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## Review

## Is fat taste ready for primetime?

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## HIGHLIGHTS

- Fat taste is not universally recognized as a primary taste quality.
- Recent evidence suggests potential fat receptors in the oral cavity of mammals.
- Gustatory signals from fats possibly drive intake and preference for fatty foods.
- The endocannabinoid system plays a key role in dietary fat intake and preference.

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## ABSTRACT

Mounting evidence suggests that gustation is important for the orosensory detection of dietary fats, and might contribute to preferences that humans, rodents, and possibly other mammals exhibit for fat-rich foods. In contrast to sweet, sour, salty, bitter, and umami, fat is not widely recognized as a primary taste quality. Recent investigations, however, provide a wealth of information that is helping to elucidate the specific molecular, cellular, and neural mechanisms required for fat detection in mammals. The latest evidence supporting a fat taste will be explored in this review, with a particular focus on recent studies that suggest a surprising role for gut-brain endocannabinoid signaling in controlling intake and preference for fats based on their proposed taste properties.

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## 1. Introduction

Flavor is comprised of several sensory modalities, which include gustatory (i.e., taste), somatosensory (e.g., tactile), and olfactory components [1]. Primary taste qualities are widely agreed to include sweet, sour, salty, bitter, and recently, umami [2]. The classification of fat as a primary taste, however, is slow to gain acceptance, largely due to disagreement on what constitutes a “fatty” taste (see for review [3, 4]). In possibly reductionist terms, primary tastes are generally defined by A) dedicated receptor mechanisms and cells (i.e., taste receptor cells) capable of transducing a discrete chemical signal into an electrical signal, B) distinct sensations unique to a particular stimuli, C) specific neural pathways that transmit electrical signals from the oral cavity to the brainstem, and recently extended by Richard Mattes to include several other elements [4] (see Table 1). Based on the evidence discussed in this review, fat is proposed to meet these basic criteria for classification as a primary taste quality.

Gustatory signals from food are detected by taste cells localized to the chemosensory organs [5], the taste buds, within distinct papillae on the tongue [3], and recent studies suggest several candidate fat taste receptors in taste cells [6–10]. Taste buds are also located in the pharynx and larynx [11]. Taste cells are further categorized [5] into three major types: type 1 cells, which, in addition to possibly functioning as taste receptor cells (see [12]), might act to maintain the local environment—similar to glial cells—by degrading neurotransmitters released from other cells; type 2 cells that contain G-protein coupled receptors for sweet, bitter and umami, and release ATP, which is likely a major source of activation of associated gustatory afferent nerves via interactions with P2X2/3 receptors located on these nerve fibers [13]; type 3 cells, which are presynaptic, respond to sour stimuli, possess conventional synapses, and release the neurotransmitters, norepinephrine, serotonin, and GABA. Fungiform and foliate papillae are located on the anterior two-thirds and lateral regions of the tongue, respectively [5]. Taste buds in fungiform papillae are innervated by the chorda tympani branch of the facial nerve (VII), while taste buds in foliate papillae are primarily innervated by the lingual branch of the glossopharyngeal nerve (IX). Circumvallate papillae located on the posterior one-third of the tongue are also innervated by the lingual branch of IX. Branches of the vagus nerve (X) carry afferent taste signals from the laryngeal epithelium to the brainstem. Gustatory neurotransmission from the oral cavity is carried by cranial nerves VII, IX, and X to the nucleus of the solitary tract in the caudal brainstem, which in turn, sends fibers to the parabrachial nucleus in the pons (in rodents) and communicates bi-directionally with numerous forebrain regions and peripheral organs [14–16]. Somatosensory (e.g., fat texture) signals from food are transmitted to the brainstem by trigeminal (V) neurons and IX [17,18]. Importantly, increasing evidence suggests that gustatory neural

pathways are critical for preferences that rodents display for fatty foods: surgical disruption of afferent neurotransmission from the tongue to the brainstem, carried by the chorda tympani or glossopharyngeal nerves, reduces intake and preference for fats [19–22]. Furthermore, gustatory signals from dietary fats and sugars are suggested to be important for maintaining food reward [15], which shares neurobiological commonalities with drug addiction [23–26], and might contribute to obesity. Indeed, obese humans, when compared to normal weight subjects, significantly prefer, crave, and consume foods containing high levels of fat [27–30].

Endocannabinoids are a class of lipid-derived endogenous signaling molecules that are synthesized in most mammalian tissues, and are proposed to drive the seeking and sensing of energy-dense foods, and the accumulation of their energy content for future utilization (see [15]). Recent studies reveal that the endocannabinoids act in concert with taste pathways to control the intake of palatable foods [15,31–33]. An increasing body of work suggests a significant role in feeding for post-ingestive cues derived from dietary fats (see for review [34–36]); however, this review will focus on preabsorptive cephalic mechanisms and evidence for fat as a primary taste quality. Moreover, new studies are discussed that support an important function for gut-brain endocannabinoid signaling in the gustatory control of dietary fat intake, preference, and possibly obesity.

## 2. Cephalic components of fat intake

### 2.1. Orosensory fat exposure and dopamine signaling

It is well established that mammals display robust preferences for fat-rich diets [37]. Several laboratories demonstrated that rodents maintain ingestion of fatty diets when post-ingestive influences are eliminated by sham feeding, which isolates the orosensory component of feeding from its post-ingestive consequences [37,38] (see Fig. 1 for details of the sham feeding paradigm in rat). Sham feeding rats with corn oil (100%) increased levels of dopamine within the nucleus accumbens [39], a brain region that is essential for processing food reward [40,41]. This effect is not isolated to the ventral striatum: recent work revealed that tasting dietary fat drives dopamine release in the basolateral amygdala (BLA) [42]. For these studies, rats were trained to develop a preference for solutions containing corn oil. After a preference was established, the rodents were presented with corn oil (100%), a low concentration of linoleic acid (18:2 FA; 1%), or non-nutritive mineral oil (100%; has similar textural properties to that of nutritive oils, but does not contain any fatty acids), and dopamine levels were measured in the nucleus accumbens and BLA via microdialysis. Oral exposure to corn oil or linoleic acid increased DA levels in both brain regions within 10 min after access to the test diets, whereas mineral oil failed to elicit a similar response. The short latency for these effects after access to the nutritive oils suggests that oral cues from dietary fats are primarily responsible for enhanced dopamine outflow, and possibly preference. Supporting this notion, earlier studies in mice confirmed that post-oral administration of corn oil into the duodenum elicited a robust place preference only when paired with concurrent oral exposure to oils [43].

