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Taste Sensation in *Drosophila melanoganster*

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Animals find nutritious foods to survive, while avoiding aversive and toxic chemicals through the chemosensory faculties of olfaction and taste. The olfaction is comparatively well characterized, but the studies of taste are only recently developing since after 2000. Genetic, immunohistochemistry, and electrophysiological studies with knock-out transgenic mice opened up the taste field in mammals. Taste in insects has been only recently been studied after mammalian taste receptors were identified. Flies also discriminate the differences of sweet, salty and sour food, while being able to detect and reject potential foods contaminated with toxins or detrimental chemicals. These discriminatory abilities indicate that flies house basic taste receptors in their taste organs like humans. For the last decade, the sweet and bitter gustatory receptors in *Drosophila* have been characterized. In this review, we compare the taste anatomy between humans and insects. We also introduce five canonical taste sensations in *Drosophila*. In addition, we introduce new taste repertoires, that fruit flies can sense water and fatty acids as well as the carbonation buffer in beverage. These studies on simple model organisms will open up a new potential for scientists to further investigate these characteristics in vertebrates.

Key Words: Taste; *Drosophila*; Gustatory Receptors; Behaviour; Electrophysiology

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INTRODUCTION

The sense of taste is one of five senses and one of two chemosensations [1]. Living organisms seek to find essential nutrients from the environment and avoid the unacceptable aversive taste as potentially harmful. All animals develop their own abilities to survive in harsh environments on Earth. Many animals ingest food from plant-derived nutrients and so many plants develop defense mechanisms, or anti-feedants, to ward off consumers, such as making toxins or repellent tastes and smells [2]. Many insects have evolved their chemoreceptors to detect detrimental chemicals as a necessary adaptation for survival.

Contact-dependent chemosensation is mainly based on the chemoreceptors on the mouthpart of fruit flies. These include Gustatory Receptors (GRs), Transient Receptor Potential channels (TRPs), Ionotropic glutamate Receptors (IRs) and Pick PocKets (PPKs). There are 68 *Grs* in the *Drosophila* genome. 6, 38, and 24 *Grs* are located on the X, 2nd, and 3rd chromosome, respectively. Recent progress regarding these ion channels will be discussed and functions in the mammalian tongue and *Drosophila* taste organs compared. In addition, we introduce five kinds of canonical tastes such as sweet, bitter, umami, sour and salty and non-canonical tastes such as fatty acids, water, pheromone and carbonation buffer in *Drosophila*.

Drosophila TASTE ANATOMY

An extraordinary feature between the taste organs in insects and mammals is that the taste system in adult insects is spread throughout the body. During the larval stage, the taste system is mainly distributed in the head region [3]. However, in adult insects, the receptors that sense tastants are present in the mouth, legs, wings and ovipositor, all of which harbor taste bristles [3].

The mouth part, which has a mainly similar role to the human tongue, is composed of external and internal taste organs. The external taste organ is connected with the proboscis, the tubular mouth-

parts used for feeding. The external taste organ consists of the labellum which contains taste bristles (sensilla) arranged in a stereotypical pattern. The taste sensilla are classified into three groups according to their length [4]. There are around 31 taste sensilla composed of nearly equal numbers of long (L), intermediate (I) and short (S) bristles. In each sensillum, there are 2-4 gustatory receptor neurons (GRNs). L-type and S-type bristles harbor 4 GRNs, while I-type bristles house only 2 GRNs. L-type respond to sugars, water, and low salt, but not aversive compounds. S-type bristles respond to sugars, high salt, sour, and aversive compounds. However, I-type bristles respond to sugars, and aversive compounds. The contact-dependent pheromone is also known to be sensed by GRNs in the mouth. However, it is not currently known if there is any relationship between pheromone sensing neurons and the other attractive or aversive GRNs. GRNs are bipolar sensory neurons that send their axons to the subesophageal ganglion (SOG) in the ventral area of the brain. There, each region of the subesophageal ganglion responds to different kinds of taste. For example, the lateral area in SOG responds to sugars while the medial area responds to aversive compounds [5]. The taste system present in the legs also regulates the intake of food prior to ingestion. There are similar GRNs to sense sugars and aversivie compounds, although the taste system in the legs is not well understood. The GRNs present in the oviposter might control the egg laying behavior on the suitable media. The specific role of the taste system present in the wings is not known.

