allowing mice to initiate a new episode of pressing beginning again at the lower ratio. Importantly, this provides two variables under the mouse's control: how hard it works during an individual bout of lever pressing and how many bouts it initiates over a 24 hour period. As before, we tested wild-type and *trpm5* KO with and without dopamine transporter knock down and combined sucralose and saccharin in analysis and included body weight as a main effect.

HCPGCC: recapitulation and novel insights

The homecage studies recapitulate key findings from the conventional progressive ratio described above but also yield differences that provide an opportunity for further insight. Despite significant differences in the conditions under which mice had access to reinforcers, the patterns observed in the conventional progressive ratio (Fig 5) and the homecage (Fig 6) are remarkably similar, with two exceptions that are discussed in the next section and bring new insights about the relationship between taste and nutrition and the role of dopamine. On the first day, there was little difference in responding between reinforcers (Fig 6A/B, left). However, as the experiment proceeded, the sucrose reinforced mice increased (sucrose only, effect of day: active lever presses, $F_{(1,93)} = 6.8$, $p = .01$; total reward, $F_{(1,93)} = 18.5$, $p < .001$) while the calorie-free reinforced mice decreased their consumption and responding (calorie-free only, effect of day: active lever presses, $F_{(1,93)} = 38.8$, $p < .001$; total reward, $F_{(1,93)} = 46.6$, $p < .001$), resulting in 2-3 fold greater responding for sucrose than calorie-free reward (breakpoint, $F_{(1,209)} = 206.6$, $p < .001$; active lever presses, $F_{(1,209)} = 199.5$, $p < .001$; total reward, $F_{(1,209)} = 328.02$, $p < .001$). The same pattern was observed after switching reinforcers (Fig 6A/B, right). The *trpm5* KO mice (both + and - DATkd) show increased responding over time (Fig 6 A/B, active lever presses, $F_{(1,46)} = 6.16$, $p = .016$; total reward, $F_{(1,46)} = 12.23$, $p < .01$), consistent with the pattern associated with sucrose rather than calorie-free reinforcement. In mice that can taste sweet, DATkd (ie., elevated dopamine) had a greater effect on sucrose than calorie-free consumption (genotype $\times$ reinforcer effect: breakpoint, $F_{(1,209)} = 6.93$, $p < .01$; active lever presses, $F_{(1,209)} = 13.29$, $p < .001$). There were no significant differences between genotypes on inactive lever pressing ($F_{(1,19)} = 1.26$, $p = .27$, data not shown), indicating the increased responding is not

attributable to generalized hyperactivity. Thus, despite the fact that (a) there was no food restriction, (b) mice had unlimited access to reinforcer (ie., 24/7) and (c) chow was freely and concurrently available, the pattern of effort, consumption and putative reinforcement yields the same observations as the conventional progressive ratio test: sucrose is more efficacious than taste alone and induces *increasing* effort and consumption over time while taste alone induces significantly less responding that *diminishes* over time. The trpm5 KO mice, ie., nutrition alone, more closely resembles the sucrose rather than calorie-free pattern, particularly showing an increase in effort and consumption over time. These data suggest that these observations of relative reinforcer efficacy hold under conditions of self-regulation and food choice.

There are also key differences between the conventional and homecage studies that brought new insights about the relations between taste and nutrition and the role of dopamine. First, although elevated dopamine increases the amount of effort expended toward sucrose (Fig 6A), it does not alter overall reinforcer consumption (Fig 6B) or preference (Fig 6C), consistent with the preference data (Fig 3). Elevated dopamine increases effort and consumption during individual bouts of sucrose seeking but this is offset by fewer total bouts (see supplemental data), resulting in similar overall consumption and preference. One of the key differences between the paradigms is that in the conventional PR only a single bout/meal in an unambiguously hungry state is being measured. In the homecage paradigm, the total food intake is under global homeostatic control and the mice can choose between foods and pattern their bouts of lever pressing for reward. These data suggest that dopamine modulates effort during episodes of goal pursuit without altering overall consumption and preference (Beeler *et al.*, 2012).

Taste: hedonic value or conditioned stimulus?

