

Diverse roles for the *Drosophila* fructose sensor Gr43a

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The detection of nutrients, both in food and within the body, is crucial for the regulation of feeding behavior, growth, and metabolism. While the molecular basis for sensing food chemicals by the taste system has been firmly linked to specific taste receptors, relatively little is known about the molecular nature of the sensors that monitor nutrients internally. Recent reports of taste receptors expressed in other organ systems, foremost in the gastrointestinal tract of mammals and insects, has led to the proposition that some taste receptors may also be used as sensors of internal nutrients. Indeed, we provided direct evidence that the *Drosophila* gustatory receptor 43a (Gr43a) plays a critical role in sensing internal fructose levels in the fly brain. In addition to the brain and the taste system, Gr43a is also expressed in neurons of the proventricular ganglion and the uterus. Here, we discuss the multiple potential roles of Gr43a in the fly. We also provide evidence that its activation in the brain is likely mediated by the neuropeptide Corazonin. Finally, we posit that Gr43a may represent only a precedent for other taste receptors that sense internal nutrients, not only in flies but, quite possibly, in other animals, including mammals.

Omnivores consume a wide selection of nutrients such as carbohydrates, amino acids, fatty acids and numerous salts to meet their needs for energy expenditure, growth, and development. Absence of a single group of nutrients can result in stunted growth, morbidity, metabolic dysfunction, and premature death. The sense of taste plays a central role for

evaluating the palatability of potential food sources, and recent progress in uncovering the molecular and cellular principle that underlie taste perception have led to a broad understanding of how mammals and insects identify and discriminate among different food chemicals and avoid the many non-nutritious, toxic chemicals which often taste bitter.¹ Intriguingly, it has been demonstrated recently in both vertebrates and insects that at least some of these nutrients can be sensed not only by the taste systems, but also by internal sensors present in the gastrointestinal system and the brain. For example, mammals appear to sense glucose (and other sugars) in the gut using the T1R2/T1R3 taste receptors,^{2,3} and the glucose transporter GLUT2 mediates glucose uptake in the pancreas and probably also in selected hypothalamic and other neurons in the brain.⁴ These glucose-sensing processes are essential for the regulation of nutrient metabolisms and behaviors via the secretion of insulin, glucagon, and numerous neuropeptides.⁵ In insects, the G-protein coupled receptor BOSS was proposed to function as a glucose sensor in the fat body to regulate insulin signaling.⁶ Gastrointestinal systems have also been implicated in sensing bitter substances, since gut endothelial cells of mammals and insects express T2R and Gr bitter taste receptors, respectively.^{7,8} Sodium is probably sensed by the mechanosensory channel TRPV1 and the atypical sodium channel NaX in the brain,^{9,10} while PKD2L1, a sour taste sensor, is expressed in the neurons surrounding the central canal of the spinal cord.¹¹ Finally, both mammals and

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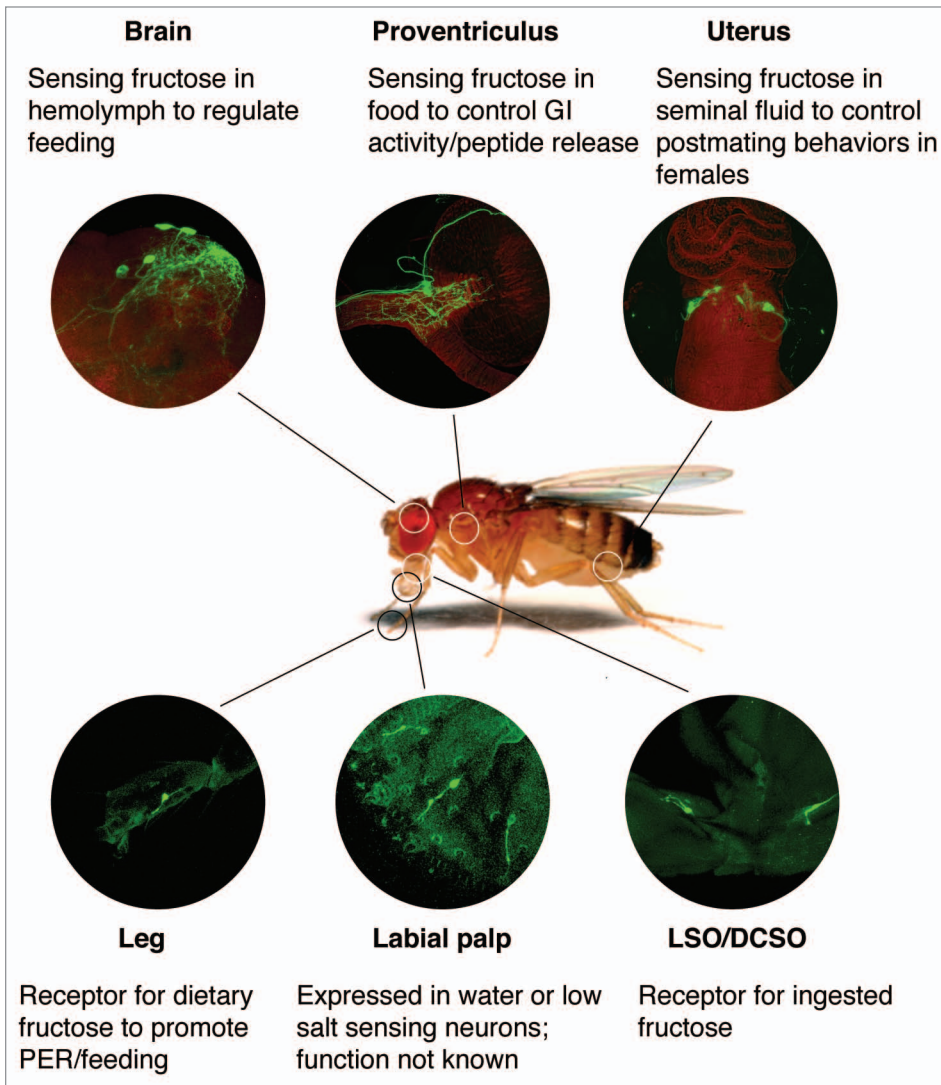