GENERAL ANATOMY OF MAMMALIAN TASTE ORGAN

The mammalian chemosensorory organ of taste is mainly the tongue. The mammalian tongue has dense taste buds that are present in three types of papillae. The apical, lateral and basal parts of the tongue are respectively called the fungiform, foliate, and circumvallate papillae [6]. Each taste bud can sense 5 different tastes, which means that all areas in the tongue have similar sensitivity to the 5 different tastes. The taste bud contains at least three distinct cell types, which have similar characteristics to the GRNs in flies. The taste bud is composed of the taste receptor cells (TRCs). TRCs are not neurons, but epithelial cells innervated by pseudounipolar neurons whose cell bodies are housed in the petrosal and geniculate ganglia. TRCs are also present in the palate, larynx, and esophagus [6]. TRCs secrete neurotransmitters to relay the taste information to the nucleus of the solitary tract (NST) and then to the gustary cortex. The information regarding the taste is transmitted via the two nerves, i.e the facial nerve (VII) and the glossopharyngeal nerve (IX). The chorda tympani is one of branches of facial nerve, transmitted the taste message from the fungiform papillae. The messages from taste buds in the circumvallate and foliate papillae are transmitted via the glossopharyngeal nerve. These nerves carry the taste information to the NST in the medulla oblongata, which projects to the thalamus. Sensory information is also passed on to the cerebral cortex. The taste message is carried in the primary gustatory cortex in the insula and to the somatosensory cortex of the post central gyrus devoted to the tongue. This information is also sent to the prefrontal cortex which is important for taste association and perception of flavor.

SUGAR SENSATION IN *Drosophila* MELANOGASTER

Carbohydrate is the main nutritional source for fruit flies. The phylogenetic tree of the *Drosophila* gustatory receptor shows a common clade, an evolutionary conserved subfamily of eight proteins as sugar receptors in *Drosophila melanogaster* (i.e. *Gr5a, Gr61a*, and *Gr64a-f*) [7]. Flies show attractive behavior to most sugars, and high frequencies of action potentials to disaccharides such as sucrose and maltose, but not lactose [8].

Among the eight sugar receptors, GR5a was first identified as trehalose receptor [9]. The expressions of *Gr5a-GAL4* and *Gr5a*-I-*GFP* represent reliable GRN markers that label attractive sugar GRNs [5]. The other seven *Gr*s are expressed in the overlapping GRNs with *Gr5a* reporters, based on enhancer-specific transgenic reporter expression [8]. Two sugar receptors such as *GR5a* and GR64a are required to distinguish most sugars, except fructose [8,10]. While GR5a is required for trehalose sensation, GR64a is required for sucrose and maltose [8,10]. In addition, GR64f might be a sugar co-receptor, because it functions with GR5a and GR64a to respond to all sugars [11]. However, the co-expression of GR64f with either two GRs in bitter-sensing GRNs in mutant background is not sufficient to induce action potentials induced by sugars. This suggests that additional GR or other kinds of components should be required [11]. Recently, the Dahunakar group succeeded in recapitulating sugar-sensing GRs in the CO_2-sensing olfactory receptor neurons (ORNs), but not other ORNs in antenna [12]. CO_2 is known to be sensed by GR21a and GR63a [13,14]. This indicates that other kinds of components besides other GRs may be required. The remarkably interesting sugar GR is GR43a. This receptor is

not only expressed in the labellum, but also expressed symmetrically in the dorsal protocerebrum in the brain [15]. GR43a is required for fructose sensation. This receptor regulates the preference of fructose when flies are starved, but inhibits feeding behavior when they are satiated [15]. The functional homolog of GR43a in silkworm, BmGR-9, is enough to be activated by fructose [16]. This study also directly shows that BmGR-9 is a cation channel rather than a G-protein coupled receptor (GPCR). This indicates that the molecular identity of insect GRs is different from mammalian taste receptors (TRs), which are GPCRs. It is suggestive that gustatory chemosensation between invertebrates and vertebrates evolved by different convergent pathways.