The second significant difference between data from the two paradigms is that in the homecage study we did not observe any effect of elevated dopamine in the trpm5 KO on responding for sucrose pellets (ie., nutrition only, active lever presses, $F_{(1,9)} = .14$, $p = .70$; rewards, $F_{(1,9)} = .69$, $p = .42$). In the conventional PR, elevated dopamine dramatically increased trpm5 KO responding for sucrose (ie., nutrition only), making them resemble mice that can taste sweet (Fig 5B, right). In the homecage paradigm, however, elevated dopamine had little effect (Fig 6A/B). Although we observe the increased responding over time associated with sucrose rather than calorie-free reward, we do not observe enhancement by DATkd.

The key difference between the paradigms is that in the conventional progressive ratio, the nutritional reward obtained by the trpm5 KO is unambiguously attributable to their lever pressing and ingestion of sucrose pellets. In the homecage paradigm, the mice eat intermittently from both chow and pellets, compromising attribution of caloric value. Wild-type mice, in contrast, benefit from a life-time of association between sweet taste and caloric value, suggesting an important role for sweet taste as a conditioned cue indicating caloric value (elaborated in discussion). Consistent with the notion that dopamine scales the incentive value of cues associated with reward, the loss of elevated dopamine enhanced responding observed in the trpm5 KO mice in the homecage could be attributed to the loss of specificity in associating the response with nutritional reward, though further studies will be required to demonstrate this conclusively.

Evoked dopamine modulated by learned nutritional value

Our central hypothesis is that a taste loses its putative reinforcer efficacy if devoid of nutritional value. If nucleus accumbens dopamine underlies reinforcing properties, as is commonly believed, then dopamine release evoked by tastants should be modulated by their

nutritional value. Crucially, previous studies showing nucleus accumbens dopamine responses to sweet taste alone (Mark *et al.*, 1991; Hajnal *et al.*, 2004; de Araujo *et al.*, 2008; Wheeler *et al.*, 2011) have not allowed animals the opportunity to learn to discriminate sweet tastes based on their nutritional value. Here, in contrast, rats were trained to retrieve both sucrose and saccharin pellets allowing them to learn the relative nutritional value associated with each. Fast-scan cyclic voltammetry was then used to measure nucleus accumbens phasic dopamine evoked by each pellet.

Food-restricted rats were trained to retrieve differently flavored sucrose and saccharin pellets on alternate days. Training sessions took place 4-6 hours before rats were fed allowing ample opportunity for an association to develop between each pellet's flavor and its nutritional value without interference from calories derived from daily chow. Over the course of training, consumption of both types of pellets rapidly increased (Fig 7A; effect of day, $F_{(4,55)} = 4.129$, $p = 0.013$). Type of pellet affected consumption only in the first session, in which more sucrose pellets were consumed than saccharin (pellet effect, $F_{(1,55)} = 8.953$, $p = 0.030$; post hoc Day 1 sucrose vs. Day 1 saccharin: $p = 0.048$). From session 2 onwards there were no statistical differences attributable to type of pellet or day as rats ate virtually all pellets available (p 's > 0.05). After 10 pre- and 2 post-surgery training sessions, phasic dopamine was measured in the nucleus accumbens core while pellets were delivered in blocks of 20 (sucrose-saccharin-sucrose). During this test phase all pellets (sucrose and saccharin) were consumed. Phasic dopamine release events, evoked by pellet delivery, varied as a function of block (block main effect $F_{(2,14)} = 12.4$, $p = .0016$). Sucrose pellets in the first block evoked robust spikes in dopamine concentration that were time-locked with pellet delivery (mean 37.3 ± 18.5 nM) (Fig 7C left). In contrast, dopamine responses to saccharin pellets in the second block were greatly attenuated (mean 10.2 ± 6.2 nM, $p < .05$) (Fig 7C middle). This attenuation to saccharin was not the result of satiety as robust dopamine responses were reinstated with the final block of sucrose pellets (mean 28.8 ± 14.0 nM, $p > 0.05$ vs. 1st sucrose block) (Fig 7C right). In summary, although dopamine release is not entirely eliminated in response to saccharin, it is greatly attenuated compared to sucrose, consistent with greatly reduced reinforcer efficacy of hedonic value alone observed throughout the behavioral experiments.

