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Function and Expression of the *Drosophila Gr* Genes in the Perception of Sweet, Bitter and Pheromone Compounds

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Introduction

Sensory perception enhances and adds dimension to life, while also serving the more primitive purpose of providing information about the environment that is necessary for survival and propagation of the individual. Two primal and essential behaviors—mating and feeding—are thought to be mediated by contact chemosensation in many animals, including *Drosophila*.

Drosophila engage in courtship before mating. A highly ritualistic and complex behavior, courtship consists of a series of stereotyped interactions between the male and female (Hall, 1994; Greenspan and Ferveur, 2000) that must occur before a successful mating event. Pheromone detection is necessary for efficient recognition of a suitable mate and therefore essential in initiating courtship. Detection of pheromones is thought to occur when the male makes contact with the female abdomen with his labellum and forelegs ('tapping'). Evidence that the contact chemosensory system may mediate pheromone detection include the observations that males have significantly more taste bristles on their forelegs (~50) compared to females (~37) (Nayak and Singh, 1983) and that female pheromones are non-volatile hydrocarbons highly concentrated on the female abdomen (Coyne *et al.*, 1994; Ferveur *et al.*, 1996).

Taste perception allows nutritionally rewarding food to be discriminated from food that is contaminated and toxic. Generally, foods that are sweet are fed upon by fruit flies, whereas bitter substances are avoided. Unlike humans, however, fruit fly taste perception is not restricted to one tissue. Instead, flies have taste bristles not only on the labellum (human tongue equivalent), but also on the wings, legs, genitalia and in the pharynx (Figure 1a).

Taste bristles are associated with two to four taste neurons, the dendrites of which extend into the bristle shaft. These taste neurons express a class of G-protein-coupled-receptors (GPCRs), called gustatory receptors (*Gr*s), that are thought to activate the neuron upon contact with a soluble ligand. Initially identified based on their structural similarity to *Drosophila* odorant receptors (*Or*s) (Clyne *et al.*, 2000), the *Gr* gene family contains ~70 members with relatively low sequence similarity (15–25%) (Dunipace *et al.*, 2001; Scott *et al.*, 2001; Robertson *et al.*, 2003). Due to their low levels of cellular expression in tissues not amenable to RNA *in situ* hybridization, the Gal4/UAS system has proven extremely valuable in determining the expression pattern of individual *Gr*s by driving the expression of a reporter gene, such as GFP or β-Gal, under the control of a *Gr* promoter. Using this system, Dunipace *et al.* (2001) and Scott *et al.* (2001) provided the first peripheral *Gr* expression map for a subset of receptors, demonstrating that *Gr*s are expressed in taste neurons of the labellum, wings, legs and pharynx.

Results

To understand how the fly recognizes and discriminates among different soluble compounds involved in taste and pheromone perception, we first created an expression map of the *Gr* genes. Of primary interest is the extent of co-expression of different *Gr* genes within the same taste neurons. For example, one might expect similarities to the mammalian taste system in which taste receptors recognizing related compounds (bitter-tasting) and mediating similar behavioral responses (repulsion) might be expressed in identical or largely overlapping neurons (Zhang *et al.*, 2003). Similarly, *Gr*s recognizing distinct compounds and thereby mediating different behaviors, might be expressed in an entirely different group of taste