Drosophila larvae also discriminate high concentrations of sugars from relatively non-nutritional food. However, most sugar *Gr*s are not expressed during the larval stage, except *Gr43a* [17]. *Gr43a* is expressed in pharyngeal GRNs to detect fructose and sucrose (disaccharide composed of glucose and fructose) [18]. GR43a is also required for sensing other sugars during the larval stages. However, the attractive kinetics (sensitivity) are much slower than fructose [18]. This indicates that other sugars may change the nutritional state and inhibit GR43a expressing neurons in the brain. Fruit flies usually prefer metabolizable sugars (sucrose or D-glucose) over non-metabolizable (zero-calorie) sugars (sucralose or L-glucose). This behavior is not affected by double mutant flies of *Gr5a* and *Gr64a* [19]. This indicates that the fly can sense the nutritional value without the influence of taste. This behavior is mediated by a sodium/solute co-transporter-like protein, called SLC5A11 [19]. This is required in a subset of the ellipsoid body R4 neurons, one of the central complex in the brain [19].

TASTE SENSATION OF AMINO ACIDS IN *Drosophila*

Amino acids are essential organic components of amine and carboxylic acid functional groups with a side-chain specific to each amino acid. It is known that vertebates sense amino acids by the heterodimer of T1R1 and T1R3 [20,21]. However, functional sensors of amino acids in fruit flies have not been reported, even though amino acids are essential nutrients. Recent report suggests that amino acid-deprived flies show enhanced preference to several amino acids [22]. This study indicates that the internal nutritional state might modulate the sensitivity of labellar taste cells, because most amino acids do not induce any action potentials from labellar sensilla [22]. Although it is not clear whether *Drosophila* are able to sense the 20 proteinogenic amino acids, *Drosophila* avoid L-canavanine, which is structurally related to L-arginine [23]. L-canavanine in certain leguminous plants makes insects avoid the plants. This compound is sensed as an aversive signal in fruit flies by GR8a and GR66a [23].

GUSTATORY RECEPTORS AND TRANSIENT RECEPTORS POTENTIAL (TRP) CHANNELS FOR AVERSIVE COMPOUNDS IN *Drosophila*

Many non-volatile detrimental chemicals including alkaloids and allelochemicals are potentially harmful to ingest. The fruit flies avoid antifeedants, because flies sense most antifeedants as aversive signals. This is innate avoidance behavior to survive in harsh environments. In *Drosophila*, I- and S-type bristles are required for sensing most bitter compounds. The representative marker of bitter-sensing GRN is *Gr66a-GAL4*, which drives GAL4 expression using Gr66a enhancer in 22 bitter GRNs in the labellum [24].

From total 68 *Gr*s in *Drosophila melanogaster* genome, 33 *Gr*s are known to be expressed in all or part of bitter-sensing GRNs, based on the reporter expression [25]. However, only 5 GRs have been genetically studied using gene knockouts [23,26-29]. From these genetic physiological studies, GR66a, GR32a and GR33a are relatively broadly required bitter GRs. This suggests that these three GRs function as co-receptors. For example, GR33a is a mostly required co-receptor to sense all bitter compounds tested except L-canavanine [23,29]. In case of sensing L-canavanine, GR66a is necessary to make the L-canavanine receptor [23]. In constrast with these three GRs, GR93a and GR8a are specific receptors for caffeine and L-canavanine, respectively [23,27]. These narrowly tuned GRs are important in defining the binding motif of chemical specificity. The specificity can be used to develop GR agonists or antagonists. However, the recapitulation of aversive GRs has not been successful using heterologous system as well as ectopic expression in sugar-sensing GRNs. If more potent agonists to aversive GR are found, the chemical might be used to ward off pests to protect animals including human as well as crops. For this reason, it is enigmatic to recapitulate aversive GRs in *in vitro* system. This will allow us to develop better insectcides or insect repellents.