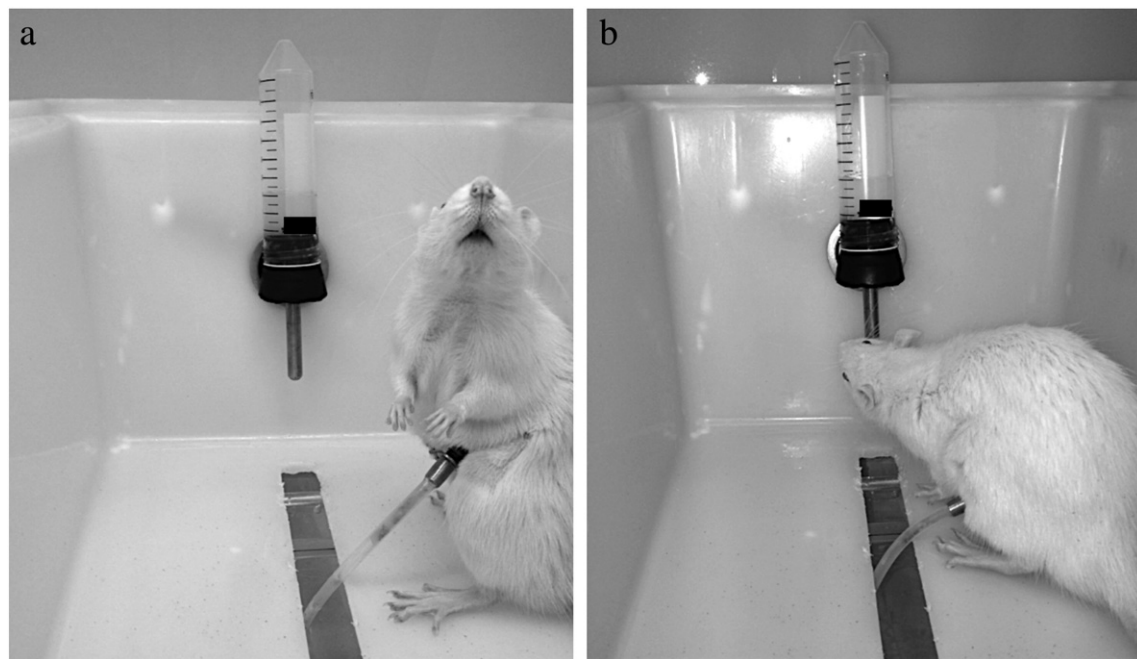
### 2.2. Cephalic-phase feeding and peripheral cholinergic signaling

Studies by Gerard P. Smith and colleagues suggest that a peripheral mechanism is responsible for maintaining intake based on the

**Table 1**

Elements of a taste. Minimal elements of a primary taste quality, as recently extended and defined by Richard Mattes [4].

Minimal elements of a primary taste quality
1) Provide some adaptive advantage
2) Have a defined class of effective stimuli
3) Have unique transduction mechanisms
4) Initiate peripheral signals conveyed by gustatory nerves that are decoded in gustatory centers
5) Be perceptible and unique
6) Evoke a functional physiological and/or behavioral response



**Fig. 1.** The sham feeding rat. Sham feeding isolates the orosensory component of feeding by eliminating its post-ingestive consequences. The sham feeding protocol is based on the methods of Gerard P. Smith [37], and recently adapted for our studies examining cephalic-phase changes in endocannabinoid signaling [38,46]. In the sham feeding paradigm, liquid diets are consumed from sipper tubes, then drain out of a reversible gastric cannulae that is surgically implanted into the stomach (see a and b). Liquids drain through a tube that is threaded into the cannulae, and placed through a longitudinal opening in the floor of the cage that allows for free movement of the animal during testing. Fluids are collected in a vessel placed below the cage, which can be weighed to determine that all consumed liquids properly drain through the gastric cannulae.

orosensory qualities of foods [44]. Treatment with the peripherally-restricted muscarinic acetylcholine receptor antagonist, atropine methyl nitrate, inhibited sham feeding of a liquid diet. Earlier studies found that atropine methyl nitrate inhibited normal food intake [45]; however, anticholinergic drugs were thought to possibly influence food intake indirectly by disrupting saliva secretion and swallowing. Smith and colleagues treated rats with atropine methyl nitrate prior to the sham feeding of water and found no changes in its sham intake [44]. Together, the results suggest that inhibiting peripheral muscarinic acetylcholine receptors selectively reduces intake of foods with nutritional value by a mechanism that does not include a general disruption in swallowing. Specific peripheral targets for muscarinic acetylcholine receptor antagonism and downstream mechanisms that influence intake remain to be determined. Recent evidence, however, suggests that endocannabinoid signals in the proximal small intestine, driven by vagal cholinergic activity, might contribute to fat sham feeding based on the orosensory qualities of fats [38,46].