DISCUSSION

The role of reward pathways in obesity has received prominent attention in recent years. The idea that highly palatable, easily available food induces dopamine mediated behavioral dysregulation, analogous to addiction, is intuitively appealing and has received some empirical support (Volkow & Wise, 2005; Kenny, 2010; Volkow *et al.*, 2010; Wang *et al.*, 2011). In these formulations, the underlying premise is that the hedonic pleasure derived from foods becomes dissociated from its nutritional value and escapes homeostatic regulation, resulting in over-consumption and weight gain, as reflected in the notion of 'hedonic hunger' (Lowe & Butryn, 2007). To assess the role that hedonic value may play in appetitive motivation, we dissociated taste and nutrition and assessed their independent contribution to behavioral responding in a number of paradigms. The data presented here suggest that palatability cannot be divorced from nutritional value. In its absence, the hedonic properties of taste alone provide greatly reduced reinforcement. In the conditioning test, although nutrition alone induces conditioning, taste alone does not, as observed previously (de Araujo *et al.*, 2008). In the operant paradigms, taste alone resulted in decreased responding over time, analogous to extinction mimicry (Wise *et al.*, 1978). This greatly reduced reinforcement efficacy is consistent with the attenuated dopamine release we observed in the voltammetry studies in response to non-caloric sweeteners. Together, these data suggest that 'wanting'—the incentive motivation generated by the dopaminergic

mechanisms in response to food— arises primarily from nutritional value associated with calories not hedonic value associated with taste.

Revisiting ‘wanting’ and ‘liking:’ distinguishing consumption from compulsion

Though both humans and rodents will consume tasty food in the absence of metabolic need, choosing to eat when sated does not equate to compulsion and is a familiar experience to many: dessert. Our preference data reflects this consumption “for pleasure” in the absence of need. When made freely available, mice increase their consumption of sucralose, demonstrating the capacity for hedonic value alone to induce consumption. The question is not whether we will eat tasty food in the absence of metabolic need but whether doing so will generate compulsive, unregulated consumption. Our operant studies suggest that when work is required, sucrose is highly reinforcing, as measured by effort expended on obtaining reward, while sweet taste without calories is not.

Berridge and Robinson (Robinson & Berridge, 2001) have famously made a distinction between ‘liking,’ the affective response of pleasure to sensory properties of stimuli and ‘wanting,’ the incentive motivation associated with stimuli that directs behavioral activity (Berridge, 2007). They have cogently argued that ‘liking’ and ‘wanting’ can become dissociated. In addition, for example, ‘wanting’ can escalate and become dissociated from ‘liking’ such that an addict continuously seeks drug despite increasingly not liking it (Robinson & Berridge, 2001).

We suggest that the hedonic properties of food support only ‘liking’ and that ‘wanting’ -- that is, the association of stimuli with incentive value that motivates and organizes future behavior-- requires nutritional value. This is most clearly illustrated in the sucralose conditioning test where mice demonstrate a preference for sucralose during training sessions (they ‘like’ it) but during testing, when both bottles contained water, only the sucrose trained mice exhibited conditioning. That is, only the cues reinforced with calories acquired incentive value to generate ‘wanting.’ The importance of nutritional value is also observed in the progressive ratio studies where both sucrose as well as nutrition alone induced increased responding over time, consistent with the notion of reinforcement—an outcome that increases the probability of the response in the future. In contrast, calorie-free rewards resulted in decreased responding over time, more analogous to the extinction mimicry observed by Wise and colleagues (Wise *et al.*, 1978). In those studies, rats were trained to stable responding and then administered a dopamine receptor antagonist that resulted in a decrease in responding over time—as observed here— that resembled extinction, which the authors attributed to a loss of reinforcement due to dopamine blockade. Here, in the case of calorie-free reinforcers, we attribute the apparent extinction mimicry to attenuated dopamine response to calorie-free reward, as observed in the voltammetry.

Interestingly, despite a broad public interest in weight loss and dieting, few low-calorie diet products have achieved widespread prominence and availability, with the obvious exception of diet soft drinks. Notably, these include caffeine—often more than non-diet versions (Chou & Bell, 2007)—known to activate the reward system (Patkina & Zvartau, 1998; Hsu *et al.*, 2009), potentially substituting for the reward value normally derived from nutrition and absent in diet soft drinks.

Dopamine and choice: does dopamine induce excessive intake of preferred food?

