The role of dopamine in the pursuit of nutritional value

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Abstract

Acquiring enough food to meet energy expenditure is fundamental for all organisms. Thus, mechanisms have evolved to allow foods with high nutritional value to be readily detected, consumed, and remembered. Although taste is often involved in these processes, there is a wealth of evidence supporting the existence of taste-independent nutrient sensing. In particular, post-ingestive mechanisms arising from the arrival of nutrients in the gut are able to drive food intake and behavioural conditioning. The physiological mechanisms underlying these effects are complex but are believed to converge on mesolimbic dopamine signalling to translate post-ingestive sensing of nutrients into reward and reinforcement value.

Discerning the role of nutrition is often difficult because food stimulates sensory systems and post-ingestive pathways in concert. Thus, in this mini-review, I discuss the various methods that may be used to study post-ingestive processes in isolation including sham-feeding, non-nutritive sweeteners, post-ingestive infusions, and pharmacological and genetic methods.

Using this structure, I present the evidence that dopamine is sensitive to nutritional value of certain foods and examine how this affects learning about food, the role of taste, and the implications for human obesity.

Keywords

Dopamine; Nutritional value; Taste; Post-ingestive; Microdialysis; Fast-scan cyclic voltammetry; Conditioning

1. Introduction

In the 1930s Curt Richter outlined the importance of behaviour as a means for animals to regulate physiological state in a homeostatic manner [1]. One focus of his research was the ability of rats to guide their behaviour towards certain nutrients in times of deficit. Potentially, the most fundamental form of such behavioural homeostasis is an organism's drive to acquire enough food to supply its energetic needs. Accordingly, a number of mechanisms have evolved to allow animals to accomplish this including sensory systems, such as taste, as well as the ability to detect nutrients post-ingestively.

Identifying the precise mechanisms that underlie the ability to detect nutritional value is challenging, particularly their neurobiological basis, because several distinct processes normally occur in concert. For example, ingestion of sugar stimulates taste receptors in the oral cavity as well as providing post-ingestive feedback via peripheral actions including activation of receptors and/or transporters, release of hormones such as insulin, and cellular glucose oxidation. Thus, the vast majority of experiments examining the neurobiology of feeding behaviour cannot separate the contribution of taste from that of post-ingestive effects. However, a number of methods have been developed that allow these processes to be studied independently of one another (see Section 3).

The neurotransmitter dopamine has long been associated with feeding behaviour, motivation, reward and reinforcement, and associative learning [2,3], all of which could be presumed important in the procurement of nutritional value. In this review, I will discuss the evidence that the dopamine system is exquisitely sensitive to certain nutrients and argue that this is an essential way in which an organism can activate behavioural plans that maintain nutrient homeostasis. I will present research using different methods of either isolating or eliminating the role of nutritional information and describe how these impact the dopamine system. In addition, the mechanisms by which this information is relayed to the brain will be examined including the possibility that different nutrients are signalled through different pathways. The role that food-associated cues play in driving feeding behaviour and the sensitivity of these cues to the post-ingestive consequences of the nutrients that they predict will be examined. Finally, the implications of this research for feeding behaviour in humans and obesity will be discussed.

2. An essential role for dopamine in feeding

The involvement of dopamine in feeding behaviour has been recognised for several decades. A dramatic demonstration of this necessity is shown in mice that are genetically engineered to lack dopamine by mutation of tyrosine hydroxylase (dopamine-deficient mice or DD mice) [4]. These mice starve to death unless supplied with an alternative source of

dopamine either via administration of the dopamine precursor, L-Dopa, or viral expression of functional enzyme [5]. What these experiments make clear is that although dopamine is essential for feeding, its precise role remains uncertain. For example, motivation to feed seems to be affected in DD mice rather than preference for rewarding foods and palatability [6]. Moreover, dopamine has been implicated in multiple aspects of feeding behaviour including sensory components, learning, relaying physiological state, providing motivation, and finally, encoding nutrient content. Importantly, however, the participation of dopamine in feeding seems to be highly conserved and has been shown in a wide range of species including *Drosophila* [7], rats [8], mice [4], non-human primates [9], and humans [10].