### 2.3. Orosensory fat exposure and peripheral endocannabinoid signaling

Increasing evidence reveals a significant role for the endocannabinoid system in controlling the intake of palatable foods [15,47], and some of our recent studies suggest that gut-brain endocannabinoid signaling controls the intake of dietary fats based on their orosensory properties [38]. Individual groups of rats were sham fed separate macronutrients, followed by collection of brain and other organs for analysis of endocannabinoid content by high-performance liquid chromatography/mass spectrometry [38]. Rats were sham fed for 30 min at a fixed concentration of 10 mL in order to ensure that all animals had equal oral exposure to the test diets. Sham feeding either a corn oil emulsion at a concentration that is readily sham fed by rats (25% vol/vol [48]), or the nutritionally-complete liquid test diet, Ensure (vanilla; 25% vol/vol), robustly enhanced levels of the two main endocannabinoids, 2-arachidonoyl-*sn*-glycerol and anandamide, in the jejunum. These effects were tissue-specific, because tasting corn oil failed to significantly affect endocannabinoid levels in other

peripheral organs included in our evaluation (i.e., tongue, stomach, ileum, liver, pancreas) or throughout the brain (i.e., parabrachial nucleus, dorsal striatum, ventral striatum, medial aspects of the hypothalamus, lateral aspects of the hypothalamus, cerebellum). Importantly, cephalic-phase gut endocannabinoid signaling was macronutrient specific: sham feeding carbohydrate or protein at concentrations (8% w/v) that are readily sham fed by rats in other studies [49] did not affect endocannabinoid levels in the jejunum [38]. Furthermore, full subdiaphragmatic vagotomy completely blocked endocannabinoid signaling in the jejunum after tasting corn oil, which suggests that critical oral signals from fat are transmitted to the gut by cholinergic neurotransmission carried by efferent vagal fibers.

We next evaluated the physiological relevance for this macronutrient- and organ-specific signaling event. Twenty minutes before presentation of the corn oil emulsion, rats were infused with low concentrations of the cannabinoid CB<sub>1</sub> receptor antagonist/inverse agonist, rimonabant (0.3–1.0 mg/kg), directly into the small intestine through an intraduodenal catheter. Rimonabant dose-dependently blocked the 30 min sham intake of corn oil. A similar reduction in sham fat intake was observed following pretreatment with the non-brain penetrant neutral CB<sub>1</sub> receptor antagonist, URB447, which supports a peripheral mechanism in the response. In contrast to corn oil, a higher dose of rimonabant (1 mg/kg) was required to affect the intake of standard rodent chow. This result is likely due to the brain-penetrant rimonabant, while administered locally into the intestine, gaining access at higher concentrations to central CB<sub>1</sub>Rs that control feeding, and thus, reducing the intake of standard chow. For this reason, peripheralized neutral CB<sub>1</sub>R antagonists were used in subsequent studies. Collectively, the results suggest that gustatory exposure to dietary fats, but not protein or carbohydrate, initiates endocannabinoid signaling in the small intestine, but not other organs, and this local signaling event might provide positive feedback to the brain to maintain the intake of palatable fatty foods.

Cholinergic signals transmitted by the efferent vagus nerve are necessary for induction of endocannabinoid signaling in the gut; however, specific constituents of the cholinergic system (e.g., muscarinic acetylcholine receptors) that are responsible for initiating production

of the endocannabinoids are unknown and currently under investigation. Furthermore, CB<sub>1</sub>R activation in the gut might drive feeding by modifying satiation and/or hunger signaling communicated to the brain by vagal afferent fibers [50,51]. Recent evidence from rodents supports this hypothesis: CB<sub>1</sub>R mRNA was found in enteroendocrine I cells of the proximal small intestine, which produce and secrete the satiation hormone, cholecystokinin [52], and CB<sub>1</sub>Rs in the stomach control the release of the hunger signal, ghrelin [53].

### 3. Orosensory qualities of fats, and the endocannabinoids

#### 3.1. Olfaction

Despite the role for olfaction in some aspects of feeding, evidence from a variety of labs suggests that olfactory cues are not necessary for the orosensory detection of dietary fats in humans and rodents. Humans reliably detect a wide range of short, medium, and long-chain free fatty acids when olfaction is eliminated through use of nose clips [54]. Rodents rendered anosmic by either zinc sulfate perfusion through the nasal cavity, or surgical ablation of olfactory signaling, resulted in only mild reductions in preference for nutritive oils at low concentrations in two-bottle choice tests [55–57]. Furthermore, zinc sulfate failed to affect conditioned place preferences that mice exhibit for corn oil [57]. Together, the data suggest that olfactory cues, while likely important, are dispensable for the detection of dietary fats.

#### 3.2. Gustation

Increasing evidence from rodents and humans suggests that gustatory signals derived from free fatty acids are important for the detection of dietary fats in the oral cavity (see for review [36,58–62]). When given the option of consuming emulsions that contain nutritive oils (e.g., corn oil or 18:2 FA), or those containing a non-nutritive mineral oil, rodents reliably prefer those containing nutritive oils [46,48,63,64]. Free fatty acids, however, do not comprise a significant portion of dietary lipids from foods. Instead, fats are typically found in the form of triacylglycerols and phospholipids. These lipids release a portion of their fatty acid component after hydrolysis by lingual lipases secreted from glands (e.g., von Ebner's glands) located in the tongue and oral cavity in rodents [65,66] and humans [67,68]. Prevention of hydrolysis with an inhibitor of lingual lipase activity, tetrahydrolipstatin, blocked preferences for triacylglycerols, but not free fatty acids, in a rodent two-bottle choice test [69], and substantially increased detection thresholds in humans [70]. The results strongly suggest that free fatty acids are detected in the oral cavity, and their liberation by hydrolysis might contribute to fat detection. Nonetheless, low levels of free fatty acids are found in some foods (e.g., nuts) and might serve as gustatory cues [71,72]. Indeed, a recent study in humans revealed that when palatable fatty foods (e.g., walnuts, olive oil) were masticated for 1 min then expectorated, free fatty acid levels in saliva reached  $\mu$ molar concentrations, which are likely adequate to drive taste receptor signaling [71]. The source of the free fatty acids, however, was not determined and could have been derived from the fat source directly, or released from the triacylglycerols contained in the food after hydrolysis by lingual lipase. Lingual lipase in humans is reported to have low activity [73] when compared to rodents [69]. Thus, these tests should be repeated with the addition of a lingual lipase inhibitor in order to more definitively ascertain the degree to which the hydrolysis of triacylglycerols is responsible for the free fatty acids found in human saliva after mastication of fatty foods.