Implicit in current theories of dopamine and obesity is the notion that the dopamine mediated reward system can drive overconsumption by motivating individuals to seek and consume more palatable foods, overriding homeostatic controls (Lutter & Nestler, 2009). However, the present data do not support this. In the homecage concurrent choice paradigm,

although the hyperdopaminergic mice do show increased responding, they do not demonstrate greater consumption of the ‘preferred’ food.

Elevated dopamine appears to enhance the vigor (see Niv *et al.*, 2007) with which the mice pursue food without altering overall consumption (Beeler *et al.*, 2012). In the present study, the DAT^{kd} mice do not consume more sucrose than wild-type mice. These data suggests that although dopamine may increase responding for a nutritionally rewarding food, including preferred foods such as sucrose, the degree to which it alters food *choice* and overall consumption cannot be simply inferred but must be empirically examined. For example, a previous study with DAT knock-out mice (Costa *et al.*, 2007) observed that elevated dopamine increased consumption of sucrose solution during one hour sessions compared to wild-type mice. One might infer from this that dopamine increases reward value and may increase consumption of a preferred food. However, the present results would argue against this. Instead, this observation is consistent with data showing that dopamine can increase goal pursuit during local episodes of effort and consumption (Beeler *et al.*, 2012), such as one hour sessions; however, as the homecage and the two-bottle preference studies demonstrate, this does not imply that overall consumption will increase. Taken in isolation, observations from time-limited sessions, such as in the Costa study and the conventional PR studies reported here, may lead to incorrect inferences.

Though the idea that dopamine mediates hedonic pleasure, the anhedonia hypothesis (Wise *et al.*, 1978), has been widely critiqued for many years (Salamone *et al.*, 1997; Berridge & Robinson, 1998; Salamone *et al.*, 2005), there nonetheless remains a pervasive notion that dopamine preferentially reinforces hedonically derived reward, that is, preferred foods. This notion forms the basis of many current theories of dopamine and obesity (Volkow & Wise, 2005; Kenny, 2010; Volkow *et al.*, 2010) and is not supported by the current data.

Taste as conditioned stimulus: organizing appetitive behavior

Dopamine release is increased in response to unexpected reward and cues that predict reward (Roitman *et al.*, 2004; Day *et al.*, 2007; Roitman *et al.*, 2008; Jones *et al.*, 2010; Wanat *et al.*, 2010; Brown *et al.*, 2011) and extracellular dopamine increases and remains elevated shortly after initiating a meal (Hernandez & Hoebel, 1988; Hoebel *et al.*, 1992; Martel & Fantino, 1996; Taber & Fibiger, 1997; Sokolowski *et al.*, 1998; Cousins *et al.*, 1999; Ostlund *et al.*, 2010), both linking dopamine to food reward. Numerous studies have demonstrated that sensory properties of sweet taste can increase dopamine release independent of postingestive signaling (Hajnal *et al.*, 2004; Norgren *et al.*, 2006; Wheeler *et al.*, 2011) suggesting that taste plays a critical role in food reward. But what role? Is sweet taste itself intrinsically rewarding? Or does it serve as a conditioned stimulus— a cue— that signals anticipated nutritional reward?

In the voltammetry experiment, by training animals prior to recording, we allowed them the opportunity to *learn* about the relative value of sucrose and saccharin pellets and achieve stable behavioral responding. Consistent with the preference tests, sweet taste alone induced consumption and rats consumed both pellets equally. However, this consumption did not correspond to dopamine release, which was greatly attenuated during saccharin compared to sucrose trials, suggesting that the dopamine response is modulated primarily by nutritional value, consistent with imaging data from human subjects in which only sucrose, not saccharin, activate the dopaminergic system (Frank *et al.*, 2008). Moreover, in a related set of studies, we recently demonstrated a similar difference in evoked dopamine in response to *cues* associated with either sucrose or saccharin where dopamine release is greatly diminished in response to saccharin reinforced cues (McCutcheon & Beeler, 2012).

