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-evoked (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.

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 repelte state, the independence of this hedonic motivation from homeostatic mechanisms has been challenged. A study by de Araujo et al. (2008) used trpm5 knockout (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. (2010) recently demonstrated that nutritional content rather than taste responsiveness determines daily intake of sweet solutions in mice. Recently, Haase et al. (2009) demonstrated that activation of various brain regions in response to pure gustatory stimuli (i.e. 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., 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 (Figulewicz & 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 responses to calorie-free sweet taste are maintained after the animal learns to discriminate the artificial sweetener from real sugar (i.e. 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 knockdown mice (DATkd) with elevated tonic dopamine (Zhuang et al., 2001), as well as DAT knockdown 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 with 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 06:00–18:00 h light cycle, except during the two-bottle preference tests and the homeocan 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 homeocan operant tests, which were conducted across a period of 30 and 7 days, respectively. Male and female mice between 8 and 16 weeks old were used, age- and sex-matched across groups within each experiment. No mice were used in multiple experiments or conditions (i.e. 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 et al., 2006a,b; Yin et al., 2006; Tilley et al., 2007). They exhibit a 85% reduction in DAT 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 et al., 2006a; Yin et al., 2006). Trpm5 KO mice were originally derived by Damak et al. (2006), and heterozygote breeding pairs were 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 polymerase chain reaction.

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 & Madsen (1963) report that the most preferred saccharin solution, 0.4%, is iso-preferred with 3.5% sucrose. Preference for sucrose clearly increases at concentrations above 3.5% (e.g. 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, sacralose and saccharin solutions were at ~100, 1 and 2 mM, respectively. From this we used a 1 : 100 ratio for sucrose and sacralose solutions, which yielded iso-preference at low concentrations (i.e. Fig. 1; small but equivalent increase at 0.2/0.002% solutions). Sacralose and saccharine pellets were at 10 and 6 mmt, 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 (15 mL 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 tantast solution, either sucrose or sacralose (Sigma Aldrich, St Louis, MO, USA), at varying concentrations in the course of the experiment (sucrose: 0.0, 5.5, 145, 290 and 435 mM; sacralose: 0.0, 0.055, 1.45, 2.90 4.35 mM). Each concentration of tantast was provided for 6 days. Bottles containing tantast 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 positions of bottles were fixed in order to provide a cue for the animals, but the positions of tantast 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 tantast solution at a constant sweetener concentration (15 and 0.15% for sucrose and sacralose, respectively) adulterated with increasing quinine hydro-
chloride (Sigma-Aldrich) concentrations (0, 0.03, 0.06, 0.1 and 0.5 mM). Each concentration was provided for 4 days. In the last part of the experiment, quinine concentration was held constant (0.5 mM), while sucrose/sucralose concentrations were decreased (10/0.10, 5/0.05 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-h food and water restriction. Animals were conditioned for 6 days with a 30-min session every day of free access to water or tantast (sucrose 0.8 M or 30 mM sucralose, replicating de Araujo et al., 2008) on alternate days, e.g. Days 1, 3 and 5: water; Days 2, 4 and 6: tantast. Water and tantast were presented in the same middle position during training in identical water bottles (15 mL 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 (counterbalanced between tantast and water). After conditioning, animals were tested for conditioned preference in a 10-min two-bottle access test, where both bottles contained water but with different black/white sipper tubes as conditioned cues. The amount of water consumed from each bottle reflects an animal’s preference for the conditioned cue previously associated with tantast or water.