In contrast to rodents, which readily consume and prefer emulsions containing free fatty acids, humans are reported to find free fatty acids unpleasant [4] despite a clear attraction to fat-rich foods [3,74]. Furthermore, it has been suggested that human preferences for fatty foods are largely supported by the textural qualities of fats (e.g., creaminess attributed to triacylglycerols), and that free fatty acids might serve as an

alarm signal for the presence of oxidized unsaturated free fatty acids (e.g., rancid meat), which are associated with several pathologies when consumed [4]. Thus, potential species differences should be noted when considering the generalization of studies in rodents to humans; however, given the evidence [71], it is plausible—but not definitive—that free fatty acids contribute to the robust preferences that humans display for fatty foods.

#### 3.3. Fat texture, free fatty acids, and peripheral endocannabinoid signaling

Tactile qualities of fats are considered important for the perception of a fat flavor in humans [75,76] and likely other mammals. We explored the role of texture and the fatty acid content of dietary fats in driving gut endocannabinoid signaling in rat [46]. Confirming our previous studies [38], sham feeding a small fixed quantity of a corn oil emulsion for 30 min (25% vol/vol, 10 mL) increased jejunal endocannabinoid levels; however, sham feeding mineral oil (25% vol/vol, 10 mL) failed to elicit the same response [46]. This result suggests that tactile qualities of oils alone are not sufficient for induction of gut endocannabinoid signaling. We next asked what specific fatty acids are responsible for cephalic-phase gut endocannabinoid signaling. We sham fed rats a variety of free fatty acids with a fixed carbon chain length of 18 carbons, and increasing levels of unsaturation. In order to mask tactile cues from the fatty acids, we maintained a fixed concentration of mineral oil (20% vol/vol) and added specific free fatty acids (5% vol/vol) to this emulsion (10 mL). Sham feeding the monounsaturated fatty acid, oleic acid (18:1 FA), or the diunsaturated fatty acid, linoleic acid (18:2 FA), increased endocannabinoid levels in the jejunum by greater than two-fold. In contrast to 18:1 FA and 18:2 FA, sham feeding the saturated fatty acid, stearic acid (18:0 FA), or the polyunsaturated fatty acid, linolenic fatty acid (18:3 FA), failed to increase jejunal endocannabinoid levels. A failure for 18:3 FA to drive endocannabinoid signaling in the jejunum was surprising and warrants further study given that this unsaturated fat drives pancreaticobiliary secretion [6] and inhibits delayed rectifying K<sup>+</sup> channels (DRK) in rat taste cells [77]. Nonetheless, the specificity for certain fatty acids to drive endocannabinoid signaling in the jejunum provides additional support for a receptor-mediated event in the oral cavity that mediates fat taste. In order to control for possible post-ingestive influences for 18:2 FA on jejunal endocannabinoid production during sham feeding (i.e., leakage of a small amount of emulsion into intestine), animals were fitted with intraduodenal catheters and infused with the same amount of 18:2 FA emulsion that drove intestinal endocannabinoid signaling during sham feeding. In contrast to sham feeding, post-ingestive exposure to 18:2 FA failed to increase endocannabinoid levels. This result strongly supports a cephalic mechanism that drives jejunal endocannabinoid signaling during sham feeding of 18:2 FA, rather than post-ingestive consequences.

An increasing source of cheap food energy in the Western diet throughout the 20th and into the 21st century includes corn and soybean based diets, which contain mostly 18:2 FA (50–60% total content; 18:1 FA, 20–30% total content) [78,79], and are readily preferred by rodents [80]. Thus, it is plausible that—similar to rodents [38, 46]—attraction to foods rich in 18:2 FA (e.g., french fries or potato chips) is also driven by gut-brain endocannabinoid signaling in humans, and might contribute to obesity. Indeed, the concentration of 18:2 FA in the Western diet has increased over the 20th century from 1% of total energy consumption to 8%, and this increase positively correlates with obesity rates throughout this time period [81]. Furthermore, polyunsaturated fatty acids (e.g., 18:2 FA) are preferentially oxidized, and when consumed, have been suggested to lead to several other pathologies, including atherosclerosis and some cancers (see for review [4]). Experiments in rodents support possible causation for consuming diets high in 18:2 FA and obesity. Mice maintained for 14 weeks on a diet in which total energy from 18:2 FA was increased from 1% to 8% exhibited significantly increased food intake and adiposity, when compared to mice that were maintained on the control diet [81]. The experimental



diet was formulated so that increases in 18:2 FA from 1% to 8% were met with reciprocal decreases in saturated fat content in order to keep the diets equicaloric. Therefore, the results suggest that the 18:2 FA content of the diet, but not absolute levels of fats, led to the obese phenotype. Early studies in humans also suggest a similar obesogenic response to high levels of 18:2 FA [82]. In contrast to elderly male control subjects maintained on a standard cafeteria diet (40% total energy from fats), subjects chronically fed an equicaloric diet enriched with 18:2 FA (fat energy mostly from the addition of vegetable oil), at the expense of saturated fats, displayed increases in the levels of 18:2 FA in adipose tissue that positively correlated with a modest body weight gain. Together, the studies suggest that diets high in 18:2 FA, but not necessarily saturated fats, might drive obesity. Other investigations in humans, however, reveal a greater obesogenic response to saturated versus unsaturated fats (see for review [83]). Furthermore, disruptions in energy balance (e.g., increased energy intake versus expenditure), rather than the specific source of energy, is widely regarded to be primarily responsible for changes in body weight (see for review [84]). Thus, further work will be critical to delineate the biological underpinnings that govern body weight, including the role for endocannabinoid signaling in these functions.

