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The convergence of psychology and neurobiology in flavor-nutrient learning

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ABSTRACT

Flavor evaluation is influenced by learning from experience with foods. One main influence is flavor-nutrient learning (FNL), a Pavlovian process whereby a flavor acts as a conditioned stimulus (CS) that becomes associated with the postingestive effects of ingested nutrients (the US). As a result that flavor becomes preferred and intake typically increases. This learning powerfully influences food choice and meal patterning. This paper summarizes how research elucidating the physiological and neural substrates of FNL has progressed in parallel with work characterizing how FNL affects perception, motivation, and behavior. The picture that emerges from this work is of a robust system of *appetition* (a term coined by Sclafani in contrast to the better-understood *satiation* signals) whereby ingested nutrients sensed in the gut evoke positive motivational responses. Appetition signals act within a meal to promote continued intake in immediate response to gut feedback, and act in the longer term to steer preference towards sensory cues that predict nutritional consequences.

1. Introduction

Sensory evaluation is an important influence in determining which foods to choose and how much of them to eat. It is practically self-evident that we prefer foods that "taste good," although that's an imprecise use of the term "taste." The complex combination of basic primary tastes plus odors, textures, and trigeminal sensations creates the experience of "flavor" (Small, 2012; Stevenson, 2009) which is a large part of what makes foods attractive and rewarding. Importantly, flavor evaluation is neither innate nor fixed. Humans (and the rodents which co-evolved with us and serve as laboratory models) are born possessing only a few general reactions to basic taste stimuli, such as a generalized liking for sweetness and dislike of bitter (Ganchrow, Steiner, & Daher, 1983; Hall & Bryan, 1981; Rosenstein & Oster, 1988). But the vast array of complex flavors in foods – the piquant zestiness of pepperoni pizza, the complex, aromatic tang of chicken tikka masala, the fruity, toothsome qualities of apple pie – take on value based on individuals' experiences with them (Capaldi, 1996; Myers, 2015; Sclafani, 2004; Yeomans, 2006). This helps explain why food preferences differ among individuals and vary so much geographically that members of different cultures enjoy foods that are unappealing or even downright revolting to outsiders. Understanding how flavor preferences are established by experience becomes increasingly important in light of the obesity epidemic, now that modern food processing brings us an array of manufactured foods with carefully engineered sensory properties and unnaturally high energy density. These learning systems may hold the key to the motivational processes driving overconsumption, but may also be used to promote choice of healthier options.

There are several ways that experience shapes flavor preference, and most of them are described in the framework of Pavlovian conditioning. A flavor can be conceptualized to act as a conditioned stimulus (CS) that, although initially arbitrary, comes to be evaluated more positively or negatively by result of its association with other biologically significant events (unconditioned stimuli, US) that occur with consumption. The powerful phenomenon of conditioned food aversions is a recognizable example for most people. When a flavor (CS) is followed by severe nausea (US), that flavor-illness association is learned and that flavor is subsequently regarded as disgusting.

While conditioned aversions had been a well-studied topic in the empirical analysis of basic learning mechanisms, Holman (1975) demonstrated that associative learning could produce strong positive reactions to flavors as well. In one experiment rats

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consumed two distinct flavors (e.g., almond and banana), one in a very sweet saccharin solution and the other in a less sweet solution. They subsequently preferred the flavor paired with higher sweetness even when it was no longer as sweet. Another experiment showed that a flavor became more strongly preferred when it was followed by delayed consumption of glucose (sweet and nutritious) compared to delayed saccharin (sweet but not nutritious). The distinction between those two experiments was crucial. Holman demonstrated two distinct types of preference learning: a flavor can become preferred by association with an already preferred taste/flavor (sweetness), or by association with nutritional consequences. These came to be called "flavor-flavor" (or, more precisely "flavor-taste") learning and "flavor-nutrient" learning (hereafter abbreviated FNL), respectively.

These two types of learning can work independently, but may also interact with one another (Capaldi & Privitera, 2007; Warwick & Weingarten, 1994; Yeomans, Leitch, Gould, & Mobini, 2008) which presents a methodological challenge. During ordinary eating, flavor-flavor and flavor-nutrient learning can presumably both occur, either independently or in combination. If an individual shows increased preference for a flavor after consuming it in a sugary food, it's not clear whether the flavor has become associated with the rewarding taste of the sugar or with its nutritive properties, or both. An experimental method for specifically focusing on the mechanisms of FNL in lab animals was developed by Tony Sclafani, called the "electronic esophagus" method (Elizalde & Sclafani, 1990). Rats' consumption of a distinctively flavored but non-nutritive solution is accompanied by direct intragastric (IG) infusion of either a nutrient (e.g., glucose) or non-nutritive solution (water) through an infusion catheter. Intake of the solution could be monitored with an electronic lick detector interfaced to a computer that in turn controlled the IG infusion pump, enabling IG infusion to be matched to the rats' oral intake. In a typical experiment, training alternated between two flavors (e.g., grape and cherry), with one flavor (CS+) accompanied by IG nutrient and the opposite (CS−) paired with IG water. Thus, if rats subsequently responded more positively to the CS+ flavor it reflected the learned association between that flavor and the postingestive effects of the nutrient.