Worldwide, N,N-Diethyl-meta-toluamide (DEET) has been the gold standard for insect repellents used worldwide during the last 60 years, despite lacking any knowledge of molecular mechanism

of action. The molecular mechanism and targets have recently been reported [26,30,31] and it is now known that DEET affects not only smell, but also taste. The dual aversive effects are very effective in repelling harmful insects such as mosquitoes and further elucidation of the DEET-specific GR chemo-receptors responsible could provide a new target to develop a new generation of repellent agonists. Other molecular sensors to detect aversive compounds are TRP channels. Three TRP channels including *trpA1, painless*, and *trpl* are required to sense detrimental compounds [32-35]. TRPA1 is expressed in a subset of bitter GRNs and required to respond to aristolochic acid [34]. Aristolochic acid is a carcinogenic, and mutagenic compound commonly found in the plant including *Aristolochia* and *Asarum* [36]. These plants had been commonly used in Chinese herbal medicine until Food and Drug Administration (FDA) advised consumers to stop using this compound in 2001. TRPA1 is also a sensor for wasabi [35]. *painless* is reported to be necessary to sense wasabi in proboscis extension response (PER) [33]. However, it is not shown for *painless* to be activated by wasabi. Also, TRPL is expressed in GRNs and responds to camphor [32, 37]. TRP Channels may be good targets to control pests through aversive GRs.

SOUR TASTE IN FRUIT FLIES

In Korea, fruit flies are called "flies that like acid". However, it is a little controversial among smell and taste studies on acid [38-40]. They suggest two different behavioral results, one study shows behavioral attractiveness to acid, but the others get behavioral avoidance to acid. This may be because acid can directly penetrate the plasma membrane and affect cellular components. Sour taste is mediated by acidic pH and by organic acids such as acetic and citric acids. Fruit flies may prefer weak acid, but avoid strong acid.

In the most recent taste report, *Drosophila* shows a repulsive behavior to four kinds of acidic foods including acetic, citric, glycolic and tartaric acids [40]. In addition, acidic solutions activate bitter-sensing GRNs and inhibit sugar-sensing GRNs like most bitter compounds, except L-canavanine [23,26]. It is enigmatic to find sour sensors in the sensilla on the labellum.

SALT TASTE IN *Drosophila*

"Salt" is sodium chloride. Animals, including humans and flies prefer low salt (< 100 mM). Sodium is an essential nutrient. This mineral cannot be generated by itself in the animal body. Sodium is necessary to regulate muscle contraction, and generate action potentials for good health. In mammals, amiloride-sensitive epithelial Na^+ channels (ENaCs) are low-salt receptors [41]. In fly larvae, two ENaC members, *pickpocket11* (*ppk11*) and *ppk19* are reported to have roles to sense low salt in the terminal organs [42]. Recently, *ionotropic glutamate receptor* (*Ir*) has been identified as a chemoreceptor in antenna and taste [43,44]. This family is composed of around 60 members. This new type of chemosensor is required for sensing a different repertoire such as ammonia, compared with the olfactory receptors (OR) in the antenna. The first role of IR in taste was characterized by using a knock-out study of *Ir76b*. *Ir76b* is required for low salt (< 100 mM), but not high salt (> 500 mM) in the sensilla on the labellum during adult stages [45]. The flies show attractive behavior to low salt concentration (< 100 mM), but aversive response to high salt concentration (> 500 mM). This antagonistic behavior is quite similar to humans. In humans, high salt diet can induce hypertension. Low salt induces action potentials from L-type bristles, but high salt from S-type bristles. Sensors for high salt are not identified so far in humans or flies.

THE MOLECULAR BASIS FOR WATER, CO_2 AND FATTY ACID TASTE IN *Drosophila*

Water is necessary for the regulation of osmotic homeostasis in every living organism. Fruit flies sense water using proximal peg GRNs as well as GRNs on the distal labellum [46]. These GRNs are marked using NP1017 GAL4 expressing neurons. The molecular sensor for water detection is a member of the degenerin/epithelial sodium channel family, PPK28 [37,47]. This mediates the cellular response to water. The loss of *ppk28* loses water sensitivity. The heterologous expression of PPK28 induces action potentials by the stimulus of hypo-osmotic solutions.

Fruit flies discriminate carbonation buffer when drinking sodas. Carbonation buffer (low concentration of CO_2) activates peg GRNs, which innervate the lateral area in SOG of the fly brain [48]. The two gustatory receptors *Gr21a* and *Gr63a* forming the same clade are responsible for the antennal detection of relatively high concentration of CO_2 [13]. These studies indicate that low concentration of CO_2 is detected by the taste system as attractive signals, although high concentration of CO_2 is mediated by the olfactory system as alarm signals [13].

