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REVIEW

Recent advances in the genetic basis of taste detection in *Drosophila*

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Abstract

The insect gustatory system senses taste information from environmental food substrates and processes it to control feeding behaviors. *Drosophila melanogaster* has been a powerful genetic model for investigating how various chemical cues are detected at the molecular and cellular levels. In addition to an understanding of how tastants belonging to five historically described taste modalities (sweet, bitter, acid, salt, and amino acid) are sensed, recent findings have identified taste neurons and receptors that recognize tastants of non-canonical modalities, including fatty acids, carbonated water, polyamines, H_2O_2, bacterial lipopolysaccharide (LPS), ammonia, and calcium. Analyses of response profiles of taste neurons expressing different suites of chemosensory receptors have allowed exploration of taste coding mechanisms in primary sensory neurons. In this review, we present the current knowledge of the molecular and cellular basis of taste detection of various categories of tastants. We also summarize evidence for organotopic and multimodal functions of the taste system. Functional characterization of peripheral taste neurons in different organs has greatly increased our understanding of how insect behavior is regulated by the gustatory system, which may inform development of novel insect pest control strategies.

Keywords *Drosophila* gustation · Feeding behavior · Taste · Chemosensory receptors

Introduction

Animals continuously receive and process massive amounts of sensory information from the surrounding environment via different sensory systems, which direct appropriate behavioral responses. Specialized sensory organs in the body are specifically tuned to various types of sensory stimuli. Sensory information is then decoded in the central nervous system, mainly in the brain. In insects, contact chemosensory cues are sensed by the gustatory system, which is critical for mating, feeding, and oviposition behaviors. *Drosophila melanogaster* has been an excellent model organism for dissecting the genetic underpinnings of behaviors driven by gustatory systems in insects, including agricultural pests and disease vectors. A wealth of behavioral and functional assays, combined with the availability of genetic tools and reagents, offer the means to probe how chemical information is encoded at different levels of the gustatory pathway in *Drosophila*. Recent years have seen significant progress in understanding sensory coding in the periphery as well as in mapping of higher-order taste circuits in the fly brain. In this review, we focus on the adult *Drosophila* gustatory system and its role in detecting food-related cues that control feeding, oviposition, and hygiene behaviors. We provide a general overview of the adult *Drosophila* gustatory system and then present recent advances in our knowledge of chemosensory receptors and neurons underlying peripheral responses to various tastants. We also discuss evidence for multimodal taste sensing properties of *Drosophila* neurons, and for functional differences between neurons across taste organs towards operating different aspects of feeding behaviors.

Anatomical organization of the gustatory system in adult *Drosophila*

In adult *Drosophila*, taste organs are distributed in different parts of the body (Fig. 1). External taste organs include the anterior wing margins (Fig. 1a), distal segments of the

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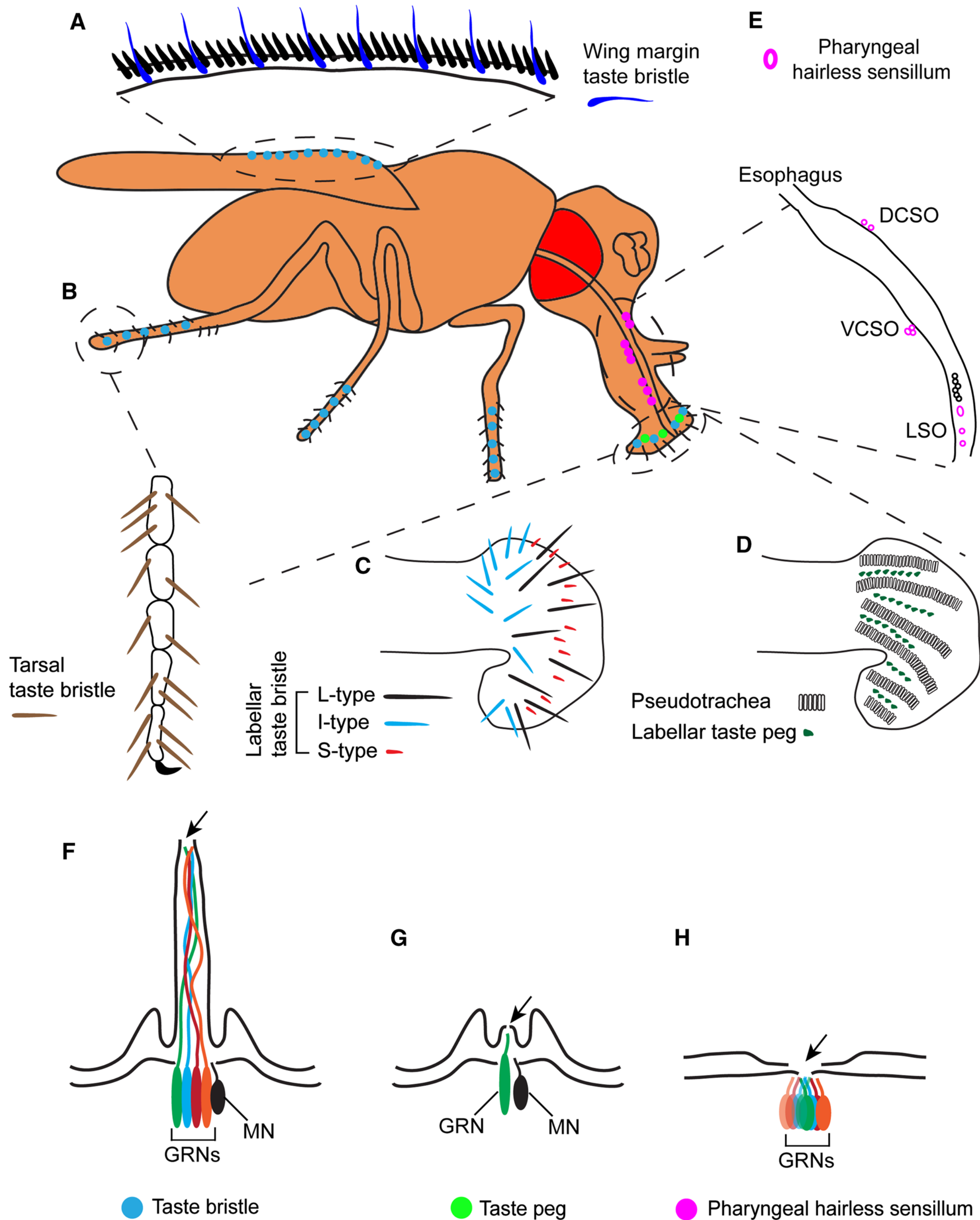