The early studies using this method (Drucker, Ackroff, & Sclafani, 1993; Drucker, Ackroff, & Sclafani, 1994; Elizalde & Sclafani, 1988, 1990; Perez, Lucas, & Sclafani, 1995) demonstrated that FNL can produce two main changes in behavior. One is conditioned *preference*: in a choice between a CS+ and CS− flavor (for which the rats had been initially indifferent before training) they strongly favor the CS+. The second is increased *acceptance*: rats learn to consume larger amounts of the CS+ flavor, mainly by taking progressively larger meals. These two behavioral outcomes of FNL reflect its adaptive value for foraging animals (and ancestral humans) who ought to preferentially seek out cues signaling potential caloric advantage. The adaptive significance of FNL is underscored by the speed of acquisition and resistance to extinction (Ackroff, Dym, Yiin, & Sclafani, 2009; Drucker et al., 1994; Myers, 2007). This learning is relevant in the modern situation by helping to explain how high-calorie foods become so attractive and capable of promoting overeating.

Though at first glance some of the findings from the Sclafani group's original electronic esophagus studies may have suggested FNL as a relatively simple mechanism for shifting flavor evaluation, work that followed revealed FNL to be quite physiologically and psychologically complex, with diverse effects on food evaluation, meal size, and meal patterning. The goal of the following sections is to outline some key areas of progress in understanding FNL, including its physiological and neurobiological substrates and the ways that the learning shapes the psychological drivers of eating behavior. My intention is to focus on areas in which our understanding of the behavioral mechanisms of FNL has converged with and illuminated the search for its underlying neurobiological signals and circuitry. This work has been chiefly led by Tony Sclafani, who, along with his many trainees and collaborators, has pursued a careful and systematically organized exploration driven by three central questions:

1) What sensor (or sensors) detect the ingested/infused nutrient post-orally to generate the reward signal for FNL?
2) How is that signal conveyed to the central nervous system?
3) How is that information integrated into the central neural circuitry governing ingestive behavior to produce lasting changes in CS flavor evaluation?

2. Central circuits in FNL

I will begin with the last of those three questions, only because that's where the focus was when I joined the Sclafani lab as a postdoc in 1999. The search for central neural circuits that process flavor-nutrient associations is conceptually linked to the psychological question of how those associations impinge on the perceptual and/or motivational controls of behavior. That is, when a CS flavor becomes associated with calories, how is it perceived differently than before? Does it actually start to "taste better?" We should expect the nature of the psychological experience to provide clues to CNS pathways mediating the behavior.

This work was heavily influenced by Berridge's model (Berridge, 1996) of "wanting and liking" which emphasized the dissociability of incentive motivation (attention towards a source of anticipated reward and focused effort towards obtaining it) from hedonic evaluation (the experience of sensory pleasure). The former is generally governed by dopaminergic signaling in mesolimbic and mesocortical pathways, while the latter is attributable primarily to opioid and endocannabinoid signaling in the limbic system. Of course this model has been continually updated to reflect the interactions between the two systems, (e.g., Berridge, Robinson, & Aldridge, 2009; Castro & Berridge, 2014; Smith, Berridge, & Aldridge, 2011), but the dichotomy between liking and wanting continues to have considerable heuristic value for understanding the controls of complex, motivated behaviors.

FNL was sometimes called "hedonic shift" learning (Mehiel & Bolles, 1988; Mehiel, 1991), although it's not necessarily the case that a nutrient-paired CS+ flavor is preferred because it becomes more palatable. Stimulation of intake could instead reflect incentive motivational effects (i.e., 'wanting' instead of, or in addition to, 'liking' in Berridge's (1996) parlance). Using the taste reactivity test, which quantifies the automatic, stereotyped orofacial reactions rats exhibit in response to small intraoral infusions as the gold-standard measure of 'liking' (see Berridge, 2000; Grill & Norgren, 1978), we found that rats did indeed react to a saccharin-sweetened CS+ that had been paired with IG glucose as more palatable than an equally-sweet CS− flavor that had been paired with water (Myers & Sclafani, 2001a). The learned shift in CS+ palatability relative to the CS− was approximately the same as seen when shifting from 3% to 16% sugar solution. A companion study showed differences in CS+ and CS− lick microstructure consistent with CS+ palatability enhancement (Myers & Sclafani, 2001b), further indicating that FNL can influence 'liking.'