Fruit flies are attracted by fatty acids. They show stage-specific

preferences for them. Larva prefer unsaturated fatty acids (UFAs), while the adults prefer saturated fatty acids (SFAs) [49]. FA are also sensed by the GRNs on the labellum. *norpA* in the sugar-sensing GRNs is required for fatty acid sensation [50]. *norpA* codes for phospholipase C (PLC). There are two PLCs in fly genomes. However, this study did not test the possible contribution of the second PLC, *plc21c*. In addition, the molecular sensor is not yet found.

TASTE TRANSDUCTION IN *Drosophila*

GRs are ionotropic receptors [16]. Each chemical activates specific GRs, but there are several studies to show that G proteins have roles to sense sweet and bitter tastants. Two studies done by Ueno in 2006 and 2008 for the sugar signal transduction in *Drosophila* found that Gsα and the adenylate cyclase gene, *AC78C*, mediate sugar signaling mainly for trehalose and sucrose in the sugar sensitive *Gr5a*-positive GRNs [51,52]. Another cyclase gene such as soluble guanylyl cyclase, *Gyc-89Da* is found to take part in signal transduction of sucrose in both the larval and adult stages [53]. Similarly *Gyc-89Db* mediate caffeine sensation [53].

PHEROMONE SENSATION IN *Drosophila*

A pheromone is generally known to be a secreted volatile chemical. Pheromones can be thought of like perfume, a compound that travels through ambient air, to act as a necessary, olfactory, social communication between members of the same species to give alarm, attractive or suppressive signals. There are also non-volatile pheromones in the animal world. These pheromones are composed of hydrocarbons. Recently, three studies suggest that *ppk23* and *ppk29* are involved in male-to-male suppression and male-to-female attraction during *Drosophila* courtship [54,55]. These studies show the direct cellular response to the pheromones in these GRNs. However, the pheromone receptor is not recapitulated *in vivo*, which indicates that other components may be required for this complex. There are both direct and indirect relationships between GRs and *Drosophila* courtship. *Gr68a* and *Gr39a* are expressed in forelegs of male flies. The knock-down or knock-out of these genes affect normal courtship behaviors [56]. The *Gr68a*, expressed in 20 male specific gustatory bristles in the foreleg controls the normal male courtship behavior. The inactivation of *Gr68a*-cells by tetanus toxin or RNAi resulted in decreased male courtship performance [56]. The loss-of-function behavioral studies with *Gr32a* and *Gr33a* also suggest that these two GRs are involved in sensing male-to-male suppressive pheromones [29,57]. However, there is no physiological evidence using pheromones in these GR studies so far.

There are at least four GRs expressed in neurons that regulate male and female reproductive organs [58]. These GRs might be required for sensing sex peptides, but it remains to be characterized. Intriguingly, these GRs are required for females to lay eggs in ideal environments.

CONCLUSION AND FUTURE PROSPECTIVE

In *Drosophila melanogaster*, there are at least four kinds of chemoreceptors that function in the GRNs. These include GRs, TRPs, IRs and PPKs. The taste receptors are first found in mammals in early 2000's. However, the development of the taste field in insects is very fast, owing to the advantages of flies as a trackable model organism. The umami, sour, and carbonation buffer receptors are not yet identified in insects. The characterization of bitter GRs is more extensively required, because only two specific GRs to caffeine and L-canavanine are identified. In addition, recapitulation using heterologous systems is highly desired in order to develop more potent drugs. To find the missing component of pheromone receptor is important to control insects in ecosystem. Moreover, the direct sensors for fatty acids and high salt remains to be identified.

The ionotropic cation channels in *Drosophila* and the GPCRs in mammals are involved in taste recognition and regulation of attraction and avoidance behavior in taste. The first IR in taste was just characterized last year, so the related studies will likely proceed rapidly over the next decade. It is possible that uncharacterized IRs are involved in mediating sour and high salt taste.

The biggest difference between mammalian TRs and insect GRs is molecular identity. TRs are GPCRs, but GRs are ion channels. Other sensors such as TRPs, IRs and PPKs are somehow conserved in vertebrates and invertebrates. This difference of GRs can be used to control insects while minimizing any side effects in mammals.

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