Fig. 1 Organization of the adult *Drosophila* gustatory system. There are three types of taste sensilla in the different taste organs: taste bristles (blue dots), taste pegs (green dots), and pharyngeal hairless sensilla (magenta dots). The taste bristles are distributed in the anterior wing margins (blue in **a**), the distal segment of the legs (brown in **b**), and the labellum (black, blue and red in **c**). The taste pegs are located between pseudotrachea in the labellum (green in **d**). The hairless sensilla are located in the three internal pharyngeal taste organs: labral sense organs (LSO), ventral and dorsal cibarial sense organs (VCSO and DCSO) (magenta in **e**). **f**–**h** Schematic diagrams showing the structures of three types of taste sensilla. All of them have a terminal pore (arrows) that allows tastants to make contact with the taste neurons in each sensillum. The taste bristle has 2–4 gustatory receptor neurons (GRNs) (4 GRNs in this schematic example) whose dendrites extend up to the tip of the taste sensillum (**f**). The taste peg has one GRN (**g**). Both taste bristles and taste pegs have one mechanosensory neuron (MN) at the base of each sensillum (black in **f**, **g**). The pharyngeal hairless sensilla usually do not have mechanosensory neurons, except for the #8 and #9 LSO sensilla. The number of GRNs in the pharyngeal hairless sensilla can vary from 1 to 8 (8 GRNs in this schematic example) (**h**)

legs (Fig. 1b), and the labellum (Fig. 1c, d). Internal taste organs include three pharyngeal taste organs located internally in the proboscis: labral sense organ (LSO), ventral cibarial sense organ (VCSO), and dorsal cibarial sense organ (DCSO) (Fig. 1e). Taste organs are covered by morphologically distinct taste sensilla, the basic functional units of taste detection (Fig. 1f–h). The *Drosophila* labellum, the most extensively characterized taste organ, consists of two types of taste sensilla: taste hairs (Fig. 1c) and taste pegs (Fig. 1d). Labellar taste hairs are located on the distal tip of the labellum. There are ~ 30 hairs on each half of the labellum that can be further divided into morphological subtypes based on the length of the hairs: L (long), I (intermediate), and S (short). Each taste hair has a single pore at the tip of the sensillum, which allows tastants to enter and make contact with the chemosensory neurons present within. All labellar taste hairs house a single mechanosensory neuron, but the number of chemosensory neurons that reside in them varies from two to four, depending on the subtype (i.e. four neurons in L- and S-hairs, two neurons in I-hairs) (Fig. 1f). Labellar taste pegs are hairless sensilla located between rows of pseudotrachea. The number of labellar taste pegs is sexually dimorphic, with females having more than males [1]. Each labellar taste peg is innervated by one mechanosensory neuron and one chemosensory neuron (Fig. 1g). During feeding, these taste pegs are thought to access food only when the flies open their labial palps. Besides the labellum, taste hairs are distributed on the five tarsal segments of all six legs as well as the anterior wing margins, all of which are innervated by one mechanosensory neuron and four chemosensory neurons [1] (Fig. 1f). Interestingly, the tarsal taste hairs on the forelegs are sexually dimorphic, with more hairs in males than in females. Perhaps not surprisingly, male-specific taste hairs on the forelegs are involved in pheromone detection during courtship behavior [2, 3]. Unlike external taste hairs, internal taste sensilla in the pharyngeal organs are hairless. They are innervated by one to eight chemosensory neurons, and may or may not be associated with mechanosensory neurons [4, 5] (Fig. 1h). In *Drosophila*, most sensory neurons are cholinergic [6], but recent studies showed that a small fraction of labellar and tarsal chemosensory neurons are glutamatergic [7, 8], suggesting neurochemical and functional heterogeneity within chemosensory neurons. However, further studies are required to systemically characterize neurotransmitters that are used in chemosensory neurons of all taste organs.

Physiological response profiles of chemosensory neurons

Single-sensillum extracellular tip recordings allow measurement of physiological responses of all chemosensory neurons in a single taste sensillum [9]. Recordings are obtained with tastant solutions in glass micropipette electrodes that are used to contact the tips of taste hairs. The stereotypical arrangement and accessibility of taste hairs in the labellum, tarsi, and wings have lent themselves to systematic surveys of tastant-evoked responses. In general, distinct responses have been recorded with stimuli representing distinct taste modalities, which include water, sugar, salt (high and low), acid, and bitter compounds [9–13]. Based on characteristic spike amplitudes and responses to tastants, neurons have been classified into water-, sweet-, salt-, and bitter-sensing populations. However, the extent to which each population is selectively tuned to tastants remains to be determined, and recent studies suggest that at least some taste neurons can respond to compounds of different taste categories (see below for details), hinting at multimodal taste detection properties in insect taste neurons. Moreover, gustatory coding information is incomplete because the same type of analysis has not been achieved for internal pharyngeal taste sensilla and hairless taste pegs of the oral surface, which are difficult to access as compared to external taste hairs.