#### 3.4. Dietary fat preference and peripheral endocannabinoids

Rodents reliably exhibit robust preferences for nutritive oils, including corn oil or 18:2 FA, over mineral oil when given the choice in two-bottle choice tests [48,63,64]. In light of our results that revealed an enhancement in jejunal endocannabinoid signaling after tasting 18:2 FA, but not mineral oil [46], we next tested the hypothesis that endocannabinoid signaling at CB<sub>1</sub>Rs in the gut contributes to the preferences that rodents exhibit for fat-rich diets containing high levels of 18:2 FA (e.g., corn oil). We confirmed that rats strongly prefer emulsions containing 18:2 FA (5% + 20% vol/vol mineral oil) to those containing only mineral oil (25% vol/vol) in the 30 min sham two-bottle choice test [46]. We then pretreated separate groups of sham feeding rats with the peripherally restricted CB<sub>1</sub>R antagonists, AM6545 [85–87], or URB447 [38,88], and presented 18:2 FA and mineral oil emulsions in the sham two-bottle choice test. Blocking peripheral CB<sub>1</sub>Rs inhibited the robust preferences displayed for 18:2 FA versus mineral oil alone. We next gave rats the choice between sham feeding two test diets that failed to increase endocannabinoid content in the jejunum, 18:0 FA (5% w/vol + 25% vol/vol mineral oil) and mineral oil (25% vol/vol) [46]. Surprisingly, rats preferred mineral oil to 18:0 FA, and this preference for mineral oil was not affected by pretreatment with AM6545. Collectively, the results support the hypothesis that tasting 18:2 FA drives endocannabinoid signaling in the gut and this signal is critical for the preferences that rats exhibit for fat-rich foods. Furthermore, the lack of effect for AM6545 on preferences for mineral oil versus 18:0 FA provides additional support for specific fatty acid signals in the oral cavity that drive gut endocannabinoid-mediated preference.

Several lines of investigation reveal that long chain unsaturated fatty acids (LCUFAs) activate taste receptor cells (see for review [4,89]). Evidence for a similar response in taste cells to saturated fats, on the other hand, is lacking. Saturated fats failed to, both, elicit endocannabinoid signaling in the gut in sham feeding rats [46], and drive pancreaticobiliary secretions following application to the tongue in rats with esophageal ligation [6]. In addition, saturated fatty acids applied to isolated rat fungiform papillae failed to induce a prolonged depolarization by the inhibition of DRK channels that occurs in the presence of most LCUFAs [90]. Nonetheless, humans are reported to detect saturated fats [91,92] and only female rats, when compared to male, were able to reliably avoid the saturated free fatty acid, lauric acid (12:0 FA), in a conditioned taste aversion paradigm [93]. The saturated fatty acid, myristic acid (14:0 FA), but not caproic acid (6:0 FA), increased intracellular Ca<sup>2+</sup> levels in cultured rat trigeminal neurons [17]. Moreover, the saturated fats, caprylic acid (8:0 FA) or capric acid (10:0 FA), induced signaling

within HEK 293T cells transfected with human GPR40 or GPR120, albeit mostly to a lesser magnitude than for LCUFAs [94]. Further studies will be critical to delineate potential differential responses to free fatty acids with differing levels of unsaturation on signaling in specific taste cell subtypes. In addition, due to discrepant results between humans and rodent studies, and male and female rodents, it will be important to address potential species and sex differences for the detection of specific fatty acids.

#### 3.5. Endocannabinoids and the tongue

Evidence from the Ninomiya group suggests that endocannabinoid mechanisms in the oral cavity control sensory neurotransmission from sweet taste [95]. CB<sub>1</sub>R protein was found in fungiform and circumvallate papillae on the mouse tongue, where almost two-thirds of type 2 cells containing CB<sub>1</sub>Rs also co-localized with the sweet taste receptor component, T1r3 [95]. Peripheral administration of endocannabinoids enhanced chorda tympani neural responses to sweeteners, but not sour, bitter, salty, or umami. This effect was also found in vitro for isolated taste cells in the presence of endocannabinoids, and was absent in mice lacking cannabinoid CB<sub>1</sub>Rs, which implicates endocannabinoid signaling at CB<sub>1</sub>Rs in these responses. Together, the evidence reveals that endocannabinoids act at CB<sub>1</sub>Rs on taste cells and might mediate the perception of sweet taste by controlling its associated neurotransmission into the brainstem.

A direct test for CB<sub>1</sub>R activation in the tongue and neural responses to fatty acids has not been reported to date. Sham feeding of corn oil, however, failed to significantly increase levels of the endocannabinoids in the whole tongue in rat [38]. The tongue contains several subtypes of taste cells [3,5]. Accordingly, it is possible that tasting fat might trigger endocannabinoid signaling at CB<sub>1</sub>Rs in distinct subpopulations of taste cells on the tongue, which might in turn, control afferent neurotransmission associated with fat taste. A recent study found elevations in endocannabinoid levels in the saliva of obese fasting humans when compared to normal weight subjects, and endocannabinoid levels were positively correlated with waist circumference and body mass index (BMI) [96]. While these results are preliminary, it is plausible that alterations in endocannabinoid signaling in the oral cavity under conditions of obesity might contribute to the dysregulation of oral sensitivities to fatty acids observed in obese rodents and humans [73, 97–100]. Further investigations, however, will be necessary to identify a direct role for endocannabinoids in the oral cavity in taste signaling and their role in obesity.

### 4. Proposed fatty acid receptors

Several proteins located on taste receptor cells are important for dietary fat detection in rodent, including the membrane glycoprotein protein CD36 [6–8,101], the G-protein coupled receptors GPR40 and GPR120 [9], and the monovalent cation-selective channel, transient receptor potential channel type M5 (TRPM5) [10]. Genetic deletion of each of these proteins attenuates preferences that rodents display for LCUFAs [6,7,9,10].

#### 4.1. Taste cell signaling

Basic criteria for a fat taste, defined by Richard Mattes (see Table 1 [4]), include a defined class of effective stimuli, and unique transduction mechanisms. Gilbertson and colleagues reported that the application of LCUFAs with two or more double bonds in the *cis* configuration (e.g., 18:2 FA, 20:4 FA), but not saturated fatty acids or LCUFAs with their double bonds in the *trans* configuration, to isolated rat fungiform papillae induced a prolonged stimulus-induced depolarization via the inhibition of DRK channels [90]. Later studies revealed that taste cells contain a variety of subtypes of DRK channels, with a particularly high expression of the Kv1.5 subtype in the anterior tongue in rat [102]. Together,

the evidence might represent one component of intracellular transduction for fat taste signaling. Sweet and sour tastes, however, also reduce  $K^+$  current and depolarize taste cells in mudpuppy and hamster [103, 104]. Therefore, DRK responses might not be unique to fatty acids, but might represent a convergent downstream mechanism involved in taste more generally. Potential differences between species, however, are possible and should be considered when interpreting the results.