While this may have seemed to settle the question of the hedonic nature of FNL, a follow-up study complicated that conclusion considerably. Instead of saccharin-sweetened flavors, which were initially moderately palatable and became more so with flavor-nutrient pairing, we studied rats' reactions to bitter or sour solutions which were initially unacceptable to rats. When a bitter or

sour solution was paired with IG glucose and the opposite paired with IG water, rats showed very strong preference learning and progressively increased their intake of the nutrient-paired solution, but taste reactivity tests revealed no change in hedonic evaluation of the CS+ (Myers & Sclafani, 2003). Despite voluntarily consuming relatively large amounts of a previously unacceptable solution, rats showed no indication they regarded it as any 'better tasting' than before. Thus apparently FNL learning *can* increase palatability but it does *not necessarily* do so, and may perhaps only do so under limited circumstances.

By showing that preferences and increased intake can sometimes result from FNL in the absence of any hedonic shift, this latter study suggests a non-hedonic, motivational process is crucial for FNL. Indeed, subsequent studies provided additional examples that nutrient-paired flavors come to exert control of incentive motivation. For instance rats will work harder to obtain small tastes of the CS+ in a progressive ratio task (Sclafani & Ackroff, 2006), a behavior the investigators interpreted as a non-hedonic, incentive effect.

At around the same time, another line of work directed by Tony Sclafani with Rich Bodnar and Khalid Touzani focused on brain pathways responsible for acquiring and using flavor-nutrient associations. Ultimately these corresponding neural investigations dovetailed with the conclusion that shifting palatability evaluation may not be the primary mechanism of FNL, instead implicating forebrain dopaminergic pathways linked to the motivational controls of "incentive salience," not sensory pleasure.

Several pharmacological manipulations of endogenous opiate signaling ultimately found no critical role for opiates in FNL. Systemic blockade of endogenous opiates is known to suppress intake of sweet solutions by decreasing perceived palatability (Kirkham & Cooper, 1988; Parker, Maier, Rennie, & Crebolder, 1992). But in the standard FNL paradigm the opiate blocker naltrexone suppressed CS intake non-specifically. That is, rats still preferred a nutrient-paired CS+ over CS− but simply consumed less of both (Azzara, Bodnar, Delamater, & Sclafani, 2000). A similar non-specific pattern was subsequently found with microinfusions of naltrexone in the nucleus accumbens shell, while naltrexone in the accumbens core was without effect (Bernal et al., 2010). The persistent CS+ preference during opiate blockade in the accumbens shell is notable given that it is evidently a critical site for opiate mediation of palatability (Peciña & Berridge, 2000). Thus, to the extent that endogenous opiate activity can be viewed as a neural currency of pleasure, this work underscored the conclusion from our taste reactivity studies that, while a palatability shift may occur under some circumstances, it is apparently dispensable and not the fundamental basis of FNL.

Dopamine (DA) plays a more critical role in FNL, although even for DA the precise mechanisms are incompletely mapped. Dopaminergic involvement was foreshadowed by an early study in which rats exhibited increased DA efflux in the striatum when tested with a taste cue that had previously been paired with IG sugar infusion (Mark, Smith, Rada, & Hoebel, 1994). Subsequent work focused on separately manipulating DA receptor sub-types with receptor-specific antagonist drugs administered systemically or microinfused into specific sites in the mesolimbic and mesocortical dopaminergic pathways. These studies taken altogether find that D1-like but not D2-like signaling is necessary for rats to learn to prefer a CS flavor when it is associated with post-oral nutrient sensing (see Sclafani, Touzani, and Bodnar (2011); Touzani, Bodnar, and Sclafani (2010a, 2010b) for detailed reviews). This is not because D1 antagonism merely prevents animals from consuming enough to learn the flavor-nutrient relationship, because strong preferences are still learned in placebo-treated animals whose training intakes are limited to the low levels consumed by the D1 antagonist group. Thus D1 antagonists appear to block learning, possibly by preventing rats from detecting the postingestive US signal, or from forming a memory of its association with the flavor.

Several brain sites participate in this effect. Blockade of D1-like receptors in the amygdala (Touzani, Bodnar, & Sclafani, 2009), nucleus accumbens shell or core (Touzani, Bodnar, & Sclafani, 2008), or medial prefrontal cortex (Touzani et al., 2010a, 2010b) blocks FNL, at least as measured by preference. Importantly, when D1 blockade is administered in any of these sites during FNL training, rats subsequently still show attraction to both CS flavors and consume substantial amounts of both, they just do not prefer the CS+.