Chemosensory receptor gene expression in adult *Drosophila* taste neurons

Almost two decades ago, the *Gustatory receptor* (*Gr*) gene family was identified as a new family encoding transmembrane proteins as candidate taste receptors expressed in taste organs [14–16]. In *D. melanogaster*, there are 60 *Gr* genes encoding 68 proteins. Although *Gr* transcript expression was typically too low to be reliably detected by in situ hybridization, a series of transgenic reporter lines using the *GAL4/UAS* binary expression system were soon developed to analyze *Gr* expression [15, 16]. Receptor-to-neuron maps based on reporter analysis were constructed for the labellum [7, 12, 17], tarsi [10], and pharynx [18, 19]. Patterns of *GAL4* reporter expression have been confirmed by independent means only in a few instances [13, 20]. Nevertheless, these reporter lines serve as excellent tools for functional analysis of molecularly defined taste neurons. In addition to members of the *Gr* gene family, recent studies have found that other chemosensory receptors, including those encoded by *Ionotropic receptor* (*Ir*), *pickpocket* (*ppk*), and *Transient receptor potential* (*Trp*) gene families, are involved in tastant detection [7, 21–47]. Transgenic reporter lines for many of these chemosensory genes, in particular the *Ir* genes, have also been constructed, and a significant fraction of them was found to be expressed in taste organs [21, 27]. In general, the expression of different chemosensory receptors showed some degree of overlap, especially in the pharynx where most pharyngeal taste neurons express more than one type of chemosensory receptor gene family [18]. In the following sections, we will discuss recent findings of chemosensory

receptors involved in detecting tastants representing canonical taste categories as well as non-canonical taste modalities (Table 1). While we have attempted to provide information that is fairly extensive, readers are also encouraged to consult other recent reviews on the general function of these chemosensory receptors [48–50].

Recent research updates on taste detection by chemosensory neurons

Sweet

In *Drosophila*, eight Grs belong to a clade of conserved sweet taste receptors that include Gr5a, Gr61a, and Gr64a-f. Based on transgenic reporter techniques, subsets of sweet taste neurons were found to express distinct combinations of sweet Grs [13, 17, 19]. Mutant analyses showed that individual sweet Grs are required for sensing multiple sugars, and each sugar response appears to be dependent on multiple sweet Grs [13, 17, 51–53]. In addition, some sweet gustatory receptor neurons (GRNs) also express Gr43a, a highly conserved Gr that is outside of the sweet clade [17, 19, 54, 55]. *Gr43a* is also expressed in nutrient-sensing neurons in the brain, which monitor fructose levels in the hemolymph [55].

Some differences in neuronal activation profiles of sweet GRNs in different taste organs have been reported. D- and L-arabinose, for example, have been found to activate tarsal and pharyngeal *Gr43a* GRNs differentially, but not *Gr43a*-expressing neurons in the brain in which both D- and L-arabinose evoke similar responses in terms of both magnitude and kinetics [56]. Instances of variation in physiological responses observed between different sweet GRNs have been attributed to distinct chemosensory receptor repertoires [13, 51, 53, 57].

Sweet GRNs originating from different organs exhibit distinct axonal projection patterns in the subesophageal zone (SEZ), the primary taste center in the central nervous system [18, 58, 59]. The organotopic map has been the basis for a model in which input from each taste organ is relayed to distinct higher-order neuronal circuits, which in turn regulate different aspects of feeding behavior. Notably, recent studies have found evidence for such differences in sweet GRN-controlled feeding behaviors. For example, two anatomically distinct classes of tarsal sweet GRNs, one that terminates in the ventral nerve cord (VNC) and a second that passes through the VNC and terminates in the SEZ, have been reported to regulate different behavioral responses to sugars. Those ending in the VNC are responsible for stopping the fly's movements upon encountering sugar, while the ones that project to the SEZ are responsible for initiating feeding [60]. In addition, pharyngeal sweet GRNs, which project to a discrete region of the SEZ, are distinct from external sweet GRNs in terms of the behaviors they regulate [18, 19]. Another study reported that sugar detection can elicit local search behavior, and this appears to be mediated primarily by pharyngeal *Gr43a* GRNs and not external GRNs [61]. A finding that confirms the presence of discrete circuit elements for internal and external taste is the identification of IN1 interneurons that are connected with pharyngeal *Gr43a* GRNs but not with external sweet GRNs. [62]. IN1 neurons integrate information about pharyngeal sweet taste and hunger to control meal dynamics. Altogether, these findings suggest that sweet GRNs in different locations can sense ligands in different ways, convey input to different regions in the CNS, and thereby control different aspects of feeding behaviors in response to carbohydrate cues in food substrates.

Given the extended focus on studies of Gr involvement in sweet taste, it was a surprise when sugar-sensitivity was found in a pair of *Ir60b*-expressing neurons in the pharynx [63]. *Ir60b* GRNs are unique in that (1) they do not express sweet *Grs*, but rather a few Irs, including *Ir60b*, *Ir94f*, *Ir94h*, and *Ir25a*; (2) their activation restricts sugar consumption rather than promotes it; and (3) they appear to be selectively involved in cellular and behavioral responses to sucrose and glucose but not to other sugars such as trehalose and fructose. These results evoke several interesting questions for follow up studies. Are there other non-*Gr* expressing neurons that detect sugars, possibly those other than sucrose and glucose? How does Ir60b confer sugar responsiveness? Is it directly involved in detecting sucrose, either alone or in combination with other Irs? How does the activation of pharyngeal *Ir60b* GRNs limit sugar consumption—by directly inhibiting *Gr*-expressing sweet taste circuits or by conveying information for integration in higher-order circuits? Finally, the ethological relevance of such narrow tuning of sugar sensitivity in *Ir60b* pharyngeal GRNs also awaits future research.