The Besnard group reported that application of LCUFAs to isolated mouse taste cells containing CD36 induced a large and rapid rise in  $Ca^{2+}$  that included the production of inositol triphosphate, which triggers the release of  $Ca^{2+}$  from intracellular pools, and suggests a G-protein coupled receptor-mediated event [105]. CD36 is not G-protein coupled, however, raising the question of how they transduce a signal that includes the activation of phospholipase C pathways (i.e., inositol triphosphate production) [10]. An influx of extracellular  $Ca^{2+}$  in response to fatty acid signaling is also thought to occur by phosphorylation of Src-protein-tyrosine kinases (Src-PTK), which opens store-operated calcium channels (SOCs) [105]. The C-terminal tail of CD36 is proposed to interact with Src-PTK, thus, providing a mechanism that couples the interactions of LCUFAs with CD36 on the taste cell membrane to subsequent intracellular signaling events [106]. Furthermore, stromal interaction molecule 1 (STIM1), which is a  $Ca^{2+}$  sensor involved in the activation of SOCs, might be critical for the opening of SOCs in taste cells in response to LCUFAs [107]. Preferences for LCUFAs were abolished in STIM-1 knockout mice, which suggests the importance for STIM-1 in the signaling cascade that drives preferences for fatty acids. Interestingly, intracellular signaling cascades initiated by LCUFAs in vitro in isolated mouse circumvallate taste cells were met with the release of the monoamine neurotransmitters, 5-hydroxytryptamine and noradrenalin, into the culture medium [105]. This event was dependent on the opening of SOC channels and required STIM-1 [107], a result that highlights possible neurotransmitter signals by which taste bud cells communicate fat taste to afferent gustatory nerves and the brainstem. Other possible signaling molecules involved in fat taste cannot be excluded [5], including glucagon-like peptide-1 (GLP-1), which is expressed by circumvallate papillae, and recently shown to be important for small quantities of LCUFAs to reinforce taste responses to sucrose in mice [108]. GPR120 was also implicated in the actions of GLP-1, which included the release of GLP-1 by circumvallate papillae into the culture medium in the presence of a specific GPR120 agonist, GSK137647A [108]. Furthermore, mice lacking GLP-1 receptors failed to elicit preferences for low concentrations of LCUFAs in a 12-hour two-bottle choice test. Additional investigations, however, will be critical to definitively identify the specific neurotransmitter(s) that link taste cell activation by fatty acids with neural signaling to the brainstem by gustatory afferents.

#### 4.2. CD36

CD36 has gained considerable experimental attention as a plausible candidate for the elusive fat receptor(s) [101]. CD36 is a multifunctional glycoprotein that is present in a variety of tissues in the body, including the tongue [101]. Immunoreactivity for CD36 was found in rodent and human circumvallate and foliate papillae, with high concentrations of CD36 localized to the apical side of taste buds in lingual epithelium [6, 8, 109]. Recent studies utilized RNAi to selectively reduce the expression of CD36 on the tongue in obesity-prone Osborne–Mendel and obesity-resistant S5B/PI rats [110]. This local genetic modification reduced preferences for the most preferred concentration of 18:2 FA in the 48-hour two-bottle choice test.

Besnard and colleagues reported that application of 18:2 FA to the tongue of anesthetized mice and rats with surgical ligation of the esophagus, which minimizes the chances for post-ingestive influences, induced increases in pancreaticobiliary secretion and protein content [6]. In contrast to wild-type, mice lacking CD36 receptors failed to exhibit both, enhanced gastric secretions following application of 18:2 FA to

the tongue, and preferences for LCUFAs. Notably, secretion mostly occurred in wild-type with application of LCUFAs (i.e., 18:1 FA, 18:2 FA, 18:3 FA), but was absent for saturated fats (18:0 FA) and short-chain saturated fatty acids (caprylic acid, 10:0 FA). A lack of effect for saturated fats under these conditions resembles our recent data showing that, in contrast to most 18 carbon unsaturated fatty acids tested, sham feeding 18:0 FA also failed to elicit endocannabinoid accumulation in the rat jejunum [46]. Together, the results suggest selectivity of cephalic-phase responses for specific fatty acids in rodents, which provides further evidence for a receptor-mediated signaling event unique to LCUFAs. Limitations of esophageal ligation to isolate taste, however, include the possibility of disrupting neurotransmission carried by branches of the vagus nerve, which run along the esophagus below the diaphragm and have recently been shown to be required for oral exposure of dietary fats to initiate gut endocannabinoid signaling [38]. Thus, the sham-feeding model in behaving rodents, which does not carry the same risk of modifying vagal neurotransmission, might be beneficial for investigating related cephalic-phase responses (see Fig. 1).

#### 4.3. TRPM5

TRPM5 are activated by elevated levels of intracellular  $Ca^{2+}$ , which results in depolarization of the cell membrane [5], and recent studies suggest that TRPM5 are important for fat taste [10]. Supporting this role for TRPM5, application of the TRPM5 channel blocker, triphenylphosphine oxide, to taste bud cells from mouse fungiform and circumvallate papillae blunted their activation by 18:2 FA [10]. Calcium increases and inward current induced by 18:2 FA were also both reduced in taste bud cells harvested from TRPM5 knockout mice when compared to wild-type controls. Furthermore, TRPM5 knockout mice failed to display preferences for 18:2 FA or soybean oil in the two-bottle choice test when compared to controls [10, 111]. TRPM5 are also important for detection of other primary tastes [111] and are likely involved in a more general role in downstream signaling mechanisms of taste transduction.