In sum, the psychopharmacological studies of opioid and dopamine signaling and the taste reactivity studies together point to a conclusion somewhat at odds with prior thinking about FNL. It had often been assumed CS+ preference and acceptance reflect altered palatability, and FNL was often assumed to represent "hedonic shift learning" (Mehiel & Bolles, 1988; Mehiel, 1991) Efforts to extend the animal model to humans by producing *de novo* FNL in controlled experiments often reflected this assumption by using liking ratings as their main dependent measure (e.g., reviews by Brunstrom (2005); Yeomans (2006, 2012)). The dopamine circuits which are essential for FNL may be only minimally involved in subjective pleasure, reprising the finding that palatability shifts are not necessary for strong CS+ acceptance and preference. "Liking" is much more strongly linked to endogenous opiates (Castro & Berridge, 2014), which appear uninvolved in FNL (Azzara et al., 2000). While these systems interact in complex ways beyond this simple dichotomy, a straightforward summary would be that FNL learning appears to drive food choice and meal patterning by making a CS+ flavor more "interesting" or more "attention-grabbing," but not necessarily more "pleasant" or "palatable."

These interpretations remain tentative, however, as the central neural circuity for FNL has only begun to be mapped. One consistent but puzzling observation is that manipulations of midbrain dopamine function reliably affect *acquisition* of FNL but have consistently failed to interfere with *expression* of FNL that had been previously acquired (Touzani et al., 2008, 2009, 2010). In other words, dopamine signaling is necessary for learning a new flavor-nutrient association, but not to remember and use that association later. This is paradoxical, since other studies have shown that increased striatal dopamine efflux is part of the ordinary response to a nutrient-paired sensory cue (Mark et al., 1994). Most studies of dopaminergic substrates of FNL have focused on preference for CS+ over CS−, and not on more subtle effects on meal size or meal patterning. It may be that conditioned DA efflux in the striatum is in fact triggered by a CS+ flavor, but affects other aspects of behavior like absolute intake or sensitivity to satiation feedback, not choice *per se*. An exciting possibility emerging from recent human fMRI work links midbrain dopamine activity to learning about "biological utility," showing that nucleus accumbens activation by a nutrient-paired flavor is not associated with reported flavor liking, but instead to the metabolic impact predicted by the flavor, measured as change in plasma glucose that followed the flavor during training (de Araujo, Lin, Veldhuizen, & Small, 2013).

Only preliminary work has considered several other central transmitter systems (GABA, glutamate, cannabanoids, NPY) that could modulate FNL. Further the ability of several neuroendocrine signals reflecting the state of bodily energy stores or recent eating (e.g., leptin, insulin, GLP-1) to modulate the acquisition or expression of FNL is largely unaddressed, although the ability of those signals to interact with central reward and motivational circuitry (Davis, Choi, & Benoit, 2010; Hayes & Schmidt, 2016) suggest that learned responses to nutrient-predictive cues may be one way these peripheral signals modulate food seeking. Recently it has

been reported that melanin concentrating hormone may play a role in learned responses to a taste cue paired with nutrient sensing (Domingos et al., 2013), yet FNL is apparently normal in MCH-1 receptor null mice (Sclafani, Adamantidis, & Ackroff, 2016). The evidence available to date suggests a complex, distributed network of circuits involved in FNL.

3. What nutrient sensors generate the US for FNL?

Another fundamental goal of FNL research has been to identify where and how the postingestive effects of ingested nutrients are detected and transduced into a "reward" signal that supports FNL. In other words, what is the actual physiological US that becomes associated with the CS+ flavor?

Meal consumption triggers a complex cascade of chemical and neural events in the gut as food is digested, and throughout the periphery as fuels are absorbed and metabolized. Just as the mouth is lined with taste receptors for various nutrient molecules, the intestinal lumen is lined with many of the same sensors, such as the receptors encoded by the TAS1R and TAS2R gene families, and receptors for various fatty acids and amino acids, e.g., (Dyer, Salmon, Zibrik, & Shirazi-Beechey, 2005; Efeyan, Comb, & Sabatini, 2015; Miyauchi, Hirasawa, Ichimura, Hara, & Tsujimoto, 2010; Rozengurt, 2006). Unlike the distinct taste sensations generated by these receptors in the mouth, there is no evidence that neural signals arising from these "gut taste" sensors produce any conscious sensory experience, but they undoubtedly serve functions in coordinating GI motility, hormonal, and metabolic responses to ingested foods. Molecules involved in active transport across the intestinal epithelium, including SGLT1 and GLUT2, may also serve signaling functions (Daniel & Zietek, 2015). Once absorbed into circulation nutrients trigger a range of biochemical reactions in peripheral tissues, most notably in the liver, as the relative balance shifts from catabolic to anabolic pathways (Efeyan et al., 2015). Conceivably any of these ways that ingested nutrients interact with the nervous system could be the source of a reward signal that becomes associated with the CS flavor. Alternatively, or in addition, FNL could result when nutrients or the products of nutrient metabolism are sensed directly in the brain (Blouet & Schwartz, 2010; Levin, Magnan, Dunn-Meynell, & Le Foll, 2011).