Progressive ratio
Tests were conducted in operant conditioning chambers with two retractable levers (Med Associates, St Albans, VT, USA). Mice were food restricted, receiving 2 h of ad libitum feeding after each session. Animals were trained to lever press at FR1 ratio overnight for 2 nights. They were then trained on an FR1 in daily 30-min sessions until reaching criteria (earning 18 pellets in a 30-min session for 3 consecutive days). Animals were then trained with 2-h sessions for 2 days on a progressive ratio schedule where after each pellet earned, the required number of presses for the next reward increases by three (PR3). Following this training, mice were tested on a PR7 schedule (cost of each subsequent pellet increments by seven) with 2-h sessions for 5 consecutive days. Twenty-microgram grain pellets (Bio-Serv, Frenchtown, NJ, USA) were used as reinforcers during FR1 and PR3 sessions, and replaced with 20 mg sucrose, saccharine 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 were available ad libitum. Upon initial placement in the homecages operant boxes, the first 50 rewards (20 mg sucrose, saccharine 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 (i.e. start the progression at the beginning) after 30 min of inactivity. Experiments lasted 7 days, during which freely available chow consumption was measured daily.

Fast-scan cyclic voltammetry (FSCV)
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 h. 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 and 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). 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 (five 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 (Enner 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 ventral tegmental area and lowered in 0.2 mm increments until electrically evoked dopamine release was detected with the carbon fiber electrode. Dopamine was measured using FSCV. In FSCV, a triangular voltage waveform (~0.4 V to ~1.3 V 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 (~0.6 V to ~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–saccharin). When these sessions ended, dopamine was evoked by electrical stimulation of the midbrain. A range of stimulation parameters was 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 (i.e. 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 induce 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, $F_{1,11} = 372.98$, $P < 0.0001$; sucralose, $F_{1,11} = 62.05$, $P < 0.0001$) in a concentration-dependent manner (both taurists, $F_{1,22} = 178.2$, $P < 0.0001$). However, sucrose induces an approximately twofold greater consumption of tastant (Fig. 1A; tastant main effect, $F_{1,22} = 42.16$, $P < 0.0001$) and exhibits greater concentration sensitivity (concentration $\times$ tastant, $F_{1,22} = 56.9$, $P < 0.0001$). At the lowest concentrations tested, both taurists 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 Materials and 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 KO mice 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, $F_{1,5} = 50.34$, $P < 0.001$). However, compared with 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, $F_{1,5} = 0.128$, $P = 0.7384$), 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 & Sclafani, 2003; Sclafani & 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 (i.e. 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. (2010) suggest that sucralose has a bitter taste that is masked by its sweet taste in B6 wild-type mice at concentrations $< 3$ mM. As only the final concentration tested here exceeds 3 mM (i.e. 0.15% = 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 with 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, $F_{1,477} = 5.15$, $P = 0.023$). 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, $F_{1,477} = 21.08$, $P < 0.001$).

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 wild-type 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 DAT 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 et al., 2006a).

Elevated dopamine (i.e. DATkd on both backgrounds) does not enhance consumption of either sucrose (Fig. 3A; wild-type, $F_{1,11} = 34.72$, $P = 0.5874$; trpm5, $F_{1,11} = 33.4$, $P = 0.46$) or sucralose (Fig. 3B; $F_{1,11} = 2.27$, $P = 0.16$). These data suggest 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 saccharose 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 6 days of training presented on alternating days with a bottle containing either water or tastant (two groups: sucrose or saccharose) for 1-h sessions. During training, only one bottle was available and always placed in the center position. The sipper tubes for tastant and water were enamelled either white or black, providing a cue to associate with the tastants (counterbalanced 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 min 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 (i.e. sipper tube color) has acquired incentive value through association with reward.
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, $t = -2.41, P = 0.0365$).

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 DAT knockdown, 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 analysed separately. Though initial body weight between genotypes is not significantly different (wild-type vs. DATkd, 23.01 and 21.1 g, respectively, $t = 0.88, P = 0.38$), all analyses include body weight as a main effect, i.e. a covariant independent variable.
The type of reinforcer (sucrose, sucrалose and saccharin) was a significant predictor of breakpoint (Fig. 5A; $F_{1,213} = 62.9, P < 0.001$). Pairwise comparison shows a significant difference between sucrose and both calorie-free reinforcers (sucralose, $F_{1,105} = 26.04, P < 0.001$; saccharine, $F_{1,105} = 39.8, P < 0.001$), but no significant difference between sucrose and saccharin ($F_{1,10} = 1.94, P < 0.17$). In the remainder of the analysis, the sucralse and saccharin data are pooled as calorie-free reward.