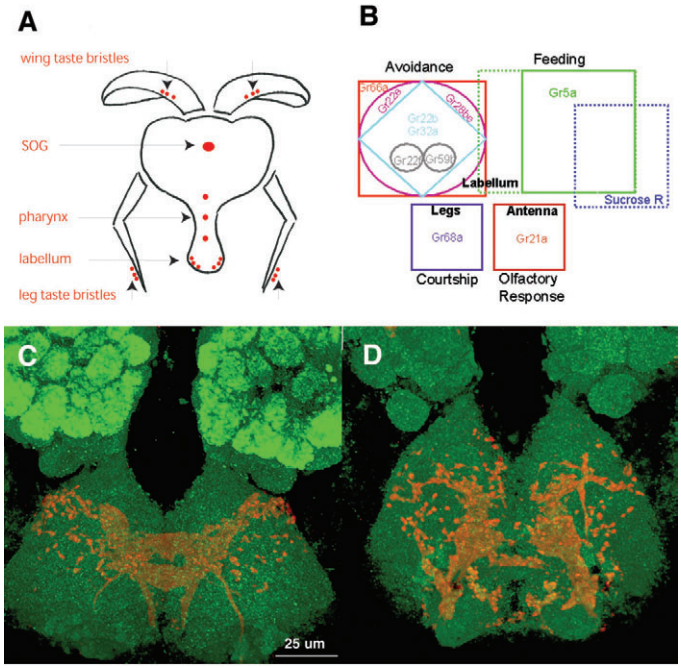

Figure 1 **(A)** *Gr*s are expressed in taste neurons located on the labellum and in the pharynx, as well as along the legs and anterior wing margins. Taste neurons of the head project their axons directly to the SOG. **(B)** *Gr* expression may functionally define chemosensory neurons. **(C)** Axonal targets of *Gr66a*-expressing neurons in the tritocerebrum/SOG as visualized using *n-synaptobrevin-GFP* as a reporter. **(D)** Axonal targets of Gr5a-expressing neurons. The qualitative difference in projection patterns between brains seen in (C) and (D) is striking.

Table 1 Expression of 13 *Gr*s in taste neurons as determined by the Gal4/UAS system

Receptor	Neurons per labial palp	Other peripheral expression					
		LSO	VCSO	1st leg	2nd leg	3rd leg	Wing
Gr5a	71	N	N	10	4	4	N/D
Gr64a	0	Y	N	0	0	0	0
Gr64e	0	N	Y	0	0	0	0
Gr22b	10	N	Y	2	0	0	N/D
Gr22e	14	Y	Y	2	8	10	10
Gr22f	3	N	N	0	0	0	0
Gr28be	13	N	Y	2	0	0	N/D
Gr32a	8	N	Y	5	3	3	N/D
Gr59b	4	N	N	0	0	0	0
Gr66a	22	Y	Y	8	7	6	0
Gr39a.a	5	N	Y	0	0	0	0
Gr39a.d	12	N	N	3	2	0	0
Gr68a	0	N	N	7*	0	0	0

Reporters used to visualize expression were β-Gal and nuclear GFP.
*Expression in males only. All cell counts are averages.

neurons. Thus, using the Gal4/UAS system, which generally allows accurate expression analysis of a gene of interest (Brand and Perrimon, 1993), we first determined the cellular expression of 13 *Gr* genes (Table 1).

To determine the functional role of neurons that express these *Gr*s, we characterized the behaviors of flies in which specific sets of taste neurons were functionally inactivated. To achieve this, we combined the *Gr-Gal4* drivers with a *UAS-tnt* reporter. *Tnt* codes for the tetanus-toxin light chain protein that inhibits synaptic transmission in the neuron in which it is expressed (DiAntonio *et al.*, 1993), thereby allowing us to inactivate neurons expressing specific *Gr*s. The expression pattern of these 13 *Gr*s, as well as functional analysis of the neurons in which they are expressed, indicate that these receptors are involved in the taste perception of bitter or sweet tastants or in pheromone detection.

Discrimination between two taste modalities—sweet and bitter—requires specific sets of gustatory receptor neurons that express different *Gr*s

Previous expression studies of *Gr*s (Clyne *et al.*, 2000; Dunipace *et al.*, 2001; Scott *et al.*, 2001; Hiroi *et al.*, 2002) have shown that most of the receptors analyzed are expressed in taste neurons of the labellum. Labellar taste bristles are arranged in a stereotyped pattern and are morphologically identified as short (S), intermediate (I), or long (L). Hiroi *et al.* (2002) associated *Gr* expression with bristle-type and found that several of the *Gr*s were expressed in a single neuron associated with the same bristle.

In an effort to understand how taste is coded at the periphery, we carried out experiments to determine whether these *Gr*s were co-expressed in the same taste neuron or different neurons associated with the same taste bristle (Thorne *et al.*, 2004). We drove expression of *nuclear-GFP* under the control of two *Gr* promoters (Gal4/UAS system) and then used immunohistochemistry and confocal microscopy to obtain an accurate count of the number of cells expressing either receptor. Based on cell counts of double *Gr*-promoter driver lines compared to single *Gr*-promoter driver lines, we found that most of the receptors analyzed were partially co-expressed in neurons associated with S-type bristles of the labellum (*Gr66a*, *Gr22e*, *Gr28be*, *Gr32a*, *Gr22b*, *Gr22f*, *Gr59b*; Table 1 and Figure 1B), indicating that labellar neurons can be defined by their *Gr* gene code.

Interestingly, *Gr5a*—a trehalose receptor—was not expressed in the neurons associated with S-type bristles in which the other receptors were expressed. In fact, neurons that express *Gr5a* had morphologically smaller cell bodies compared to the S-type bristle-associated neurons that express the other *Gr*s. Additionally, *Gr5a* was expressed in a relatively large number of labellar neurons (~70 per palp) and many neurons expressing *Gr5a* were clustered, with their dendrites converging to enter the same bristle shaft. Thus, our results indicated that neurons sensitive to sweet taste, in particular trehalose, may be distinct from those associated with a different taste modality, represented by *Gr66a* neuronal expression.