Some clues to identifying a particular sensor first came from the observation that not all macronutrients are equally effective in producing FNL. This line of work (much of it performed by Karen Ackroff in Tony Sclafani's group, see (Ackroff, 2008)) demonstrated that glucose and glucose polymers (maltodextrins like Polycose) generate strong FNL whereas fructose is largely ineffective despite having equivalent energy value. Rats do acquire a preference for a flavor paired with IG fat, but it appears to produce a weaker reward signal than equicaloric glucose, such that learning is slower and requires more training (Ackroff et al., 2009; Lucas & Sclafani, 1999; Revelle & Warwick, 2009). This further demonstrates that the oft-used descriptor "flavor-calorie learning" is not really accurate, as it is not energy value that produces FNL, but some physiological action(s) of only some nutrient molecules.

While much work on this question by Sclafani's group systematically evaluated various candidates for the reward signal using physiologically manipulations, in my own lab I took a complementary (I hoped) behavioral approach. My goal was to help identify which post-oral sensing mechanisms might produce the US signal in FNL by asking *when* rats appeared to be detecting it. These studies trained rats with one flavor occurring in the first half of the meal and another in the second half, with both accompanied by IG glucose infusion. This experiment relied on the principal of temporal contiguity in Pavlovian learning: if the postingestive US signal arising from the glucose infusion was acting late in the meal or even after it ended, it should become most strongly associated with the flavor in the second half of the meal. But contrary to that prediction, rats learned a strong preference for the early flavor, suggesting that the US signal was detected within 10 min of meal initiation (Myers & Whitney, 2010).

The picture for fat was different than for glucose, as rats learned a stronger preference for flavors occurring at the end of the meal, consistent with the view that the rewarding postingestive effects of fat arise more slowly (Myers, 2013). This finding was consistent with other evidence suggesting fat is a weaker US for FNL, but helped clarify the possible reason. The differential effectiveness of various nutrients in producing FNL could be explained by a single nutrient-sensing mechanism primarily responsible for the reward signal for FNL, with different nutrients being differently effective at stimulating it. But the alternative which better fits the data is that there are several sensing pathways involved, with different pathways being stimulated at different points in time during or after a meal, and with different nutrient molecules stimulating a different subset of these pathways. Conceivably, then, postingestive glucose sensing is a powerful US for FNL because it stimulates several redundant pathways, whereas fat is less effective because it stimulates only some, and only the ones occurring after some delay.

The findings from these studies that for glucose, the critical post-oral reward signal for FNL is acting within the first several minutes of the meal coincided with the parallel demonstration in mice (Zukerman, Ackroff, & Sclafani, 2011; Zukerman, Ackroff, & Sclafani, 2013a, 2013b) and my own subsequent studies in rats (Myers, Taddeo, & Richards, 2013) that when animals were accustomed to drinking a neutral CS− flavor accompanied only by IG water, upon their very first encounter with a CS+ flavor paired with IG glucose infusion they increased their licking rate (relative to the control flavor baseline) by about the 6–7 min mark of that first meal.

This behavioral evidence for a rapidly-onsetting *within*-meal feedback signal which promotes ongoing intake fits well with several physiological observations about gut nutrient sensing in FNL. Confining the infused nutrient to the stomach by closing a pyloric cuff makes it ineffective for producing FNL (Drucker & Sclafani, 1997), as does bypassing pre-absorptive sensors altogether by infusing nutrients intravenously (Ackroff, Yiin, & Sclafani, 2010). Thus some pre-absorptive sensor within the intestinal lumen appears to be critical. This sensor is likely concentrated in the proximal portion of the intestines, as nutrient infusion into the duodenum or ileum is superior to infusion into the jejunum (Ackroff et al., 2010). This narrows the field of candidates since nutrient-sensitive receptors vary across the length of the GI tract. Further, the fact that some non-metabolizable sugar analogs can be effective in FNL (Zukerman et al., 2013a, 2013b) provides additional evidence for a pre-absorptive site of action.

The ongoing work in Sclafani's lab has used several physiological approaches to identify the critical post-oral sensor(s) supporting FNL, especially taking advantage of several newly available genetically-manipulated mouse models. The genetic knockout method was initially successful in unambiguously ruling out some prime candidates. For instance, discovery that the T1R2+T1R3 heterodimeric receptor known to act as the sugar taste receptor in the mouth is also expressed in the intestinal lumen (Margolskee et al., 2007) had the tantalizing implication that it could generate the US in FNL, analogous to how sugar tasted in the mouth is the US for flavor-flavor learning. However, mice lacking functional T1R2+T1R3 receptors are quite normal in tests of FNL, acquiring a strong preference for a flavor paired with IG sugar infusion (Sclafani, Glass, Margolskee, & Glendinning, 2010). Intact FNL abilities are also found in mice lacking the Trpm5 element of the signaling pathway used in taste-like cells (Zukerman, Glendinning,

Margolskee, & Sclafani, 2013).