Bitter

Bitter taste is mediated by members of the Gr family. Initial analyses of *Gr* mutants as well as *Gr-GAL4* reporters revealed that bitter GRNs expressing several bitter *Grs*, including *Gr32a*, *Gr33a*, *Gr66a*, *Gr89a*, and *Gr93a*, are required for physiological and behavioral responses to bitter compounds [20, 64–66]. A number of observations also suggested that multiple Grs are likely to come together in heteromeric complexes to detect various bitter substances, however, a minimum Gr subunit composition remained unclear until 2015, when a combination of Gr8a, Gr66a, and Gr98b was reported as a full receptor repertoire for detection of L-canavanine [67]. All three receptors are required for L-canavanine response in bitter GRNs and co-expression of the three receptors is sufficient to confer

Table 1 Receptors, neurons and taste responses in adult *Drosophila*

Tastant	Receptor	Transgenic reporter	Example ligands	Taste organs	Physiological measurement	Behavioral measurement	References
Sweet	Gr5a, Gr43a, Gr61a, Gr64a-f		Trehalose, sucrose, glucose, fructose, glycerol and other sugars	Labellum	Tip recording	PER, food choice	[9, 13, 51, 52, 105–107]
	Gr5a, Gr61a, Gr64a-f			Labellum	None	PER	[17]
		Gr5a-GAL4		Labellum	Ca^{2+} imaging	Food choice	[108]
		Gr5a-GAL4			None	PER	[58]
	Gr43a			Brain	Ca^{2+} imaging	PER, CAFE	[17, 55]
		Gr61a-GAL4		Tarsi	Ca^{2+} imaging	PER	[57, 109]
		Gr43a-GAL4		Pharynx	Ca^{2+} imaging	Food choice, food consumption	[19]
		Tub-GAL4		Wing	Ca^{2+} imaging	Aggregation	[110]
		Gr21a-GAL4, *Gr63a-GAL4*		Ectopic expression system in olfactory sensilla	Tip recording	None	[111]
				Tarsi	Tip recording	None	[10]
	Ir60b		Sucrose, glucose	Pharynx	Ca^{2+} imaging	Food consumption, FLIC	[63]

L-canavanine response in sweet GRNs as well as in *Drosophila* S2 cells. Subsequently, several other members of Grs have been reported to be involved in detection of specific bitter compounds, such as strychnine, coumarin, umbelliferone, chloroquine, saponin, and nicotine [68–73]. Two recent studies have further elucidated the molecular basis of bitter detection by characterizing differences in responses of bitter GRNs that have distinct molecular profiles of bitter Gr expression [74, 75]. One study found that Gr32a, Gr59c, and Gr66a together are sufficient for sensing lobeline, berberine, and denatonium, whereas Gr22e, Gr32a, and Gr66a are sufficient for sensing the same three bitter compounds as well as strychnine. Given that the two combinations differ only in one Gr and show overlapping but distinct bitter response profiles, it was suggested that a selected bitter compound could activate molecularly distinct receptor complexes, and a selected heteromeric receptor complex could detect multiple bitter compounds. Thus, the observed heterogeneity of *Gr* expression in bitter GRNs would contribute to an even greater diversity in cellular responses to bitter tastants [74]. Consistent with these observations, the presence or absence of a single bitter Gr can alter endogenous responses of bitter GRNs by increasing or decreasing responses to selected bitter tastants or by conferring novel responses to bitter tastants [75]. These findings complicate evaluation of the functional roles of single Grs using mutant or ectopic expression analyses. Extensive studies have been focused on labellar bitter GRNs while leaving other taste organs unexplored, except one pharyngeal GRN labeled by *Gr9a-GAL4* shown to be responsible for behavioral avoidance of L-canavanine [18]. An understanding of behavioral roles of various classes of bitter GRNs in different organs, and how inputs from various bitter GRNs are integrated to mediate selected behaviors, will be facilitated by further elucidation of the molecular profiles and cellular responses of bitter GRNs in different taste organs.

Salt

Salt is an essential nutrient for many physiological processes, including reproduction. However, salt elicits opposite behavioral responses depending on its concentration: low salt (< 100 mM) is attractive while high salt (> 200 mM) is aversive in binary choice assays [23]. The gustatory response to salt is also sexually dimorphic and mating status dependent—mated females show higher proboscis extension upon stimulation of either the labellum or the tarsi as compared to virgin females or males [76]. Ir76b was first identified as a salt receptor functioning in labellar taste neurons that mediate salt attraction [23] but was subsequently also reported to be involved in avoidance of high salt [39]. Besides *Ir76b*, *Gr2a* and *Gr23a* expressed in the pharyngeal L7-3 GRN of the LSO have been implicated in feeding avoidance of

Table 1 (continued)