#### 4.4. GPR40 and GPR120

The G-protein coupled receptors, GPR40 and GPR120, are located on type 1 and type 2 taste bud cells, and mice lacking these receptors exhibit reduced preferences for LCUFAs and nerve (i.e., chorda tympani and glossopharyngeal) responses to lingual application of LCUFAs [9]. Furthermore, LCUFAs activate  $Ca^{2+}$  signaling in vitro in HEK 293T cells transfected with mouse or human GPR40 or GPR120 [94]. Scalfani et al., however, recently reported that GPR40 and GPR120 might not be necessary for the cephalic component of fat preference [112]. Mice lacking GPR40, GPR120, or double knockouts, exhibited similar preferences to wild-type mice for intralipid versus water in a two-bottle choice test [112]. In contrast to oral exposure, knockout mice trained in the conditioned flavor preference paradigm to drink a non-nutritive sweet flavor (conditioned stimulus +) paired with intragastric infusion of intralipid, and another non-nutritive sweet flavor (conditioned stimulus –) paired with intragastric infusion of water, exhibited reduced intakes and preferences for the CS + solution in subsequent one-bottle intake or two-bottle choice tests when compared to wild-type mice. Studies from another group showed that non-fatty acid agonists of GPR40 and GPR120 initiated glossopharyngeal nerve activation in wild-type, but not mice lacking GPR40 or GPR120; however, these same agonists failed to elicit any discernable preferences in two-bottle choice tests in wild-type [113]. Collectively, these studies suggest that GPR40 and GPR120 are not necessary—but are possibly sufficient—for preferences associated with tasting dietary lipids, and their importance might lie in the post-oral regulation of preference [112].

## 5. Obesity and dietary fat detection

Recent studies in humans and rodents suggest that the oral detection and perception of dietary fats might be dysregulated under conditions of obesity. Several laboratories report that obese rats [97] and mice [98], when compared to lean, require higher concentrations of nutritive fats (i.e., corn oil or 18:2 FA) in order to effectively engage in consumption. The data could be interpreted to suggest a dysregulation in the sensory perception of fats in DIO rodents; however, the authors' measurements of intakes do not reflect taste thresholds as psychophysically defined [114]. Thus, changes in, for example, the motivation to consume under conditions of DIO, rather than changes in detection thresholds, are possible and should be noted. Nonetheless,  $\text{Ca}^{2+}$  signaling in isolated taste cells from mice fed a high fat diet for four weeks was diminished in the presence of LCUFAs, when compared to control mice fed a standard chow [98]. Stewart et al. reported a similar phenotype in obese humans when compared to DIO rodents. Obese men required higher concentrations of 18:1 FA for detection, when compared to lean subjects [99], and detection thresholds for 18:1 FA positively correlated with BMI [115]. Furthermore, subjects ranked as hyposensitive to 18:1 FA, when compared to hypersensitive subjects, reported increased energy intake, and reductions in the ability to distinguish minor changes in the fat content of custard [115]. Hypersensitivity to 18:1 FA, in contrast, was associated with lower BMI, lower total energy consumption, and increases in the detection of custards with lower fat content [73].

Recent evidence suggests that the quantity of fats consumed might be a predictor of sensitivity to fatty acids [116]. Lean or overweight/obese human subjects (mean BMI 23 and 28, respectively) were maintained for four weeks on a high-fat (greater than 45% of caloric intake) or low-fat diet (less than 20% of caloric intake) and assessed for taste sensitivities to 18:1 FA. All subjects maintained on the low fat diet exhibited increased sensitivities to 18:1 FA when compared to baseline measurements obtained prior to the onset of testing. Lean subjects maintained on the high-fat diet exhibited reduced sensitivities to 18:1 FA, while overweight/obese subjects did not show any changes. The authors suggest that obese subjects might already be “adapted” to chronically consuming a high-fat diet, and thus, do not show any further differences in their oral perception of fatty acids following four weeks on a regimented high-fat diet. Lean or overweight/obese subjects, however, did not differ in dietary intake and detection of 18:1 FA at baseline before experimental intervention, raising the question of the generalization of the experimental data to normal feeding conditions. Furthermore, other studies in human also revealed no correlation between the quantity of fat normally consumed by the test subjects and their detection thresholds for 18:1 FA [71].

### 5.1. CD36 expression in obesity

Recent studies in human and rodent suggest that the expression or function of the proposed fatty acid receptor, CD36, might be dysregulated in obesity. Pepino et al. reported that obese human subjects (mostly African American women included in the study) homozygous for a common single nucleotide polymorphism in the CD36 gene (rs1761667 A-allele), which associates with reduced CD36 expression, displayed eight-fold increases in the detection threshold for 18:1 FA and triolein versus obese carriers of the CD36 rs1761667 G-allele [70]. Moreover, Wistar rats maintained on a high-fat diet for eight weeks displayed reduced CD36 mRNA levels in circumvallate papillae and reduced number of cells containing immunoreactivity for CD36 protein, when compared to rats maintained on a standard chow [117]. Other studies found that mice fed ad-libitum a no-fat diet had comparable levels of CD36 mRNA in circumvallate papillae when compared to fasted controls; however, mice fed ad-libitum a high-fat (30% w/w) or a low-fat diet (0.5%) exhibited reductions in levels of CD36 mRNA, with no changes to GPR120 mRNA in any condition [118]. Furthermore, application of soybean oil to the tongue in anesthetized mice fed ad-libitum a standard

chow resulted in reductions in CD36 mRNA and protein expression within 1 h after exposure. The authors of this study suggested that small amounts of fat are sufficient to decrease CD36 gene expression and might contribute to a reduction in the oral detection of dietary fats. In support of this hypothesis, mice maintained on a high-fat diet, when compared to controls, displayed impairments in the detection and preference for low concentrations of rapeseed oil in a two-bottle choice test. Furthermore, DIO mice failed to exhibit the normal reduction in CD36 protein [98] that occurs within 1 h after refeeding in fasted control mice maintained on a standard chow [98,118], and have impaired calcium signaling in isolated CD36-positive circumvallate papillae [98]. CD36 protein expression in taste bud cells of fasted mice fed a high-fat diet for four or 23 weeks, however, was not different when compared to those maintained on a standard chow. Thus, given the similar expression of CD36 protein in diet-induced obese (DIO) and lean mice under control conditions, coupled with the previously discussed data in humans in which no correlation was found between the quantity of fats normally eaten and the oral detection of 18:1 FA [71], it is preliminary to conclude that a dysregulation in CD36 expression in obesity contributes to altered fat detection, and further studies will be required.