A recent finding suggests (at least when the nutrient is a glucose-containing carbohydrate) an important role of the SGLT1 transporter that carries glucose from the intestinal lumen into enterocytes. SGLT1 knockout mice do not learn to prefer a flavor paired with IG glucose infusions (Sclafani, Koepsell, & Ackroff, 2016). Importantly, these mice do not act as if glucose in the gut is aversive, which suggests a muting of the normal postingestive reward effect. But the role of SGLT1 is still not entirely clear cut, as the SGLT1 inhibitor phizordoin does not entirely abolish glucose-mediated FNL (Zukerman et al., 2013a, 2013b). Nonetheless this lack of post-oral reinforcement by glucose in SGLT1 knockout mice is noteworthy, in that no other genetic manipulation or physiological disruption of peripheral nutrient-sensing pathways tested to date has had the effect of entirely abolishing FNL for glucose. As is the case with the central neural circuitry, identification of peripheral sensors involved in FNL has seen much progress in recent decades, but is still incompletely understood.

4. Route of gut-brain communication for FNL reward signals

A third guiding question is closely related to the previous one and concerns how the US signal for FNL is communicated to the brain. The evidence described above implicates one or more pre-absorptive sensing mechanisms in the proximal intestines, as opposed to direct sensing of circulating nutrients or energy balance by the brain itself. There are a several routes such a gut-brain signal could take, the main alternatives being neural or humoral. There is now strong evidence that gut nutrient sensing (or some other metabolic event that closely follows) stimulates mesolimbic DA signaling independently of any contribution of taste or oral sensation (De Araujo et al., 2008; Tellez et al., 2016). DA efflux in the dorsal striatum in particular appears to reflect the energy content of a gut carbohydrate infusion. Since lesion studies have found mesolimbic DA activity to be essential for FNL during acquisition, this “gut-brain dopaminergic axis” (de Araujo, Ferreira, Tellez, Ren, & Yeckel, 2012) could play a critical role in the rapid, within-meal feedback effect described in the previous section. But no specific route has been identified for how that information reaches the brain.

The key evidence pointing to a humoral rather than neural signal comes from experiments showing that the vagus nerve is apparently uninvolved. Reducing afferent vagal communication from the viscera to the brain with capsaicin treatment, or completely abolishing it with total or selective afferent vagotomy have virtually no effect on FNL for a flavor paired with intraduodenal carbohydrate or with fat infusion (Lucas & Sclafani, 1996; Sclafani & Lucas, 1996; Sclafani, Ackroff, & Schwartz, 2003). Further, for a vagal signal about viscosensory information to reach associative circuits in the forebrain it would presumably relay from the caudal nucleus of the solitary tract through the parabrachial nucleus, but parabrachial lesions leave FNL intact (Sclafani, Azzara, Touzani, Grigson, & Norgren, 2001).

It is more likely, then, that one or more humoral signals from gut nutrient sensing are involved. Several possibilities remain to be explored but would need to coincide with existing evidence about the importance of intralumenal sensing in the proximal intestines as well as the relatively rapid within-meal time course of the feedback effect on intake.

Some recent work has pointed to prandial insulin as an important factor. Based on the observations that peripheral insulin may act to stimulate striatal dopamine release, and that striatal dopamine is essential for acquisition of FNL, Catherine Woods (Woods et al., 2016) investigated whether manipulating prandial insulin would affect FNL. This work confirmed that insulin receptor signaling is detected in the nucleus accumbens following intragastric glucose infusion at roughly the same time course as the acceleration in licking previously reported during animals' first meal of a novel flavor accompanied by IG glucose. Further, administering an insulin antibody into the nucleus accumbens prior to flavor-nutrient training prevented preference for the CS+ flavor. This exciting finding does not, however, demonstrate the insulin itself is acting to convey the US value of the nutrient, and it is challenging to identify a role for insulin considering that streptozotocin-diabetic rats show significant FNL, albeit weaker than rats with normal insulin function (Ackroff, Sclafani, & Axen, 1997). Instead insulin could be playing a modulatory role, for instance through an incentive or attentional effect that makes the flavor CS easier to associate with some other nutrient-related US signal from the gut, or altering the response to signals from shifts in glucose trafficking occurring throughout the periphery. Similarly, human fMRI studies demonstrate a change in accumbens response to a flavor cue that is correlated with blood glucose dynamics during the flavor+nutrient pairing (de Araujo et al., 2013).

Though the precise role of insulin and other potential signals requires further exploration, the effects seen with insulin are consistent with observations about the time course of behavioral changes seen during flavor-nutrient training. As mentioned previously, learned preference for a flavor that occurred in the early half of a nutritive meal (Myers & Whitney, 2010) along with the increased licking observed within the first several minutes of flavor+IG nutrient pairing (Myers et al., 2013; Zukerman et al., 2011, 2013, 2013) all indicate a rapid feedback mechanism. But importantly, further behavioral observations also indicate that the feedback signal rapidly endows the flavor with motivational significance, consistent with a mesolimbic DA effect.