Convincing demonstrations of dopamine's role in modulating specific aspects of feeding have been obtained using methods that allow activity of the dopamine system to be measured in real-time during feeding behaviour. As such, electrophysiological recordings of dopamine neuron activity or measurement of dopamine concentration in terminal fields with either microdialysis or fast-scan cyclic voltammetry have shown that presentation of food stimuli reliably evokes neuronal activity and dopamine release across the striatum [11–14]. Such responses have been observed to a wide range of different food stimuli and under different experimental conditions. The magnitude and probability of occurrence of such signals can be modulated by properties of the food such as its novelty, and the physiological state of the subject [3,11,15,16]. In this short review, my focus will be one specific question: how relevant is the nutritional content of a food stimulus for the dopamine activity evoked by food or food-associated stimuli?

3. Methods for isolating the contribution of nutritional content

A major challenge in studying the neurobiology underlying post-ingestive signalling is that ingestion of food engages multiple sensory components and elicits post-ingestive effects. Therefore, the vast number of studies examining neurobiology of feeding inherently confound contributions from several sensory modalities (e.g. taste, smell, somatosensation) and post-ingestive processes, including nutrient-sensing. Thus, specific experimental manipulations have been devised in an attempt to study each process in isolation (Fig. 1).

One such method, which has been used to remove post-ingestive processes from the study of feeding behaviour, is the sham-feeding paradigm (Fig. 1A) [17]. Here, animals are typically implanted with a gastric fistula, which allows them to feed while any substance consumed leaves the stomach before being absorbed or processed. Thus, the effect of orosensory stimulation in the absence of post-ingestive effects can be determined.

Another method of isolating post-ingestive processes is to use non-nutritive artificial sweeteners (Fig. 1B). Examples are acesulfame K, saccharin, and sucralose. The sweetness of these products can, in theory, be matched to real sugars, such as sucrose or glucose, and responses to each can be compared. However, care must be taken with these studies as precisely matching the orosensory component is challenging, if not impossible. Additionally, differences in preference for these sweeteners varies across species and individuals such that matching the taste or flavour of a real sugar to a non-nutritive sweetener might not be possible [18,19].

Direct infusions of nutrients into various compartments of the gastrointestinal (GI) tract or vasculature can be used to probe the post-ingestive effects of nutrients in isolation from other components of feeding behaviour (Fig. 1C). Such infusions may be made in the absence of any behaviour or be triggered by an operant response. These techniques have formed the basis for a pioneering collection of flavour-nutrient conditioning studies by Sclafani and colleagues, which have provided a great deal of fundamental knowledge regarding how post-ingestive effects interact with behaviour [20]. Moreover, infusions into different sites may be used to probe the exact mechanisms by which nutrients are signalled to the brain.

Pharmacological manipulations may provide insight into whether a specific biological mechanism relating to nutrient signalling is involved in feeding (Fig. 1D). For example, the use of the nonmetabolizable glucose analogue, 2-deoxyglucose (2-DG), has been used to demonstrate a role for glucose utilization in several processes [21].

Finally, genetic methods may be used to remove a certain component of feeding behaviour (Fig. 1E). One example is that knockout of the TRPM5 channel, which is essential for the transduction of sweet taste (and bitter and umami), has been used to generate mice that are 'sweet-blind'. This mutant line has allowed study of the post-ingestive effects of ingested sugars in isolation from taste-mediated effects [22].

It should be noted that these methods do not need to be used in isolation and, in fact, often are combined. For example, in flavour-nutrient conditioning paradigms, post-ingestive infusions are regularly used in combination with artificial sweeteners so that sweet-tasting food stimuli can be combined with varying degrees of nutrient provision.

3.1 Sham feeding

The use of the sham-feeding paradigm to decouple orosensory stimulation from post-ingestive mechanisms has provided invaluable information on the relationship between taste, reinforcement value, and dopamine signalling. If rats are allowed to sham feed

sucrose then the amount of sucrose ingested and number of licks increases as sucrose concentration increases [17]. Microdialysis measurements during sham feeding show that dopamine efflux increases in a concentration-dependent manner and that this increase is independent of the greater motor activation produced by high concentrations of sucrose, relative to low concentrations [23]. Similarly, sham-feeding of corn oil increases dopamine efflux in nucleus accumbens [24]. Thus, dopamine efflux may be produced by the orosensory properties of nutrients even in the absence of their post-ingestive effects. Moreover, in the sham-fed paradigm the behavioural and neurochemical effects of sucrose consumption may even be enhanced, relative to real feeding, due to the removal of satiation-related signals that are normally produced post-ingestively [25].

That dopamine is an important mediator of the enhanced behavioural response to high concentrations of sugar has been demonstrated by blocking dopamine receptors, which reduces licking for high sucrose concentrations [17,26]. This effect is seen with blockade of D1-like or D2-like dopamine receptors and occurs without affecting motor output [26].