Tastant	Receptor	Transgenic reporter	Example ligands	Taste organs	Physiological measurement	Behavioral measurement	References
Bitter			Caffeine, quinine, denatonium, DEET, 7-tricosene, and other bitter compounds	Labellum, tarsi	Tip recording	Food choice	[9, 12, 112, 113]
	Gr32a, Gr33a, Gr66a, Gr93a			Labellum	Tip recording	Food choice, courtship	[20, 64–66, 74]
	Gr2a, Gr10a, Gr22b, Gr28a, Gr28b.a, Gr36a, Gr58c, Gr59c	*Gr89a-GAL4*		Ectopic expression system in labellum	Tip recording	None	[75]
		Gr66a-GAL4		Labellum	Ca^{2+} imaging	Food choice	[108]
		Gr66a-GAL4	Strychnine, L-canavanine	Labellum, tarsi	Tip recording	Food choice, CAFE, PER	[114]
		Gr66a-GAL4	Caffeine, quinine, denatonium, berberine		None	PER	[58]
	Gr8a, Gr66a, Gr98b		L-canavanine	Labellum	Tip recording	Food choice	[67]
		Gr9a-GAL4	L-canavanine	Pharynx	None	Food choice	[18]
	Gr47a		Strychnine	Labellum	Tip recording	Food choice, PER	[71]
	Gr22e		Strychnine, chloroquine	Labellum	Tip recording	Food choice, PER	[68]
	Gr33a, Gr66a, Gr93a		Umbelliferone, coumarin	Labellum	Tip recording	Food choice, oviposition	[69, 70]
	Gr28b		Saponin	Labellum	Tip recording	Food choice, PER	[72]
	Gr10a		Nicotine	Labellum	Tip recording	PER	[73]
	TrpA1		N-methylmaleimide	Labellum	Tip recording	CAFE	[115]
	TrpA1		Aristolochic acid	Labellum	Tip recording	Food choice	[37]
	TrpL		Camphor	Labellum	Tip recording	Food choice	[38]
	Painless		Isothiocyanate	Labellum, tarsi, pharynx, wing	None	Food choice, PER	[116]
Salt	Ir76b		Sodium chloride	Labellum	Tip recording	Food choice	[23, 39]
		Ir76b-GAL4		Tarsi	None	PER	[76]
	Gr2a			Pharynx	None	Food choice	[77]
				Labellum	Tip recording	Food choice	[9]
		Gr64f-GAL4, *Gr66a-GAL4*, *ppk23-GAL4*, *Ir94e-GAL4*		Labellum	Ca^{2+} imaging	Food choice	[7]

Table 1 (continued)

Tastant	Receptor	Transgenic reporter	Example ligands	Taste organs	Physiological measurement	Behavioral measurement	References
Acid	Ir7a		Acetic acid	Labellum	Tip recording	Food choice, PER	[46]
		Gr64f-GAL4, *Gr66a-GAL4*	Acetic acid	Labellum	Ca^{2+} imaging	PER	[78]
	Ir25a, Ir76b		Carboxylic acids, HCl	Tarsi	Ca^{2+} imaging	Oviposition	[42]
		Gr89a-GAL4	Carboxylic acids, HCl	Labellum	Tip recording	Food choice, PER	[11]
Amino acids/Yeast	Ir76b		Serine, threonine, phenylalanine, alanine, glycine, yeast extract	Tarsi	Ca^{2+} imaging	Food choice	[25]
		Ir76b-GAL4	Yeast	Labellum, taste pegs	Ca^{2+} imaging	FlyPAD	[24]
		Gr66a-GAL4	Tryptophan, phenylalanine	Labellum	Tip recording	Food choice	[86]
		AstC-, *Npf-*, and *Dh31-GAL4* (*EE-GAL4*)	Amino acids/yeast	Gut	CaLexA	None	[117]
			Amino acids	Labellum, Tarsi	None	Food choice, PER, CAFE	[118]
Carbonated water		*E409-GAL4*	Sodium bicarbonate, cesium bicarbonate (pH 5–6.5)	Taste pegs	Ca^{2+} imaging	Food choice	[91]
	Ir56d			Labellum, taste pegs	Ca^{2+} imaging	Food choice, Positional preference, PER, Expresso, FlyPAD	[21]
Fatty acids	Ir56d		Hexanoic acid, octanoic acid, and other fatty acids	Taste pegs, tarsi	Ca^{2+} imaging	PER	[43, 44]
	Gr64e			Labellum	Tip recording	PER	[90]
		Gr33a[GAL4]	Hexanoic acid	Tarsi	Ca^{2+} imaging	PER	[43]
Polyamines	Ir76b	*Gr66a-GAL4*	Putrescine, cadaverine	Labellum	Ca^{2+} imaging, tip recording	Oviposition	[45]
UV/H_2O_2	TrpA1	*Gr66a-GAL4*	UV/H_2O_2	Labellum	Ca^{2+} imaging	Oviposition	[34]
	TrpA1	*Gr66a-GAL4*	UV/H_2O_2	Labellum	Tip recording	Food choice	[36]
LPS	TrpA1	*Gr66a-GAL4*	LPS	Pharynx	Ca^{2+} imaging	Food choice, PER, oviposition	[35]
		Gr64f-GAL4, *Gr5a-GAL4*, *Gr33a-GAL4*, *Gr66a-GAL4*, *Ir76b-GAL4*	LPS	Wing	None	Grooming	[95]
		Gr33a-GAL4	LPS	Wing, tarsi	Tip recording	Grooming	[96]
Ammonia		*Gr66a-GAL4*	Ammonium chloride	Labellum	Tip recording	Food choice, food consumption	[98]

Table 1 (continued)

Tastant	Receptor	Transgenic reporter	Example ligands	Taste organs	Physiological measurement	Behavioral measurement	References
Calcium	Ir62a	*ppk23-GAL4*	Calcium chloride	Labellum	Tip recording	Food choice	[99]

CAFE capillary feeder assay, *CaLexA* calcium-dependent nuclear import of LexA, *DEET N,N*-Diethyl-meta-toluamide, *EE-GAL4 enteroendocrine-GAL4*, *Expresso* an automated feeding assay for quantification of real time food ingestion, *FLIC* fly liquid food interaction counter, *flyPAD* fly proboscis and activity detector, *LPS* lipopolysaccharide, *PER* proboscis extension response