This is illustrated by an experiment in which hungry rats consumed a flavored saccharin solution accompanied either by IG glucose infusion or water (Myers et al., 2013). After the first few minutes of the meal the bottle was removed and replaced with another, which contained either the same flavor or a different flavor. When no gut nutrient sensing was occurring (IG water infusion) rats appeared rather indifferent to the flavor switch, consuming a similar amount from the second bottle irrespective of flavor. But, when gut nutrient sensing accompanied the first bottle, the flavor began to matter. Intake of the second bottle increased if it was the same flavor as the first, but decreased if the flavor was changed. In other words, rats acted as if they were already “attributing” nutrient in the gut to the flavor in the first bottle. It remains to be determined if that rapid motivational shift attached to the flavor truly represents the initial formation of the flavor-nutrient association that is remembered across subsequent encounters. But it contributes to a view of FNL as a rapidly-acting process that acts within the span of a single meal to promote ongoing ingestion when nutrients are sensed in the gut.

5. Future directions and caveats

In light of an obesity epidemic that fundamentally involves the powerful attraction to cues for energy density, it is of interest to determine how FNL acting on individual meals is related to overeating in the long run, and perhaps ultimately to obesity. A motivating assumption has long been that FNL evolved as a mechanism guiding adaptive behavior in animals foraging for food that was scarce and of relatively low energy density, but that in the modern environment it is now acting as an obesogenic influence. By fundamentally steering preference and motivating increased intake of high-energy foods, it seems obvious that FNL inherently promotes weight gain, or at least biases appetite control in that direction. Yet the links between obesity and the neural substrates of

FNL may be more complex. Only recently has there been direct investigation in animal models of whether flavor-nutrient learning is altered in diet-induced obesity. The results so far have been conflicting, with studies finding both enhanced and impaired FNL in obese animals (Wald & Myers, 2015; Woods et al., 2016). Furthermore, studies linking FNL and obesity face an inherent causality dilemma, with individual differences in FNL potentially reflecting a pre-existing influence or a consequence of the obese state.

As research continues on the three central questions identified in previous sections, there are a few caveats that will be useful to heed, especially in connecting the psychological and behavioral impacts to underlying physiological substrates.

First, there appear to be notable species differences in some of the gut sensing pathways. Basic FNL effects have been demonstrated across diverse phylogenetic taxa, from fruit flies to domestic livestock to humans (Burritt & Provenza, 1992; Figueroa, Solà-Oriol, Borda, Sclafani, & Pérez, 2012; Fujita & Tanimura, 2011; Ralphs, Provenza, Wiedmeier, & Bunderson, 1995; Yeomans et al., 2008). In recent decades much work has shifted from rats to mouse models to make use of genetic techniques. But there are several indications that the mechanisms of flavor-nutrient learning may differ substantially even between rats and mice, especially in the gut sensors that respond to different nutrients. For example, the monosaccharide galactose is an ineffective reinforcer in rats and can even produce learned avoidance of a paired flavor (Sclafani & Williams, 1999), while it appears to have at least moderately rewarding effects in mice (Zukerman et al., 2013a, 2013b). Mice show a rapid response to IG fat infusions (Zukerman et al., 2011) whereas rats' responses to fat are weaker and much slower to appear, requiring several training sessions (Ackroff et al., 2009; Lucas & Sclafani, 1999; Revelle & Warwick, 2009). In general it appears mice respond positively to a wider variety of macronutrient molecules in FNL paradigms than rats do, suggesting differences in peripheral sensing mechanisms. Given that the physiological and psychological mechanisms of flavor-nutrient learning were presumably adapted to the dietary and metabolic demands of different species, caution is warranted when generalizing even between mouse and rat models.

Another caveat also concerns cross-species generalization: further experimental attention is required to address the translation to human behavior. Despite animal work that finds consistent effects on food choice and meal patterning, analogous effects in well-controlled laboratory studies of adult humans have proven a bit more elusive (see reviews Brunstrom (2005, 2007); Yeomans (2012)). Examples of experimentally produced *de novo* shifts in flavor preference/acceptance by controlled flavor-nutrient training are relatively rare, and there are several null results, which is indeed puzzling when effects in animal models are so robust. While some of the difficulty is surely attributable to the considerable methodological limitations in controlling experience and measuring outcomes precisely, those same limitations have not hampered progress in other areas of human eating research. For basic animal work on FNL to have translational value, those of us who study animal models must take this dilemma seriously. It has been proposed that the modern environment which includes supernormal food variety and considerable flavor-nutrient inconsistency acts to impair the basic mechanisms of flavor-nutrient learning. There is some evidence of such effects in learned controls of satiety (Martin, 2016), but in terms of acceptance/preference learning evidence for this view is still lacking and some evidence contradicts it (Palframan & Myers, 2016). An alternative is that the extreme dietary variety already encountered by most Western adults makes it near impossible for an experimenter to provide anything genuinely novel to learn about. In any case, not only should researchers remain mindful of this limitation, it is likely that work aimed directly at resolving it will yield useful insights.