Interestingly, prior experience with sucrose, whether sham-fed or real-fed, increases subsequent sham feeding responses showing that the taste-mediated effect is subject to experience-dependent plasticity [25,27]. In fact, experience with a high concentration of sucrose in a sham-fed state (i.e. in the absence of the post-ingestive effects of sucrose) increases subsequent sham-fed responses, which is suggested to be due to extinction of a conditioned satiety effect specific to hypertonic carbohydrate solutions [27]. If there are circumstances in which sham-feeding responses and dopamine release evoked by weak carbohydrate solutions will decrease due to decoupling of orosensory properties from rewarding post-ingestive consequences remains to be determined.

3.2 Artificial sweeteners

Artificial, non-nutritive, sweeteners can be used to examine responses to taste in the absence of post-ingestive processes. In general, these substances are readily consumed by mice and rats and elicit appetitive orofacial reactions if infused intraorally, suggesting a positive hedonic evaluation [28,29]. Moreover, mice and rats show increased dopamine efflux and dopamine release in response to free consumption or intraoral delivery of non-nutritive sweeteners [22,30,31]. However, the extent to which these non-nutritive compounds participate in reinforcement/conditioning and how they interact with dopamine signalling during these processes is less clear.

Certain conditioned responses are observed when non-nutritive substances are used as stimuli. For example, if a novel flavour is paired with the non-nutritive sweetener, saccharin,

a conditioned preference will develop to the novel flavour. This is termed flavour-taste conditioning and is observed with most palatable compounds including nutritive sugars such as glucose. However, if experiences with the novel flavour and with saccharin are separated in time, in a so-called delayed procedure, then no conditioned response develops [32,33]. This differs from the situation with most nutritive sugars in which delayed conditioning is observed. Flavour-taste conditioning with non-nutritive substances also differs in that it is not modulated by the physiological state of the subject (e.g. hunger) [34]. Conditioned responses to other cues are also reduced or abolished to non-nutritive sweeteners. For example, mice fail to condition to bottle position associated with the non-nutritive sweetener, sucralose, whereas if sucrose is used in its place they do learn the association [22,35]. Finally, in tests of reinforcement and motivation we found that food pellets sweetened with non-nutritive sweeteners, sucralose and saccharin, yielded lower break-points in progressive ratio tests, relative to sucrose pellets, and did not sustain responding over time. Moreover, whereas mice increased responding for sucrose with repeated experience, this escalation was not seen when non-nutritive sweeteners were used [35].

We reasoned that a reduction in dopamine evoked by the non-nutritive compounds as animals learned that they did not predict nutritional content might underlie these behavioural effects. To examine this possibility, we trained food-restricted rats over a number of sessions with exposure to differently-flavoured sucrose and saccharin pellets occurring on alternate days. In this way, we maximised the rats' opportunity to associate each pellet with a different post-ingestive outcome. On a single test day we delivered both types of pellet in a single recording session while we measured phasic dopamine release using voltammetry. Consistent with our hypothesis, we found that, although phasic dopamine release was evoked by delivery of both sucrose and saccharin pellets, the response to non-nutritive pellets was greatly attenuated, relative to the response to nutritive pellets [35]. We further hypothesized that the dopamine responses that develop to food-associated cues [36] would be weakened if the predicted food stimulus was non-nutritive. Indeed, we found that, if rats are trained to associate a distinct cue with each type of pellet then the cue-evoked phasic dopamine responses also differed between nutritive and non-nutritive rewards with sucrose cues evoking greater dopamine release than saccharin cues [37] (Fig. 2B). Results obtained with pellets are recapitulated when food stimuli were presented in liquid rather than solid form. Separate cohorts of rats were trained that a cue predicted either intraoral infusion of sucrose solution (0.3 M) or saccharin solution (1.1%). Importantly, intraoral infusions of these concentrations in the same rats produced similar orofacial movements in the taste reactivity paradigm (see Fig. 2 legend). Despite this similar hedonic evaluation, voltammetry

measurements revealed that cues that predicted sucrose infusions evoked greater phasic dopamine responses than saccharin-predictive cues (Fig. 2C).