salt in a specific behavioral context, in which mildly starved flies were tested with a moderate level of salt (150–450 mM) [77]. The complex view of salt coding emerging from these studies was tackled by a recent comprehensive functional imaging analysis of salt responses in labellar GRNs [7]. To begin to decode taste responses to different concentrations of salts, the authors first gathered molecular tools for labeling subsets of taste neurons in all labellar hairs. First, the authors identified a driver, *Ir94e-GAL4*, which labels a single GRN that is distinct from previously characterized *ppk28*, *Gr64f*, or *ppk23*-expressing GRNs neurons in L-type hairs, thus completing a molecular genetic toolkit for accessing all four GRNs in these hairs. The authors then identified two subpopulations of *ppk23*-expressing neurons by labeling either glutamatergic or cholinergic neurons (ppk23^{glut} and ppk23^{chat}), which represented distinct taste neurons in the S-type labellar hairs. Imaging of salt responses in these GRN subpopulations revealed that most if not all types of GRNs respond to salt at some range of the tested concentrations. Specifically, weak calcium activity in response to low concentrations of salt was observed in *Gr64f* and *Ir94e* neurons, while response to high salt was observed in *Gr64f*, *Gr66a*, and *ppk23* neurons. Notably, previous electrophysiological recordings had found high salt-induced activity in two neurons in labellar L-type hairs [9]. Since there are no *Gr66a*-labeled neurons in these hairs, one possibility is that the L-type responses are derived from *Gr64f* and *ppk23* neurons. In I-type hairs, tip recordings have identified high salt sensitivity in both taste neurons that innervate them [9], which are labeled by *Gr64f* and *Gr66a*, respectively. Interestingly, only the salt response in *Gr66a* neurons is independent of *Ir76b* function, although it is partially dependent on *Ir25a*, suggesting potential heterogeneity among salt receptor complexes as well [7]. This appears to go hand-in-hand with functional diversity in salt-sensing circuits—although both *ppk23*^{glut} and *ppk23*^{chat} GRNs respond to high salt, only the *ppk23*^{glut} subset is involved in mediating internal state-dependent modulation of high salt avoidance. Altogether, it is conceivable that different concentrations of salt activate distinct populations of GRNs, many of which express Ir76b, which explains the previously observed roles of this receptor in both low and high salt detection.

Acid

Carboxylic acids are detected via both olfactory and gustatory systems in adult *Drosophila* to mediate appropriate selection of food and oviposition sites [11, 46, 78–83]. Although flies are attracted to vinegar, they avoid high concentrations of acetic acid detected via *Ir64a* neurons in olfactory sensilla in the antennae [81]. In the gustatory system, several carboxylic acids have been shown to activate labellar bitter GRNs and also to suppress sugar responses in sweet

GRNs [11]. In contrast to the overlap between bitter and acid detection in labellar GRNs, acid sensing in tarsal hairs occurs via two separate groups of GRNs that do not respond to either sugars or bitter compounds: one is broadly tuned to various carboxylic acids, while the second is narrowly tuned to glycolic and malic acids and to high concentrations of salt [42]. Acid responses in both these classes of tarsal GRNs require two broadly expressed *Irs*, *Ir25a* and *Ir76b*. Given that *Ir25a* and *Ir76b* are widely expressed in both olfactory and gustatory neurons, the identity of additional *Irs* that may confer ligand specificity remains to be determined. Interestingly, one recent report identified another member of the Ir family, Ir7a, which is only expressed in a subset of labellar bitter GRNs as a receptor for acetic acid [46]. A high concentration of acetic acid (5%) was found to evoke feeding aversion in binary choice feeding assays. Although feeding avoidance of acetic acid was disrupted in *Ir7a* mutants, it was not dependent on *Ir25a* or *Ir76b*. The observed defects in feeding avoidance were selective for acetic acid and responses to other carboxylic acids were not affected in the absence of *Ir7a*, consistent with the idea that different receptors with distinct ligand-binding specificities may be involved in sensing various carboxylic acids. Ectopic expression of *Ir7a* in sweet GRNs conferred acetic acid response as measured with tip recordings [46], an observation that needs to be reconciled with acetic acid-evoked calcium activity in endogenous sweet GRNs [78]. Moreover, the restricted expression of *Ir7a* in bitter GRNs indicates that the molecular mechanism of acetic acid detection in sweet GRNs is yet to be determined.

Amino acids/yeast

Yeast is the primary source of dietary proteins and amino acids for *Drosophila*. Yeast feeding is modulated by mating status and prior yeast feeding experience [84, 85]. Recent reports suggest that amino acids are the principal gustatory cues in yeast extract [25], and cellular and behavioral responses to amino acids are mediated via Ir76b [25, 26], which is broadly expressed in peripheral GRNs. Although Ir76b may act alone for salt detection [23], it is likely to serve as a co-receptor for amino acid detection given that taste neurons in labellar hairs, many expressing Ir76b, have limited responses to amino acids [25, 86]. An RNAi screen identified one putative amino acid co-receptor, Ir20a. Ectopic expression of *Ir76b* and *Ir20a* together in labellar sweet GRNs conferred amino acid response but not salt response and expression of *Ir20a* in labellar *Ir76b*-expressing salt GRNs reduced salt responses but did not confer amino acid response, invoking the contribution of additional receptors/factors present in sweet GRNs but not in salt GRNs in mediating amino acid response. Since *Ir20a* shows a considerably limited domain of expression in comparison with *Ir76b*, and *Ir20a* mutants do not phenocopy the *Ir76b* mutant, it is expected that additional amino acid receptors in other GRNs will be involved in taste detection of amino acids.

Although amino acids might be salient components in yeast extract, another recent study indicates that flies might have distinct pathways for sensing amino acids and yeast [24]. Using yeast rather than yeast extract, the authors showed that yeast feeding requires *Ir76b*-expressing GRNs in labellar taste hairs and taste pegs but not in tarsal taste hairs. Further, *Ir76b* GRNs in labellar taste hairs are responsible for the initiation of yeast feeding (i.e. PER responses), while those in labellar taste pegs are involved in sustaining yeast feeding, providing additional insight into taste organ-specific roles in controlling feeding behavior. Interestingly, yeast-evoked activity in GRNs of both labellar hairs and pegs is modulated by internal amino acids, suggesting that consumption of amino acids and yeast is tightly integrated even though peripheral neuronal detection pathways may be distinct. Future experiments identifying receptors for yeast taste in the two types of labellar GRNs would provide the means to compare mechanisms of amino acid and yeast sensing in peripheral GRNs. In addition to taste-sensing mechanisms, there is evidence that three specific dietary amino acids are detected by brain DH44 neuroendocrine cells which innervate the gut [87, 88]. The proposed fast-acting, post-ingestive mechanism of amino acid detection is independent of *Ir76b* and requires putative amino acid transporters in the DH44 cells.