To determine what substrates neurons expressing *Gr66a* could recognize, we conducted behavioral studies—specifically, feeding preference assays—on flies with neurons functionally ablated by TNT. For example, flies lacking functional *Gr66a*-expressing neurons were given a choice to feed on 6 mM caffeine with 2 mM sucrose versus 2 mM sucrose alone. We found that these flies were significantly less sensitive to caffeine—a bitter substance—compared to control flies not expressing TNT in these neurons. However, these flies did not have reduced sensitivity to either 2 mM sucrose or 25 mM trehalose. These results indicated that neurons expressing *Gr66a* were sensitive to a bitter compound—caffeine—but did not play a role in sweet taste perception. Alternatively, flies in which TNT was driven by *Gr5a* could not discriminate 25 mM trehalose from water. These flies did not have an altered sensitivity to any bitter compound tested or to sucrose. Thus neurons expressing *Gr5a* appear to mediate sensitivity to trehalose and do not confer sensitivity to bitter compounds or sucrose.

Our behavioral data combined with peripheral expression analysis indicate that neurons sensitive to repellants may be discrete and separate from neurons sensitive to attractants and that these neurons express different *Gr*s. Therefore, we postulate that the *Gr*s expressed in a given neuron define its substrate specificity and sensitivity.

Since the majority of neurons sensitive to the repellant caffeine were distinct from the trehalose sensitive neurons expressing *Gr5a*, we expected that the axonal targets in the CNS for these two sets of neurons would be different. Not surprisingly, analysis of the axonal projection patterns of neurons expressing the *Gr66a* group of co-expressed receptors are qualitatively different from the pattern seen for *Gr5a* in the fly's primary taste center in the brain—the tritocerebrum/SOG (Figure 1). Thus, bitter and sweet taste modalities may have different neural circuits that translate into different behaviors.

A male-specific *Gr, Gr68a,* is involved in female pheromone detection

We have recently discovered a *Gr* whose expression is associated with ten male-specific taste bristles in the foreleg (Bray and Amrein, 2003). This male-specific expression of *Gr68a* suggested a role in female pheromone detection, especially during the tapping step of courtship. Males expressing TNT exclusively in *Gr68a*-expressing neurons were significantly impaired in their ability to mate: 41% of these males failed to mate within 30 min compared to an ~5% failure rate for control males.

Although mating success appeared to be significantly affected in males lacking *Gr68a*-expressing neurons, our hypothesis would predict that *Gr68a* is directly involved in pheromone detection—a process that plays an important role in courtship. To this end, we have analyzed courtship behavior of males lacking functional *Gr68a* neurons and found that not only did they spend significantly less

time courting virgin females compared to control males, but that these males were less successful in proceeding to the wing extension/vibration step of courtship. Thus it appeared that these males were hindered moving through the tapping step of courtship—the step that is thought to involve the detection of female pheromones. It is worth mentioning that these males were not found to court, or attempt to mate, other males.

Our data therefore indicate a functional role for *Gr68a*-expressing neurons of the male forelegs in female pheromone detection. A direct role for *Gr68a* in this process was found by using RNA interference—males were made that expressed double-stranded *Gr68a* RNA in an effort to degrade endogenous *Gr68a* RNA through RNA interference. We found that these males had a very similar, albeit slightly weaker, courtship phenotype compared to males lacking functional *Gr68a* neurons. This weaker mating phenotype could indicate expression of additional putative pheromone receptors in these neurons. Possible candidates include genes closely related to *Gr68a*, such as *Gr32a* or members of the *Gr39* gene cluster. Regardless, *Gr68a* appears to mediate the detection of an unknown female-specific pheromone required for efficient courtship in *Drosophila melanogaster*.

Conclusion

Members of the *Gr* gene family appear to play a significant role in *Drosophila* chemosensory perception, including taste perception, pheromone detection and olfaction. These receptors, therefore, must detect a large number of distinct compounds.

Future experiments will address whether flies are able to discriminate between different bitter compounds, as suggested by the partial co-expression of many labellar *Gr*s. An effort will also be made to assign ligand specificity to the pheromone receptor *Gr68a*. It will also be interesting to determine if other members of the *Gr* gene family code for pheromone receptors.

Drosophila thus provides an elegant model for coupling the molecular basis of sensory perception (*Gr*s) to behavioral output (feeding and courtship), perhaps giving us insight into how sensory perception influences our apparently sophisticated interactions with our environment and the people around us.

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