In an attempt to identify differences in nutritional content as the cause of the differential dopamine release between sucrose and saccharin, we masked nutritional differences between the pellets by exposing rats to both types of pellet on the same day [37]. In this way, the source of the post-ingestive signal was blurred because experience of each pellet was separated by less than 2 minutes. In this condition, the difference in dopamine release evoked by each cue was reduced, strongly suggesting that cue-evoked dopamine signals are sensitive to the nutrient content of the food reward predicted by the cue. However, it should be noted that a residual difference in dopamine remained even under these conditions. Our hypothesis is that this residual difference reflects preference for sucrose pellets over saccharin pellets as rats showed a strong preference for sucrose pellets over saccharin pellets even when the difference in nutrient content was masked [37]. Interestingly, a positive correlation exists between each individual rat's preference for sucrose over saccharin pellets and the difference in dopamine evoked during each trial (Fig. 3). This implies that in the absence of nutritional differences, preference is the key contributor driving differential dopamine release. This relationship does not exist for cohorts in which the caloric difference between pellets was emphasized by training with each pellet on alternate days.

Recent studies from the Friedman lab have used activation of the dopamine system during consumption of non-nutritive sweetener to offer support for dopamine's role in encoding the reinforcement value of nutrients. The normal preference for sucrose (110-140 mM) over sucralose (0.5 mM) was reversed by pairing sucralose consumption with optogenetic stimulation of either dopamine neurons or a subset of inputs to dopamine neurons from melanin concentrating hormone (MCH)-expressing neurons of the lateral hypothalamus [38,39]. These neurons are excited by glucose via closure of ATP-gated potassium channels providing a mechanism by which changes in blood glucose could be rapidly relayed to the mesolimbic system [40]. Stimulation of these neurons is shown to increase dopamine efflux in the striatum [39]. Importantly, while direct stimulation of dopamine neurons will generate a preference for water over sucrose [38], stimulation of MCH inputs does not generate a preference for water over sucrose [39]. Thus, the ability of MCH neurons to drive feeding and dopamine release seems dependent on a palatable taste input is required as well as neuronal activity.

3.3 *Post-ingestive infusions*

Infusions of nutrients into various parts of the GI tract or vasculature provide a powerful means of testing the contribution of post-ingestive signalling independently of taste. This technique was pioneered by seminal work on flavour-nutrient conditioning conducted by the Sclafani lab [41–43]. In a typical experiment of this kind, rodents are allowed access to two flavoured solutions (often cherry or grape flavoured saccharin). Consumption of one flavour results in infusions of a nutrient (e.g. glucose) into the stomach while consumption of the other flavour results in infusion of water. This procedure results in a preference forming for the nutrient-paired flavour over the water-paired flavour (flavour preference) and also increased absolute consumption of the nutrient-paired flavour (flavour acceptance) [44]. This type of conditioning is supported by most sugars, carbohydrates, and fats [45,46]. Glucose is the most commonly used nutrient in experiments of this kind and other sugars that support conditioning include sucrose, maltose, and polycose [41,46]. Interestingly, in most situations fructose does not support conditioning [47–49] suggesting that it is not simply caloric value that drives this process, but rather nutrient-specific signalling mechanisms [50,51]. A number of flavours have been used in this paradigm and flavour-nutrient preferences even develop to innately aversive flavours, such as sour- or bitter-tasting compounds, after pairing with nutrients [43].

Post-ingestive infusions are associated with activation of dopamine signalling. Intragastric infusion of nutrients in the flavour-nutrient conditioning paradigm was first shown to evoke dopamine release by the Hoebel lab [52]. Subsequently, post-ingestive infusion of nutrients has been shown to activate dopamine signalling under a number of different conditions [21,53–55]. Intragastric glucose infusions increase dopamine efflux in dorsal and ventral striatum of mice, relative to infusions of the sweet-tasting amino acid, L-serine [21]. Moreover, intragastric nutrients can evoke dopamine efflux and drive behaviour even in the absence of taste input, as has been shown in a “dry licking” paradigm in which mice lick at an empty spout in order to obtain self-infusions of fat emulsions [53,55]. In this paradigm, mice match their lick rate to the concentration of fat emulsion being self-infused, thereby regulating their caloric intake (i.e. more licks were produced for lower concentrations) [53]. Dopamine efflux was proportional to fat concentration rather than lick rate showing that dopamine was sensitive to caloric density of the emulsion rather than motor output. In addition, passive infusions of fat modulated dopamine efflux in a concentration-dependent manner. Finally, infusion of glucose in anesthetized rats stimulates dopamine release as measured by voltammetry providing more evidence of the sensitivity of dopamine signalling to post-ingestive nutrients in the absence of taste input [54].