During the initial 5 days with either sucrose or calorie-free sweeteners, sucralse induced greater responding that increased across the five sessions, while the lower rate of responding with calorie-free reward remained flat (Fig. 5B, left; sucrose vs. calorie-free reward, $F_{1,209} = 141.8, P < 0.001$; reinforcer × session, $F_{1,209} = 31.99, P < 0.001$). Switching from sucralse to calorie-free reward resulted in a decline in responding (Fig. 5B, right). In contrast, switching to the sucralse from calorie-free pellets resulted in increased responding (Fig. 5B, right). There was no significant main effect of genotype ($F_{1,10} = 1.9, P = 0.17$), but a significant genotype and reinforcer interaction ($F_{1,209} = 20.12, P ≤ 0.001$), 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 and B; breakpoint, genotype main effect, $F_{1,19} = 5.42, P = 0.044$; active lever presses, $F_{1,19} = 11.6, P < 0.01$), with the trpm5 KO:DATkd mice exhibiting the same progressive increase across sessions (genotype × session, $F_{1,41} = 6.80, P = 0.012$) observed with sucralse 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: (i) sucrose is more efficacious than taste alone as a reinforcer and induces ‘increasing’ effort over time; (ii) taste alone induces less responding that does not increase over time; and (iii) 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 sucralse 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 & Trifunovic (1999) have reported that saccharin and sucralse 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 sucralse and calorie-free pellets, the sucralse 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 sucralse pellets are consumed equally, again suggesting the fiber used in calorie-free pellets does not induce a significant aversive response.

**Homemage progressive ratio and concurrent choice**

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 and concurrent choice (HCPRCC) 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 homemaches, and have a continuous choice of consuming a freely available standard chow or working for a reinforcer (either sucrose, sucralse 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-h period. As before, we tested wild-type and trpm5 KO with and without DAT knockdown, and combined sucralse and saccharin in analysis and included body weight as a main effect.

**HCPRCC – 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 and B, left). However, as the experiment proceeded, the sucralse-reinforced mice increased (sucrose only, effect of day: active lever presses, $F_{1,93} = 6.8, P = 0.01$; total reward, $F_{1,93} = 18.5, P < 0.001$), while the calorie-free-reinforced mice decreased their consumption and responding (calorie-free only, effect of day: active lever presses, $F_{1,93} = 38.8, P < 0.001$; total reward, $F_{1,93} = 46.6, P < 0.001$), resulting in two–threefold greater responding for sucrose than calorie-free reward (breakpoint, $F_{1,209} = 206.6, P < 0.001$; active lever presses, $F_{1,209} = 199.5, P < 0.001$; total reward, $F_{1,209} = 328.02, P < 0.001$). The same pattern was observed after switching reinforcers (Fig. 6A and B, right). The trpm5 KO mice (both + and – DATkd) show increased responding over time (Fig. 6A and B; active lever presses, $F_{1,146} = 6.16, P = 0.016$; total reward, $F_{1,146} = 12.23, P < 0.01$) consistent with the pattern associated with sucralse rather than calorie-free reinforcement. In mice that can taste sweet, DATkd (i.e. elevated dopamine) had a greater effect on sucrose than calorie-free consumption (genotype × reinforcer effect: breakpoint, $F_{1,209} = 6.93, P < 0.01$; active lever presses, $F_{1,209} = 13.29, P < 0.001$). There were no significant differences between genotypes on inactive lever pressing ($F_{1,19} = 1.26, P = 0.27$, data not shown), indicating the increased responding is not attributable to generalized hyperactivity. Thus, despite the fact that: (i) there was no food restriction; (ii) mice had unlimited access to reinforcer (i.e. 24/7); and (iii) 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, i.e. 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

**Fig. 6.** HCPRCC effort and reward. (A) Effort: left panel, total active lever presses across days for wild-type (filled symbols) and dopamine transporter knockdown (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 (i.e. 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 < 0.001, **P < 0.01, *P < 0.05. N = 6 (wild-type background) and = 5 (trpm5 background).