A third caveat concerns the largely unaddressed issue of conditioned satiation. It has long been recognized that associations between sensory properties of food and its postingestive consequences can be manifest in learned control of both appetite (preference and increased intake) and satiety. But little work has directly addressed how these two apparently opposing systems interact (Warwick & Weingarten, 1996). It's not obvious how or why two independent, parallel learning systems – one promoting intake and the other limiting intake – would be simultaneously engaged during eating. Presumably some principles are yet to be discovered to explain which system predominates in different circumstances. For instance, they could be differentially involved with foods of relatively high vs low energy densities, or when individuals are in different states of energy balance. In recent years, research on learned satiation has primarily focused on how sensory-nutrient inconsistency in the diet leads to overeating, ostensibly due to impaired learned satiety (Davidson & Swithers, 2004; Hardman, Ferriday, Kyle, Rogers, & Brunstrom, 2015). But the findings from those paradigms are difficult to directly relate to flavor-nutrient preference/acceptance learning, in which inconsistent predictive relationships between a flavor CS and nutrient US would cause decreased intake, not increased intake. It is especially important to pursue the interactions between these two parallel learning systems given the implications for energy balance and weight gain.

6. “Appetition” within meals and beyond

The work on FNL has consistently demonstrated it to be a powerful influence on food choice. In recent years the highly replicable demonstrations of rapid, within-meal positive feedback effects from the gut on meal microstructure and sensory evaluation require that we reconsider some ideas about the biopsychology of meals. A basic observation about the physiology of appetite is that ingested nutrients entering the gut trigger several negative feedback signals that progressively inhibit further eating and eventually bring the meal to an end. These *satiation* signals have dominated research on gut-brain communication, yet even as we come to understand more about the mechanisms of satiation, an obesity epidemic grows unabated. It appears that the central dogma of gut feedback – that signals arising from nutrient sensing in the gut are *exclusively* inhibitory – had overlooked something important. The effort led by Tony Sclafani to combine physiological, genetic, and behavioral approaches to characterize FNL has shown it is not merely a nudge that makes one food more attractive than another. That's just one output of a robust and extensive motivational system that powerfully influences food choice, meal size and meal patterning on a number of levels. The term “appetition” as a converse to “satiation” (Sclafani, 2012) refers to these positive, intake-promoting influences of gut nutrient sensing.

Since first establishing that FNL was a mechanism by which the energy content of food influences choice and intake, Sclafani's work has convincingly shown that the well-known satiating signals from nutrients in the gut were not the source of the reward signal that produced FNL. The satiating potency of different macronutrients is broadly unrelated to effectiveness in FNL, and increasing the concentration of a carbohydrate infusion accelerates satiation but can weaken preference learning (Lucas, Azzara, & Sclafani, 1997; Lucas & Sclafani, 1999; Sclafani, Fanizza, & Azzara, 1999; Sclafani & Ackroff, 2004). Several physiological manipulations that impair satiation such as vagal deafferentation leave FNL unaffected (Lucas & Sclafani, 1996; Sclafani & Lucas, 1996; Sclafani et al., 2003). These findings support the view of a gut-brain appetition system wholly separate from satiation.

Until recently, none of the findings on FNL necessarily contradicted the conventional view of exclusively inhibitory gut feedback, because FNL was interpreted as nutrients in one meal affecting intake in subsequent meals. That is, upon the next encounter with a CS+ flavor, meal size may be increased by retrieved *memory* of the nutrient's positive effects. But several recent demonstrations of within-meal appetition responses to ingested or infused nutrients do challenge the conventional view, showing direct intake stimulation by nutrient sensing (Sclafani & Ackroff, 2012). Because these immediate feedback signals specifically affect flavor evaluation (Myers et al., 2013), it may be that flavor-nutrient associative memories are formed within minutes of meal onset. That remains to be explored, as the neurophysiological overlap between within-meal appetition effects and memory-mediated effects on subsequent meals are unknown. But nonetheless the evidence is now clear that nutrients in a meal produce appetition signals that can promote ongoing intake and can increase meal size by enhancing evaluation of orosensory stimuli.

In sum, the major findings on appetition which have emerged from work on FNL serve to underscore, first, the importance of sensory evaluation in the orchestration of ingestive behavior, and, second, that the hallmark of sensory evaluation is its experience-dependent plasticity. When considered in addition to classic satiation signals, the short- and long-term *positive* post-oral effects of nutrients on intake provide a fuller explanation of how psychological responses to the flavors of specific foods are adjusted based on energetic and metabolic impacts. As it represents one major nexus between energy influx and the motivational controls of food intake, further study of the appetition system promises new insight into the causes of overeating. As has been the case to date, future work on the appetition system will benefit from considering the underlying physiological and neural substrates along the gut-brain axis in conjunction with the psychological manifestations of appetition which incorporate perception, motivation, and memory.

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