The behavioural significance of activation of dopamine signalling has been demonstrated by showing that both systemic injections and central infusions of the dopamine D1-like receptor antagonist, SCH23390, disrupt the acquisition, but not expression, of flavour-nutrient conditioning [56–59]. Central sites in which D1 receptor blockade is effective include nucleus accumbens, prefrontal cortex, amygdala, and lateral hypothalamus. In contrast, antagonism of dopamine D2-like receptors has no effect on this conditioning procedure. In the dry-licking paradigm, behavioural modulation of lick rate by caloric density is disrupted when the dopamine D2-like receptor antagonist, haloperidol, is used [53]. Thus, promotion of associative learning and ongoing regulation of caloric intake by dopamine may be mediated by distinct dopamine receptor subtypes, D1-like and D2-like, respectively.

Infusions into different sites of the GI tract or vasculature have been used to determine the mechanism by which dopamine signalling is sensitive to nutrients. In particular, there has been an attempt to distinguish between a pre-absorptive or post-absorptive mechanism of action. It is suggested that flavour-nutrient conditioning, at least to glucose, is dependent on pre-absorptive mechanisms as infusions of glucose into the stomach, duodenum, and mid-jejunum conditioned a preference whereas infusions into distal ileum or hepatic portal vein did not [42]. In this situation glucose may be sensed by intestinal sodium-glucose co-transporter proteins (SGLT1 and/or 3) to drive conditioning. In support of this is a study showing that conditioned responses develop to intragastric infusions of the nonmetabolizable sugar analogue, α -methyl-D-glucopyranoside, which is a substrate for SGLT3 and that this effect is blocked by a SGLT1/3 antagonist [60]. As fructose is not a substrate for SGLTs this may explain its failure to induce conditioning in this paradigm. In contrast, in another study when bottle position, rather than flavour, was used as a cue, conditioned responses were observed after post-absorptive glucose infusions into the hepatic portal vein and the jugular vein; the former was more efficacious [54]. This study argues that post-absorptive nutrient-sensing mechanisms can also play a role in certain conditioning procedures. These authors also showed corresponding changes in dopamine release, measured via voltammetry in nucleus accumbens shell of anesthetized rats. As such, infusions of low (5%) concentrations of glucose into hepatic portal vein elicited conditioned responses and an increase in dopamine transients, whereas infusions in jugular vein did not. More support for a post-absorptive mechanism was provided by using 2-DG to block glucose-evoked dopamine release [21]. The discrepancies between these studies have yet to be resolved but it seems that the existence of multiple mechanisms is likely. Thus, a different complement of these overlapping mechanisms might be required by any particular experimental set-up in order to observe an effect on conditioning and/or dopamine signalling.

In a link between nutrient-evoked dopamine release and obesity, intragastric infusions of fat did not increase dopamine efflux in high-fat fed mice, relative to low-fat fed mice. This effect was mediated by reduced release of the gut-derived satiety factor, oleoylethanolamine (OEA) [61]. Thus, one potential mechanism underlying obesity might be the inability of post-ingestive signals such as OEA to activate dopamine signalling and induce satiety. In more general terms, disruption of gut-brain signalling, whether diet-induced or due to a genetic predisposition, could contribute to aberrant feeding behaviour.

3.4 Pharmacological manipulations

Administration of drugs that disrupt or enhance specific physiological pathways may allow the reinforcement value of different nutrients to be explored. A prominent example is the use of the glucose analogue, 2-DG, which interferes with glucose utilisation. This compound has been used to demonstrate an importance for glucose utilization in behaviour and carbohydrate-evoked dopamine release [21,54]. In addition, specific components of a pathway involved in nutrient-sensing may be targeted. For example, the pathway linking fat intake and sensing in the gut to increased dopamine efflux has been determined by blocking components of this signalling cascade [61]. Finally, the precise role for dopamine in any behavioural or neurochemical response has been tested by using antagonists to dopamine receptors allowing the specific receptor subtype involved to be resolved [57].

3.5 Genetic models

Genetically modifying an organism provides opportunities for studying components of feeding behaviour such as nutrient sensing. In particular, knockout of various elements in the taste transduction pathway have been used to eliminate the contribution of orosensory components while leaving intact post-oral processes.