Figure 1. Overview of *Gr43a* expression and putative function in a female fly. The 6 sites of *Gr43a* expression in an adult female fly are depicted and actual expression sites are shown in individual images. Established (brain, forelegs), proposed (VSO/DCSO, proventriculus, and uterus), or unknown (labial palps) are indicated.

insects can sense internal levels of amino acids, which is used to modulate their feeding behavior; specifically, uncharged tRNAs are suggested to be mediators of amino acid sensing in brain neurons of mammals.¹²

We recently discovered that the *Drosophila* gustatory receptor 43a (*Gr43a*), one of the most conserved insect taste receptors, is expressed not only in taste neurons, but also in neurons associated with internal organs such as the brain, the proventricular ganglion, and the uterus.¹³ Expression in these organs was established with a *GAL4* knock-in allele (*Gr43a^{GAL4}*), in which the *Gr43a* coding sequence

was replaced with that of the *GAL4* gene. Using Ca^{2+} imaging, we found that *Gr43a* functions as a narrowly tuned receptor for fructose. These observations raised the intriguing possibility that fructose is not only sensed as a dietary component by the taste system, but also serves as a carbohydrate component in the hemolymph to reflect the internal nutrient status. Here, we discuss potential functions of *Gr43a* expressing neurons in each of the organ system where its expression has been established (Fig. 1). We also show for the first time that the *Gr43a* expressing uterus neurons respond to fructose in a manner similar to the

brain neurons. Finally, we provide evidence that *Gr43a* expressing neurons use distinct modes of neurotransmission in different organs to propagate stimulation by fructose. Specifically, the *Gr43a* expressing brain neurons co-express Corazonin, a highly conserved insect neuropeptide, suggesting that downstream neurons, which mediate *Gr43a* activity, express the Corazonin receptor.

The Adult Taste Sensory System

Fly taste neurons, referred to as gustatory receptor neurons (GRNs), are organized in taste sensilla and taste pegs, located in various appendages.¹⁴ The 2 labial palps harbor close to 80 taste sensilla and taste pegs, and there are ~30 to 40 taste sensilla on each leg and about 20 on each anterior wing margin; while the function of labial and tarsal taste sensilla in mediating feeding responses is well established, the contribution to taste of sensilla on the wing margin are largely unknown.

Most taste sensilla contain 4 GRNs, each thought to detect distinct groups of food and other chemicals: the sweet neuron senses various sugars, the bitter/high salt neuron responds to various non-nutritious and often harmful organic chemicals, as well as high concentration of salt (> 400 mM), a third neuron responds to low salt solutions and the last neuron responds to water.¹⁵ In addition, the fly harbors internal taste neurons, located in 3 pharyngeal structures, the labral, dorsal, and ventral cibarial sense organs (LSO, 18 neurons; DCSO, 6 and VCSO, 8).¹⁶

Function of *Gr43a* as a Taste Receptor

Tarsal taste sensilla

The most prominent expression of *Gr43a* in the taste system is observed in the legs: *Gr43a^{GAL4}* is expressed in a single GRN of 2 taste sensilla located on the 5th tarsal segment of each leg (Fig. 1).¹³ These GRNs also express several members of the sugar gustatory receptor (sugar Gr) subfamily consisting of *Gr5a*, *Gr61a*, and