In the voltammetry studies, all pellets were consumed despite differential dopamine response. Thus, dopamine release did not predict intake nor did differences in dopamine release arise as a consequence of different behavioral responses to the two pellets. What is the behavioral significance of the differential dopamine release to nutritive sucrose and calorie-free saccharine pellets? Much evidence demonstrates that dopamine release affects behavior under conditions requiring work/effort (for review, Salamone, 2009). Thus, the differential dopamine response observed here in response to sucrose would suggest the animal would work harder for delivery of the nutritional pellets, as we observe in the operant studies. Another possibility, not mutually exclusive, is that the greater dopamine response would drive greater approach behavior towards pellet-associated cues, consistent with our observations in the two-bottle conditioning study. Additionally, we have recently shown that cues predicting sucrose pellets evoke greater dopamine responses than cues predicting saccharin pellets (McCutcheon & Beeler, 2012) and others have shown that such cue-evoked dopamine correlates with approach behavior (Flagel *et al.*, 2010). In general, we conclude that the increased dopamine release associated with sucrose delivery would enhance incentive associated with sucrose and that such dopamine mediated incentive would be greatly attenuated for calorie-free reward, as observed throughout the behavioral studies reported here. Interestingly, a recent study by Sclafani and colleagues observed a similar divergence in response between sucrose and calorie-free reward over time (Sclafani *et al.*, 2010). Though they do not elaborate, the authors note this may reflect a learning process, consistent with our interpretation of the present data.

Using sham-feeding techniques, Hajnal et al (Hajnal *et al.*, 2004) demonstrated that the sensory properties of sucrose alone can induce dopamine release. Importantly, the Hajnal study did not include a control for comparison in which the mice received both sensory *and* post-ingestive (ie., nutritional) stimulation, precluding the ability to interpret the magnitude of the sensory only dopamine response. Both our experiment as well as the studies by de Araujo et al (de Araujo *et al.*, 2008) directly compare calorie-free sweetener and sucrose. In both cases, dopamine increases approximately 100% in response to sucrose but only ~40% in response to calorie-free sweet taste. Importantly, in the Hajnal study, the dopamine response was approximately 40%, consistent with the present data, though the lack of a control group in that study precludes observing this as an *attenuated* response.

Traditionally it has been thought that taste provides positive feedback that promotes consumption while postingestive signaling provides negative feedback—satiety signals—that terminates consumption (reviewed in Sclafani, 2001; Davis & Smith, 2009). Accumulating data, however, demonstrate that postingestive signaling can promote consumption (current data, Sclafani, 2001; 2006; de Araujo *et al.*, 2008) and conversely, that taste, as a Pavlovian cue associated with satiety, can contribute to termination of consumption (Van Vort & Smith, 1987; Swithers & Davidson, 2008; Swithers *et al.*, 2009). Collectively, these data suggest an alternative relationship between taste and postingestive signaling: taste serves as a conditioned stimulus to *organize* appetitive and consummatory behavior by anticipating the consequences of ingestion (Woods & Ramsay, 2000; Swithers *et al.*, 2010; Davidson *et al.*, 2011)—based on learned associations between taste and metabolic events—rather than directly motivating ingestion as a primary reward. The present results are consistent with this view and suggest that in the absence of continued association with nutritional value, hedonic value loses its capacity to significantly drive or ‘organize’ behavior. We suggest that dopamine responds to primary nutritional reward and to taste stimuli that *predict* nutritional reward—possibly integrating associated costs (Ostlund *et al.*, 2010)—but not to sensory-hedonic properties independent of nutritional value.

Accumulating evidence demonstrates that dopamine does not modulate hedonic value (Cannon & Palmiter, 2003; Berridge, 2007). The present data indicate further independence between hedonic and motivational systems suggesting that, conversely, hedonic value in the absence of nutrition does not significantly modulate dopamine. Interestingly, Touzani et al demonstrate the reverse: that blocking opioid signaling, linked to hedonic processing, does not impair reinforcement and conditioning *despite modulating consumption* (Touzani et al., 2010).

In our experiment, we measured dopamine release in the nucleus accumbens core, which has long been proposed as a primary site for modulating incentive associated with both Pavlovian and instrumental behavior (Bassareo & Di Chiara, 1999; Kelley, 2004; Nicola et al., 2004; Balleine, 2005; Everitt & Robbins, 2005; Day et al., 2006). In this region, we observe a decreased dopamine response to calorie-free sweet taste as discussed above. However, the sensory pathways that carry gustatory information are widely distributed throughout the brain and taste is relayed through multiple channels (Saper et al., 2002; Norgren et al., 2006), likely serving multiple functions. We cannot rule out a role for dopamine in other potential functions of taste information in other regions and pathways. For example, in the NAc shell, the dopamine response to sweet taste (Mungarndee et al., 2008) may be independent of nutritional value and may contribute to preference and “non-reinforcing” consumption such as we observed in the preference tests. Regardless of the role taste may play in other regions, the present data clearly indicate that the reinforcement efficacy of taste alone is limited, consistent with the diminished dopamine response observed here in the nucleus accumbens core in response to saccharin. Examining the role of taste signaling in other brain regions represents an important area for further study.