The transient receptor potential channel M5 (TRPM5) ion channel is a critical step in the transduction of taste for sweet compounds, bitter compounds, and many amino acids [62,63]. Mice generated to lack this channel (*trpm5*^{-/-} mice) have been used to study the post-ingestive consequences of carbohydrate ingestion in the absence of sweet taste. Whereas wild-type mice show a strong preference for sucrose over water if given short access in a two bottle taste test, *trpm5*^{-/-} mice show no preference [22]. In this study, given multiple two bottle training sessions with sipper position stable across session, mice did develop a preference for the sipper associated with sucrose. However, the authors also showed that *trpm5*^{-/-} mice were conditioned to the spatial location of the sipper and not the non-sweet orosensory qualities of sucrose; when trained over repeated sessions with a single sipper, *trpm5*^{-/-} mice failed to develop a preference for sucrose. This result contrasts with other studies that show *trpm5*^{-/-} mice are indeed capable of developing a preference for

the non-sweet orosensory cues associated with sucrose, as well as other sugars, if given access over 24 h rather than in brief (10-30 min) sessions [64,65]. Thus, different sensory information (visuospatial vs. taste) seems to interact with the reinforcement value of nutrients in distinct ways and the amount of experience with stimuli seems to be an important factor. The ability of sugars to drive conditioning in long access tests is thought to be due to the post-ingestive consequences of sugar ingestion exerting a reinforcing effect. In progressive ratio tests, *trpm5*^{-/-} mice respond for sucrose pellets and responding is further increased in double knockouts that also have knockdown of the dopamine transporter (DATkd) and so are hyperdopaminergic [35]. If sucrose is paired with malaise, *trpm5*^{-/-} mice develop an attenuated, but not abolished, conditioned aversion suggesting that in the absence of taste, the post-ingestive effects of sucrose may serve as a conditioned cue [66].

In *trpm5*^{-/-} mice ingestion of sucrose is associated with increased dopamine efflux, relative to baseline or ingestion of the non-caloric sweetener, sucralose [22]. The rise in dopamine in response to sucrose was slower in *trpm5*^{-/-} mice than in wild-type mice but eventually reached the same levels suggesting that post-ingestive stimulation of brain dopamine circuits is robust but takes longer to be manifested than when taste is available as a predictor.

The receptor for sweet taste is a heterodimer constituted of two subunits, T1r2 and T1r3 [63]. Knockout of each subunit abolishes preference for non-nutritive sweeteners and sugar in brief taste tests [67,68]. However, in T1r3 knockout mice, prolonged experience with sugar allows a preference to develop, presumably due to rewarding post-ingestive effects [65,68]. As T1r3 is expressed in the GI tract as well as in the mouth, it may contribute to post-oral nutrient sensing as well as oral sensory responses. To examine this, post-ingestive infusions may be used to identify a role for gut receptors. For example, flavour-nutrient conditioning induced by intragastric sucrose is unaffected in T1r3 knockout mice demonstrating that these receptors are not involved in conditioning [69].

Mice engineered to lack the other components of the taste transduction pathway including the G-protein, α -gustducin, or calcium homeostasis modulator 1 (CALHM1), show initial insensitivity to sweet taste but develop a preference with experience reflecting the action of a post-ingestive reinforcing mechanism [70,71].

Mice lacking functional P2X receptors through double knockout of P2X2 and P2X3 are 'taste blind', due to the importance of ATP as a signalling molecule for taste receptor cells. These mice still develop preferences for carbohydrates (maltodextrin) and fat, likely due to post-ingestive reinforcing effects [72].

In addition to targeted disruption of molecular components underlying taste or nutrient-sensing, genetic models that allow signalling pathways to be probed with optogenetic and chemogenetic tools (e.g. Cre recombinase-expressing lines) provide a further ability to manipulate or record from neuronal populations that may encode the reinforcement value of nutrients [39].

Finally, the genetic background of different mouse strains can interact with taste transduction and nutrient sensing. Thus, studying these naturally occurring differences could be a powerful way of elucidating mechanisms underlying taste perception and nutrient sensing [73]. For example, C57BL/6 mice are more sensitive to sweet taste than 129P3/J mice due in part to differences in the expression of the T1r3 subunit of the sweet taste receptor [74]. Differences in post-ingestive mechanisms may also exist. The ability of intragastric infusions of fructose to induce flavour-nutrient conditioning is enhanced in FVB mice, relative to C57BL/6 mice [49].