**Fig. 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 FSCV. (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 ± SEM. Right-hand panel: histological verification of electrode placements in nucleus accumbens core. Numbers are mm anterior to Bregma. (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 the 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 ± SEM of all rats is shown for each trial, and mean for each block is shown as a horizontal line.
individual bouts of sucrose seeking, but this is offset by fewer total bouts (see Supporting Information), 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 (i.e. nutrition only, active lever presses, effect of elevated dopamine in the trpm5 KO on responding for 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 (mean 28.8 ± 14.0 nM; Fig. 7C, right). In the homecage paradigm, however, elevated dopamine had little effect (Fig. 6A and 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. 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. FSCV 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 h 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 ($P > 0.05$). After 10 pre- and two 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, $F_{2,14} = 12.4, P = 0.0016$). 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, $P < 0.05$; 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 with 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 overconsumption 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 reflect this consumption ‘for pleasure’ in the absence
of need. When made freely available, mice increase their consumption of sucrose, 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. 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 addiction, 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 sucrose conditioning test, where mice demonstrate a preference for sucrose 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 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 DATkd mice do not consume more sucrose than wild-type mice. These data suggest 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 KO mice (Costa et al., 2007) observed that elevated dopamine increased consumption of sucrose solution during 1-h sessions compared with 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 1-h 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, 2005; Berridge & Robinson, 1998), 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, 2008; Day et al., 2007; 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 post-ingestive 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 with 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, see 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 release 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. (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 (i.e. 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. (2008) directly compare calorie-free sweeter 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 post-ingestive signaling provides negative feedback (satiety signals) that terminates consumption (reviewed in Sclafani, 2001; Davis & Smith, 2009). Accumulating data, however, demonstrate that post-ingestive 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 post-ingestive 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 ‘organized’ 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. (2010) demonstrate the reverse – that blocking opiod signaling, linked to hedonic processing, does not impair reinforcement and conditioning despite modulating consumption.

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 nucleus accumbens shell, the dopamine response to sweet taste (Mungambedu 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 post-ingestive 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 comment on 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 arise 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 overconsumption will require understanding of the relationship between taste and nutritional value, and dopamine’s role in linking the two and shaping appetitive behavior.

Supporting Information

Additional supporting information can be found in the online version of this article:

Fig. S1. Patterning of behavioral effort and reinforcer consumption in HCPRCC. (A–C) Left panels: average number of bouts of responding per day; middle panels: average reinforcers earned per bout; right panels: dotplot of bout size (i.e. number of reinforcers) against number of meals per day with a separate regression line for with and without DATkd (dashed, DATkd; solid, wild-type or trpm5 KO without DATkd). ***P < 0.001, **P < 0.01, *P < 0.05. N = 6 (wild-type background) and =5 (trpm5 background).

Fig. S2. HCPRCC consumption and preference. (A–C) Left panels: average daily consumption of reinforcers (color-coded portion of bars) and free chow (ivory portion of bars). Right panels: boxplots of preference for reinforcer as percentage of total consumption (reinforcer + chow) for (A) sucrose, (B) sucralse/saccharin and (C) sucrose with trpm5 KO mice. Lighter color in boxplots represents DATkd for either wild-type (A and B) or trpm6 KO (C) backgrounds. ***P < 0.001, **P < 0.01, *P < 0.05. N = 6 (wild-type background) and =5 (trpm5 background).

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Abbreviations

DAT, dopamine transporter; FSCV, fast-scan cyclic voltammetry; HCPRCC, homecage progressive ratio concurrent choice.

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