## 6. Humans and cephalic-phase lipid release

Cephalic-phase biochemical responses in the periphery are widely reported and viewed as anticipatory signals for the arrival of nutrients [119,120]. Early work in humans from the 1950s found that consuming a meal after direct loading of the highly fat soluble vitamin A into the intestine led to a rapid increase in circulating vitamin A content, which is an indirect indicator of elevated triacylglycerol levels [121]. This effect was reproduced by the authors in a modified sham-feeding paradigm in humans that involves mastication of a palatable food, then expectoration without swallowing. The results suggest a cephalic mechanism for the rise in circulating triacylglycerol levels. Moreover, pretreatment with the muscarinic acetylcholine receptor antagonist, atropine, delayed the rise in plasma vitamin A levels after a meal, which suggests that cholinergic signals carried by the efferent vagus nerve are required for the response [122].

Richard Mattes further explored the role for fat taste in driving increases in circulating triacylglycerol levels in the modified sham-feeding paradigm in humans [123]. Oral exposure to fats, despite wearing nose plugs to eliminate olfactory cues, led to increases in serum triacylglycerol levels that were maintained at all time points, while odor-only exposure increased triacylglycerol levels at 4 h only. Additional studies showed that tasting butter, but not fat substitutes (i.e., Olestra), elicited a rise in serum triacylglycerol levels, a result that highlights the ability for gustatory signals from dietary fats, but not non-nutritive fats, to initiate changes in circulating triacylglycerols [124]. Interestingly, tasting non-fat cream cheese triggered increases in serum triacylglycerol levels; however, the effect was more pronounced with full-fat cream cheese [125]. Further studies revealed that oral fat exposure for as little as 10 s led to an initial spike in triacylglycerol levels [125], then a more prolonged postprandial rise at later time points [125, 126]. Thus, it is possible that a portion of lipids from a high-fat meal are retained in enterocytes and mobilized following subsequent meals, leading to a rapid initial spike in serum triacylglycerols (i.e., second meal effect) [127]. Other investigations in humans support this possibility. For these studies, subjects were provided a high-fat meal, then 5 h later, given water or glucose, followed by biopsies of jejunum mucosa via esophageal-gastro-duodenoscopy [128]. Subjects that drank water retained significantly more lipid droplets in enterocytes and displayed lower levels of circulating triacylglycerols than those that consumed glucose, a result that further supports the possibility that lipids are stored in enterocytes and rapidly released by a cephalic mechanism after a subsequent meal. It would be of interest, however, to perform the same test in subjects after tasting dietary fats in order



to evaluate the selectivity and magnitude of the response to different macronutrients.

Our recent work suggests that tasting a fatty liquid meal drives endocannabinoid signaling at cannabinoid CB<sub>1</sub>Rs in the rat jejunum through efferent vagal neurotransmission, and this peripheral signal is required to maintain intake and preferences for dietary fats [38,46]. It is possible that this cephalic-phase signal initiated by fats might serve, in addition to the promotion of feeding, a broader role in lipid processing. Several studies support this hypothesis. Pharmacological inhibition of endocannabinoid degradation in mice with the non-selective inhibitor, isopropyl dodecylfluorophosphonate, induced hypertriglyceridemia through a mechanism that included disrupting apolipoprotein-E-mediated clearance of plasma triacylglycerols [129]. This effect was blocked with the CB<sub>1</sub>R antagonist, AM251, and absent in CB<sub>1</sub>R or apolipoprotein-E knockout mice. Furthermore, inhibiting CB<sub>1</sub>Rs in DIO rats with rimonabant significantly reduced levels of circulating triacylglycerols and non-esterified fatty acids, and triacylglycerol content in skeletal muscle [130]. A similar response was found in weight-stable baboons: rimonabant treatment significantly increased turnover of free fatty acids and triacylglycerol-rich lipoproteins in circulation [131]. Endocannabinoid mechanisms in peripheral organs, such as the liver [132,133] or adipose tissue [134–137], are important for energy storage. The localization of fat-induced endocannabinoid signaling in the gut [15, 46], however, is suggestive of an anticipatory signal that might regulate the processing of dietary lipids, and their release into, or clearance from, circulation.

## 7. Conclusions and future directions

Studies from the past two decades have substantially expanded our understanding of the physiological underpinnings of dietary fat detection in mammals, and support fat taste as a primary taste quality. Many knowledge gaps, however, remain. The evidence suggests that gustation, texture, and olfaction are all to varying degrees involved in the detection of fatty foods in the oral cavity, with gustatory signals from free fatty acids likely being a critical component. This diverse set of characteristics contributes to the difficulty in defining fat as a primary taste quality, and at the same time, highlights the unique nature of these molecules and our possibly dogmatic interpretation of what constitutes a basic taste quality. Some of the more immediate key elements that need to be addressed in terms of adequately defining a specific fat taste are 1) a more definitive identification of specific “fat receptor(s)” that interact with free fatty acids in the oral cavity of humans and other mammals, 2) characterization of key intracellular mechanisms that transduce the receptor-mediated signal from fatty acids into an electrical current, 3) the specific neurotransmitter(s) that communicate signaling from taste bud cells to the afferent gustatory nerves, 4) how fat taste is represented in forebrain structures, 5) how specific signaling mechanisms in the periphery, including the endocannabinoid system, contribute to fat taste and preference, and more generally, 6) how these systems are dysregulated in obesity.

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