4. Summary and implications

4.1 Role of taste and post-ingestive signals in reinforcement and learning

The importance of post-ingestive consequences of food for driving behaviour, certain forms of learning, and dopamine signalling questions whether or not taste alone is sufficient to support reinforcement and reward-related processes. Animals will express a preference for and work to acquire delivery of non-nutritive sweeteners, although these responses may be attenuated relative to real sugars [35,75]. Moreover, even when given the opportunity to learn about the absence of calories in these substances, responses do not extinguish. In our hands, rats will lever press to obtain intraoral infusions of saccharin (1.1%) over multiple sessions on different days (J. McCutcheon and M. Roitman, unpublished observations). In addition, in sham-feeding studies, consumption of carbohydrate solutions over several sessions does not decrease and in many circumstances increases with additional experience [27]. Thus, available evidence suggests that palatable taste alone is sufficient to support behaviour.

Non-nutritive, but palatable, substances will also support the development of preferences to novel flavours that they are paired with [32,76]. As such, both saccharin and mineral oil will condition a flavour preference provided these stimuli are presented at the same time (i.e. in the same solution). Importantly, however, in a delayed conditioning paradigm these non-nutritive substances fail to produce conditioning. Moreover, another situation in which non-nutritive sweeteners appear ineffective, or at least weakly effective, is in conditioning responses to non-flavour cues associated with their delivery. As such, wild-type mice fail to

condition responses to the bottle position associated with sucralose, if tested in the absence of the sweetener [22,35]. As dopamine is an essential mediator of associative learning, this failure of non-nutritive sweeteners to support conditioning in certain situations may be reflected in the decreased dopamine release that these substances evoke, compared to real sugar (Fig. 2) [35,37].

These findings may be explained with reference to the mesolimbic dopamine system's ability to encode stimuli that predict rewards, such as food [77]. If animals learn that distinct environmental stimuli reliably predict the delivery of food then these stimuli, (cues) will drive dopamine neuron firing and dopamine release [9,11,36]. In fact, reward-evoked increases in dopamine seem essential for associative learning and reinforcement to take place [78,79]. Therefore, a distinct possibility is that reduced and/or temporally restricted dopamine release evoked by non-nutritive sweeteners fails to provide a sufficiently strong signal to induce associative learning.

4.2 Mechanisms of dopamine neuron activation and plasticity

The precise mechanisms that link food intake to activation of dopamine signalling are still under active research but seem to be numerous and varied. These mechanisms certainly involve activation by proximal cues including orosensory stimulation, pre-absorptive mechanisms, and post-absorptive mechanisms [21,23,42,54,60]. Multiple hormones, among them ghrelin, insulin, amylin, and glucagon-like peptide 1, that are released peripherally interact either directly or indirectly with the mesolimbic dopamine pathway [15,80–84]. Cells located both peripherally and centrally are able to respond to changes in glucose concentration including intestinal cells and dopamine neurons and their inputs [60,85,86]. Thus, there are many routes to activation and these mechanisms are likely engaged in concert to exert synergistic effects on feeding. For example, for stimulation of glucose-sensitive MCH neurons to evoke dopamine efflux and induce feeding the presence of a taste input is required [39]. Observations that neutral cues associated with high ingestion of certain nutrients can drive learning and behaviour predicts the existence of neuroplasticity mechanisms that can induce alterations in subset of synapses in a highly specific manner.

The dopamine system is heterogeneous in terms of its anatomy, its responses to different stimuli, and its receptor signalling [87]. The ability of dopamine D1-like receptor blockade to disrupt flavour-nutrient conditioning when performed in several different forebrain sites suggests that dopamine's roles in such behaviours are widespread [57]. The majority of data discussed in this review involves measurement of dopamine in either the dorsal or ventral striatum of rodents. Although most studies have found similar changes in dopamine efflux evoked in both regions there have been some nuanced differences that could potentially be

important. For example, while intragastric infusions of glucose increase dopamine efflux in both dorsal and ventral striatal subregions, the time course of this effect is different [21]. In addition, while serine infusions decrease dopamine efflux in ventral striatum, they have no effect in dorsal striatum. It is worth noting that in many studies, different regions have not been systematically studied and, in addition, dopamine projections outside the striatal complex have received little attention. These factors are of particular interest given that reward-related dopamine signals vary greatly across the striatum [88] and different neuronal subpopulations and projection targets may be activated by rewarding and aversive stimuli [89].

4.3 Implications for obesity and human feeding behaviour

Changes in dopamine signalling may underlie the onset of obesity and contribute to the increase in prevalence of the disease [90]. In animals and humans, obesity and intake of high-fat or high-sugar diets are associated with decreased dopamine D2 receptor expression and binding, decreased dopamine transporter function, and reduced thresholds for brain stimulation reward [91–94]. These changes may decrease the reward value of food, relative to control subjects, and this may drive people to overconsume to compensate.