Fatty acids

Fatty acid taste elicits an appetitive or aversive response depending upon the concentration [43]. Recent studies have largely focused on the positive behavioral valence of low concentrations (< 1%) of short to medium chain fatty acids (hexanoic, octanoic), which is mediated by a subset of labellar and tarsal sweet GRNs [43, 44, 89]. Notably, a number of studies have found fatty acid taste to be dependent on several members of the *Ir* family, including *Ir56d*, *Ir25a*, and *Ir76b*. In the labellum, there are two subpopulations of *Ir56d* GRNs: one is a subset of sweet GRNs in taste hairs that responds to both sugars and fatty acids, and another is a subset of GRNs in taste pegs that responds to fatty acids but not to sugars. Fatty acid-stimulated proboscis extension requires *Ir56d* GRNs in the labellar taste hairs, but not in taste pegs [44], consistent with distinct behavioral roles for the two GRN populations. Tarsal stimulation-evoked proboscis extension response (PER) is also mediated by *Ir56d*-labeled sweet GRNs, whose function is dependent on *Ir56d*, *Ir25a*, and *Ir76b* [43]. Notably, tarsal PER to hexanoic and octanoic acids is significantly higher in octuple mutant flies lacking all 8 sweet Grs [43], indicating a possible role in

for one or more of these receptors in regulating fatty acid response. Consistent with this idea, a recent study reported that one sweet Gr, Gr64e, is involved in mediating fatty acid taste in the labellum [90]. Whether Ir56d and Gr64e act independently or together for mediating fatty acid signaling is still unclear. However, all studies have found that NorpA, which encodes a phospholipase C, is essential for fatty acid signaling in sweet GRNs [43, 44, 89, 90].

Interestingly, hexanoic acid shows dose-dependent activation of tarsal GRNs that express Gr33a, a receptor that broadly marks bitter-sensing GRNs. At concentrations of hexanoic acid exceeding 1%, control flies exhibit a reduction in proboscis extension, which is not the case in flies in which *Gr33a* GRNs are functionally ablated [43], consistent with the idea that tarsal bitter GRNs mediate an aversive response to fatty acids. Whether or not labellar bitter GRNs also respond to fatty acids has not been reported. Notably, tarsal bitter GRN sensitivity to fatty acids does not require *Ir25a* and *Ir76b*, suggesting that other as yet unidentified receptors are involved in fatty acid taste aversion. As in the case of salt, which elicits opposing behaviors at low and high concentrations, it will be of interest to decipher fatty acid coding at the sensory level and dissect how appetitive and aversive fatty acid-sensing pathways are integrated to shape feeding behaviors.

Carbonated water

Gustatory responses to carbonated water in *Drosophila* were found to be mediated by *E409-GAL4*-labeled GRNs that innervate labellar taste pegs [91]. Surprisingly, a suite of chemosensory receptors involved in fatty acid taste (Ir56d, Ir25a, and Ir76b) is also required for sensing carbon dioxide dissolved in fluids [21]. Unlike fatty acids that can activate *Ir56d* GRNs in labellar hairs, labellar pegs, and tarsal hairs, carbonated water mainly activates GRNs in labellar taste pegs. GRNs in taste hairs of the labellum but not tarsi show a weaker response to carbonated water; however, *Ir56d*, *Ir25a*, and *Ir76b* are unlikely to be involved in these responses according to mutant and rescue analyses [21]. Although the three *Irs* are necessary for carbonated water detection in taste peg neurons, combined ectopic expression, which was tested in labellar bitter GRNs, did not confer carbonated water sensitivity, indicating that additional factors may be involved. How does carbonated water taste affect feeding behavior? It was first reported that carbonated solutions trigger mild behavioral attraction in a position-based preference assay [91]. However, no behavioral relevance for carbonated fluid has been observed in consumption-based feeding assays such as flyPAD (solid food) and Expresso (liquid food), in which several high-resolution micro-feeding parameters are monitored, including total number of sips, number of sips per feeding burst, feeding success, latency to the first bout, total consumption per fly, number of meal bouts and average bout volume [21]. Thus, carbonated water may be used as a gustatory cue for behaviors other than food ingestion.

Polyamines

Taste input is important not only for food consumption and choice but also for egg-laying site selection by female *Drosophila*. Polyamines, such as putrescine or cadaverine, are important nutrients for reproductive success and have been shown to activate both olfactory and gustatory pathways for long-range positional attraction and short-range oviposition site selection, respectively [45]. Interestingly, both short-range and long-range behaviors require a common chemosensory receptor, Ir76b. A more narrowly expressed antennal chemoreceptor, *Ir41a*, is also necessary for polyamine attraction. In fact, *Ir76b* expression in *Ir41a* olfactory neurons is sufficient to rescue polyamine attraction in *Ir76b* mutants. In the gustatory system, there are at least two classes of polyamine-sensing GRNs that mediate oviposition site selection: *Ir76b* GRNs in labellar taste hairs and taste pegs, and *Gr66a* GRNs in labellar taste hairs. *Ir76b* GRNs in taste pegs exhibit stronger responses to polyamines than those in taste hairs, but *Ir76b* is required for the responses in both. However, polyamine response in *Gr66a* GRNs is independent of *Ir76b*, invoking a distinct mechanism for polyamine detection in these GRNs. In dissecting the behavioral contributions of various polyamine-sensing GRNs in controlling egg laying, the authors found that polyamine avoidance during egg-laying behavior relied on labellar input. Silencing of *Ir76b* or *Gr66a*-expressing neurons reduced polyamine avoidance to different extents, implicating roles for both classes of neurons. In fact, silencing of *Gr66a* GRNs caused a slight attraction to polyamine substrate, which was lost upon silencing both *Ir76b* and *Gr66a* neurons, indicating some positive behavior component in the *Ir76b* pathway for egg-laying site selection. Functional heterogeneity in *Ir76b* and *Gr66a* neurons might provide multiple substrates for modulation, which could be important for the highly context-dependent egg-laying site selection behavior [92].