Interactions between nutritional signaling and dopamine

Several candidate mechanisms have been proposed to convey nutritional information to the reward system (for review see de Araujo et al., 2012). The present data do not favor any particular mechanism but do highlight the necessity for a form of neuroplasticity that takes into account the temporally diffuse nature of nutritional signals, i.e. acting on a time course of minutes rather than milliseconds. As such, how a diffuse signal becomes integrated into a system that is activated by cues on a subsecond temporal scale is an essential question that remains to be answered. By providing discrete stimuli with which to associate postingestive consequences, taste may provide an efficient set of cues for organizing future behavior; that is, nutritional information is transferred onto discrete taste stimuli. In this view, however, the incentive properties of taste are derived from the prediction of nutritional value rather than from hedonic value itself.

Limitations and conclusions

The studies reported here have several limitations. First, we examine only sugar and sugar substitutes and do not investigate the reinforcing properties of other macronutrients or their associated hedonic value. Notably, the primacy of nutritional value observed here suggests an obvious reason why fats, extremely dense in energy, are highly rewarding. Second, we examine the effects of elevated but not diminished dopamine. In some theories of dopamine and obesity, diminished rather than enhanced dopamine function plays a critical role (Kenny, 2010). We cannot speak to these hypotheses, though notably the work of Salamone over many years has repeatedly demonstrated that reduced dopamine diminishes rather than augments effort toward obtaining a reward (Salamone et al., 2009). Third, we use rodents as experimental subjects. It is possible that hedonic value is much more important in humans. However, much of the data supporting the importance of hedonic value also arises from rodent work (but see Frank et al., 2008; Hursh & Silberberg, 2008; Haase et al., 2009). This highlights the potential importance of conducting studies to assess the degree to which the

present findings may be replicable in human subjects. Finally, in these studies we use normal, lean mice rather than obese mice. Not a limitation *per se*, we wish to draw a distinction between studying ‘normal’ reward processes and understanding how these may or may not contribute to overconsumption and the *development* of obesity and the study of reward processes in obese subjects, which may reflect pathological function arising as a *consequence* of obesity. Both types of studies are critical to understanding obesity; we focus here on the former.

The dramatic rise in obesity in recent years is a complex, multifaceted phenomenon. Understanding its causes and developing strategies to reverse the trend represents a significant social and scientific challenge. Recent efforts to step outside of the traditional domain of homeostatic regulation to examine non-homeostatic neural substrates shaping consummatory behavior are providing unique insights. The present report highlights a critical interaction between the putatively non-homeostatic reward system and the traditional homeostatic system suggesting that reward value is, ultimately, dependent upon metabolically derived nutritional value. Understanding the role of palatability and reward in over-consumption will require understanding the relationship between taste and nutritional value and dopamine's role in linking the two and shaping appetitive behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Effects of nutritional and hedonic value on consumption under conditions of free access. Throughout figures, colors code type of value: nutritional/metabolic only (blue), hedonic/taste only (red) or combined (purple). (A) Average daily consumption of tastant and water in wild-type mice provided a choice of either sucrose (filled squares) or sucralose (filled triangles) and water (open circles). (B/C) Average daily consumption of sucrose or sucralose (B, C respectively) and water (open symbols) in wild-type (filled squares) and trpm5 homozygous (filled triangles) mice. *** $p < .001$, ** $p < .01$, * $p < .05$, vertical dashed lines indicate main effects. $N = 12$ (A/B), $N = 6$ (C/D).

Figure 2.

Effect of quinine adulteration on consumption of sucrose or sucralose solution. Wild-type (A) consumption of tastant and water at various ratios of quinine and sweetener (dotted horizontal lines represent tastant consumption at baseline without quinine) and (B) preference for tastant over water (dotted horizontal line represents no preference mark). N = 8. Statistics in text.