Changes in appetite, satiety, and learning evoked by post-ingestive influences can be observed in humans, although these effects are often complex [95]. How might the post-ingestive effects of our diet contribute to the onset of obesity and is dopamine involved in this interaction? One potential outcome is increased appetite, which in the modern obesogenic environment, could lead to overconsumption, weight gain, and addiction-like responses to food [55,96]. In addition, in obese patients, obesity might be exacerbated by a reduction in the ability of post-ingestive nutrients to activate dopamine signalling. This has been suggested by the link between gut-derived signals and dopamine in high-fat fed mice [61]. Finally, decoupling of taste from nutritional content may also be involved in behavioural and metabolic disturbances. It is suggested that the negative health outcomes resulting from artificial sweetener intake are linked to aberrant learning about the relationship between taste and nutritional content. Taste normally prepares the gut for the onset of certain nutritional components allowing effective processing to occur. Artificial sweeteners disrupt this and lead to a decoupling of taste from physiological consequences so that actual carbohydrate intake is not dealt with efficiently [97].

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Figure legends

Fig. 1. The role of nutritional content in driving behaviour can be studied by isolating it from taste or by removing its influence. (A) In sham feeding experiments a gastric fistula allows ingested solution to be removed from the stomach so that there is minimal absorption by the GI tract. (B) Artificial sweeteners provide stimulation of taste pathways without producing post-ingestive consequences. (C) Post-ingestive infusions of nutrients can be made into the GI tract or vasculature to study nutrient sensing in the absence of taste input. (D) Pharmacological agents may be used to disrupt either post-ingestive or taste processes. (E) Genetic methods can be used to alter signalling in pathways necessary for either taste or nutrient sensing.

Fig. 2. Phasic dopamine signals evoked by sucrose and sucrose-predictive cues are greater than those evoked by saccharin. Dopamine was recorded using fast-scan cyclic voltammetry in adult rats. Top panels show average dopamine concentration traces aligned to delivery of unpredicted sucrose (blue) or saccharin (orange) pellets (A), cues predicting pellet delivery (B), or cues predicting delivery of an intraoral infusion (0.3 M sucrose, blue; 1.1% saccharin, orange) (C). Bottom panels show average dopamine concentration evoked in 1 s following pellet or cue presentation for individual rats (circles) and group mean (bars). Greater phasic

dopamine is evoked by sucrose pellets (A), and sucrose-predictive cues (B, C) than by saccharin pellets and saccharin-predictive cues. Hedonic evaluation of sucrose and saccharin infusions used in (C), as measured by taste reactivity, was similar (positive: sucrose, 59.00 ± 2.68 vs. saccharin, 53.75 ± 3.97 ; negative: sucrose, 2.00 ± 1.00 vs. saccharin, 1.00 ± 0.58 ; $n=4$; p 's > 0.05). (A,B) are within-subjects comparisons; (C) is between-subjects. $n=4-6$. Data in (A) were originally published in Beeler et al. (2012) and data in (B) were originally published in McCutcheon et al. (2012).

Fig. 3. Preference for sucrose over saccharin is correlated with the difference in dopamine evoked by each sweetener only under conditions when nutritional differences are masked. (A) In rats given the opportunity to learn about the caloric difference between sucrose and saccharin pellets, sucrose pellets are strongly preferred and there is no correlation between preference and dopamine evoked by cues predicting each pellet ($R^2=0.0223$). (B) In contrast, when rats are trained such that the caloric difference between each pellet is masked, dopamine ratio correlates with preference such that dopamine discriminates less in rats with a weak preference than in rats with a strong preference ($R^2=0.7302$). Sucrose preference = $1 - (\text{saccharin pellets eaten} / \text{sucrose pellets eaten})$; dopamine ratio = $1 - (\text{saccharin cue-evoked dopamine} / \text{sucrose cue-evoked dopamine})$. Data extracted from McCutcheon et al. (2012).

A

Sham feeding

Taste

Yes

Nutrient-sensing

No

B

Artificial sweeteners

Yes

No

C

Post-ingestive infusions

No

Yes

D

Pharmacology

Maybe

Maybe

E

Genetics

Maybe

Maybe

A **B** **C**

A

nutritional differences
emphasized

B

nutritional differences
masked