H_2O_2/bacterial lipopolysaccharide

Recent research has uncovered functions for *Gr66a* bitter GRNs in detecting other types of aversive stimuli such as H_2O_2, which can be induced by UV [34] or by microbial infection [93, 94], for example, bacterial lipopolysaccharide (LPS) is a known substance from bacteria that induces H_2O_2 [35]. These chemicals are detected by TrpA1, one of the transient receptor potential (Trp) channels, which is expressed in a subset of *Gr66a* GRNs in labellar taste hairs [38] and in pharyngeal L8 and L9 GRNs of the LSO [35,

41]. UV-induced H_2O_2 -sensing bitter GRNs in the labellum were found to promote egg-laying avoidance of strong UV. In addition, the nucleophile-sensitive TrpA1 (A) isoform expressed in I-labellar hairs was found to play an important role in suppressing intake of food sources with reactive oxygen species produced by strong UV exposure [36]. Another study reported that pharyngeal L8 and L9 GRNs detect bacterial LPS via TrpA1 and mediate feeding aversion [35]. Flies also sense LPS via GRNs in the legs and wing margins that mediate grooming behaviors [95, 96], but a requirement of TrpA1 for LPS sensitivity in these organs has not been tested. Since TrpA1 is a highly conserved channel in many species, the recent observations raise the possibility that it may be an ancient chemoreceptor for various aversive stimuli.

Ammonia

Similar to acid, ammonia has been reported to activate both olfactory and gustatory neurons. While olfactory detection of ammonia as an attractive cue depends on *Ir92a*-expressing olfactory neurons [97], gustatory responses to ammonia depend on *Gr66a* GRNs in labellar hairs [98]. In addition, ammonia elicits weak responses in L-labellar hairs in which there are no *Gr66a* GRNs. Given that *ppk23* GRNs that respond to high salt are the only known GRNs to detect aversive stimuli in L-labellar hairs, it is possible that they are the ones that sense ammonia. Experiments with *ppk23-GAL4* would help to resolve this question. However, identification of the molecular basis of ammonia taste will need further investigation.

Calcium

High levels of calcium activate *ppk23* GRNs in S- but not L-type labellar hairs and stimulate aversive behaviors [99]. At least three *Irs*, *Ir25a*, *Ir62a*, and *Ir76b*, were found to be required for the neuronal response to calcium but ectopic expression of the three in sweet GRNs did not confer calcium sensitivity, suggesting that additional factors may be involved. Similar to the activity of bitter compounds and acids, calcium also inhibits sweet GRNs, providing an additional mechanism for behavioral avoidance of calcium–laced mixtures. The report of calcium taste invites many interesting questions. For example, what is the ligand specificity of Ir62a, since the *ppk23* GRNs in S-labellar hairs also respond to high salt (NaCl and KCl)? Do multiple neurons in S-type hairs respond to calcium? An *Ir62a* reporter is expressed in tarsal GRNs [27], which raises the question of whether GRNs in other organs respond to calcium, and if so, how they contribute to behavioral avoidance of calcium. Finally, is it possible that the mechanism underlying calcium detection is one common to various salts? The answers to these questions will provide insight into how flies distinguish different salts and mount appropriate feeding responses.

Concluding remarks and future perspectives

Taste neurons in adult *Drosophila* exhibit complex molecular signatures in terms of chemosensory receptor expression. Accumulating evidence suggests that members of *Gr*, *Ir*, *ppk*, and *Trp* gene families contribute to the detection of various tastants. Overlapping expression patterns of these different chemosensory receptors could be the underlying basis of multimodal taste sensing that has now been reported for many taste neurons (Table 1). In many cases, tastant-evoked responses rely on Ir25a and Ir76b, which might serve as co-receptors for various categories of tastants. Although transgenic chemosensory reporters have presented valuable tools for interrogating the functions and response profiles of taste neurons, it should be noted that there might be further functional sub-division within these molecularly defined groups of taste neurons.

For example, *Ir76b-GAL4* and *Ir56d-GAL4* label both labellar taste hairs and pegs that respond to polyamines and fatty acid, respectively. Projection patterns of GRNs originating in these two areas can be prominently distinguished by their positions in the SEZ (posterior vs anterior), but calcium activity observed in termini of GRNs from taste hairs cannot be assigned to one type (L-, I-, or S-), from among the types that are labeled. Development of genetic tools for further defining subgroups of GRNs, possibly at single-neuron resolution, will be helpful to understand the extent of molecular and functional heterogeneity in GRNs. Single sensillum recordings can be used to better target types of sensilla that are measured, but analysis can be complicated by the fact that this method simultaneously gathers activity from all neurons in a sensillum, and also that direct comparisons between tip recordings and calcium imaging are complicated by the presence of interneurons in the SEZ that modulate pre-synaptic activity from other taste input or internal state [100–104]. Finally, since GRNs appear to detect multiple compounds of distinct taste modalities, the idea that population coding mechanisms may be involved in discrimination between tastants has some appeal. In the future, not only will it be of interest to determine how taste neurons in different organs control different aspects of feeding behaviors and connect to different higher-order neuronal circuits, but to understand how input from GRNs is integrated and evaluated for more complex taste-associated behaviors.

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