Figure 3.

Effect of elevated dopamine on consumption of tastants under conditions of free access.

Average daily consumption of (A) sucrose or (B) sucralose for wild-type (filled squares), DATkd (open squares), trpm5 KO (filled triangles) and trpm5 KO:DATkd (open triangles). N = 6.

Figure 4.

Conditioning induced by sucrose and sucralose in wild-type mice. Average consumption of (A) sucrose, (B) sucralose compared to water during conditioning sessions. (C) Relative consumption of sucrose or sucralose compared to water during each of three pairs of conditioning sessions. (D) Preference for bottle previously paired with either sucrose or sucralose compared to bottle previously paired to water. Dashed line marks no preference (ie., 50%). * $p < .05$, $N = 6$.

Figure 5.

Effect of elevated dopamine on progressive ratio responding for hedonic and metabolic reward. (A) Average breakpoint across all sessions of PR7 for each reinforcer (blue 'nutrition' reflects sucrose with *trpm5* KO mice) for mice with DATkd (light bars) and wild-type littermates (dark bars, either wild-type or *trpm5* KO background). Dotted horizontal line represents median breakpoint. (B) Active lever presses for each day of the experiment for wild-type (filled symbols) and DATkd (open symbols) reinforced with sucrose (purple), calorie-free (combined sucralose and saccharin, red) or nutrition only (blue, sucrose tested with *trpm5* KO background). In the left panel, the arrow represents where reinforcers were

switched with the color continuing to indicate the reinforcer (ie., color will switch for same group/trace). *** $p < .001$, ** $p < .01$, * $p < .05$, $N = 6$.

Figure 6.

Homecage progressive ratio concurrent choice effort and reward. (A) Effort: left panel, total active lever presses across days for wild-type (filled symbols) and DATkd (open symbols) reinforced with sucrose (purple), calorie-free (combined sucralose and saccharin, red) or nutrition only (blue, sucrose tested with *trpm5* KO background). The arrow represents where reinforcers were switched with the color continuing to indicate the reinforcer (ie., color will switch for same group/trace). Right panel, average breakpoint for each reinforcer (sucrose, purple; calorie-free, red; sucrose with *trpm5* KO, blue) split by DATkd (light bars) and wild-type or *trpm5* KO (solid bars). (B) Consumption/rewards earned: same as in A. (C) Boxplots of preference for reinforcer as percentage of total consumption (reinforcer + chow) *** $p < .001$, ** $p < .01$, * $p < .05$. $N = 6$ (wild-type background) and $= 5$ (*trpm5* background).

Figure 7.

Phasic dopamine release evoked by saccharin pellets is attenuated relative to sucrose pellets. A. Experimental timeline. After 10 days training (starting with either sucrose or saccharin, counterbalanced) rats were surgically prepared for voltammetry recordings. After recovery, rats received two additional days training to habituate them to the recording headstage. During a single day of voltammetry recordings, rats received sucrose and saccharin pellets in blocks while phasic dopamine release was measured in nucleus accumbens core using fast-scan cyclic voltammetry. B. Left-hand panel, Behavioral data from training sessions showing that after several days of training rats retrieve and consume both types of pellet readily. Bars show mean $\pm$ SEM. Right-hand panel, Histological verification of electrode

placements in nucleus accumbens core. Numbers are mm anterior to Bregma. Adapted from Paxinos and Watson (2008). C. Left-hand panel, Average heat map from all rats showing trial-by-trial analysis of pellet evoked dopamine with dopamine concentration coded by color, hot colors indicating high concentrations. Pellet evoked responses are strong during first block of sucrose pellets, 'fade out' during delivery of saccharin pellets, and return during the second block of sucrose pellets. Dashed lines indicate transitions between blocks. Right-hand panel, Single trial traces of dopamine concentration from a representative animal. D. Mean dopamine concentration traces for each block of trials (sucrose-saccharin-sucrose) with data normalized for each rat to peak dopamine concentration in block 1. Dotted lines show SEM. E. Peak pellet-evoked dopamine response as a function of trial number. Data are taken from maximum value in the 2 s after pellet delivery, indicated by white vertical lines in C. Mean $\pm$ SEM of all rats is shown for each trial and mean for each block is shown as horizontal line.