Gr64a-f and are broadly tuned to and activated by most sugars, as determined by Ca^{2+} imaging.¹³ Lack of *Gr43a* reduces the response specifically to fructose, while absence of the sugar *Gr* genes abolishes the response to all sugars except fructose and sucrose (disaccharide of fructose and glucose). Finally, lack of all sugar *Gr* genes and *Gr43a* completely abolishes fructose and sucrose response, and transgene expression of *Gr43a* alone is sufficient to restore both responses. Thus, *Gr43a* functions as a secondary tarsal fructose receptor. We note that the *Gr43a* expressing neuron housed in the 5V1 sensillum is significantly more sensitive to sugars—especially to fructose and sucrose—than other sweet sensing GRNs that do not express *Gr43a*.¹⁷ A possible explanation for the large contribution of *Gr43a* to fructose sensing in this sensillum is its high level of expression: analysis from tarsal tissue shows that *Gr43a* transcripts are represented approximately 10 times more than transcripts of any of the classical sugar receptor genes (data not shown), even though the former is expressed in fewer cells than the latter.^{13,17,18} Behavioral relevance for the high sensitivity of *Gr43a* expressing neurons has yet to be established, as the standard behavioral proboscis extension reflex (PER) response is not significantly affected for any sugars in *Gr43a* mutant flies when compared with wild type flies.¹³

Labial palp neurons

Gr43a is expressed in ~8 GRNs in the labial palp (Fig. 1).¹³ Surprisingly, *Gr64f*, a marker expressed in virtually all sugar neurons, is not co-expressed with *Gr43a* in these neurons, and *Gr66a*, a receptor for caffeine and a marker for bitter sensing GRNs, is not co-expressed with *Gr43a* either.¹³ Thus, by default, these *Gr43a^{GAL4}* expressing neurons appear to correspond to water or low salt sensing neurons. Single neuron Ca^{2+} imaging has not yet been possible on sensilla located in the palps and, hence, the response properties of these *Gr43a* expressing neurons are not known. Compared with other taste organs (i.e., tarsal neurons or pharyngeal neurons; see below), the expression level of *Gr43a^{GAL4}* in the labial palp is much lower,¹³ and it is therefore also possible that *Gr43a* has no obvious function in these neurons.

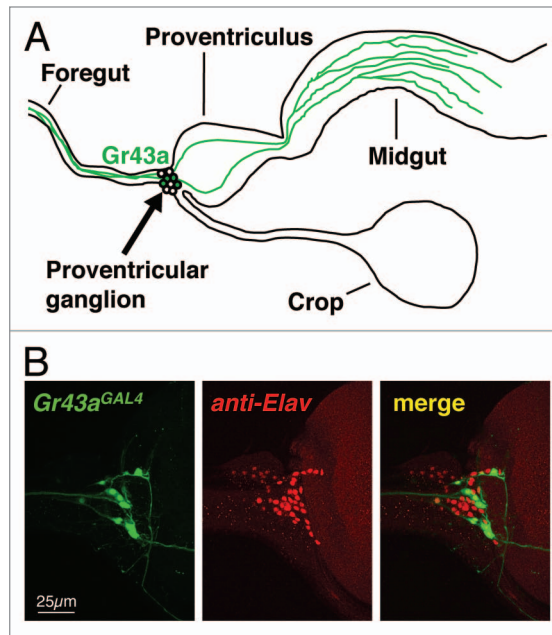


Figure 2. *Gr43a* expressing neurons in the proventricular ganglion. (A) Schematic diagram of *Gr43a* expressing neurons. All cell bodies are in the proventricular ganglion. One group sends axons along the foregut to the SOG, the other projects axons to the midgut. The dendrites innervate the lumen of the foregut. (B) The proventricular ganglion consists of 30–40 neurons (pan-neuronal marker, *Elav*), *Gr43a^{GAL4}* drives *UAS-mCD8GFP* expression in 4–8 of them (*Gr43a^{GAL4}*).

LSO and VCSO neurons

Gr43a is expressed in 2 neurons located in the LSO and the VCSO¹³ (Fig. 1). A putative sugar receptor gene, *Gr64f* is also co-expressed in 2 of the *Gr43a^{GAL4}* positive LSO neurons, but not in the VCSO neurons.¹³ The only established role for pharyngeal taste neurons has been reported for the VCSO, where bitter sensing (*Gr66a* expressing) neurons contribute to egg laying preference on lobeline containing food substrates.¹⁹ This is interesting, because bitter chemicals sensed by labial or tarsal neurons suppress feeding responses in proboscis extension reflex (PER) assays.²⁰ Regardless, we observed no co-expression between *Gr43a* and *Gr66a* in any pharyngeal taste neurons. Based on partial co-expression with *Gr64f*, the role of *Gr43a* is likely to be related to sensing sugars while food is ingested, but new behavioral paradigms will have to be developed to assess their specific role in feeding.

Functions of *Gr43a* as an Internal Nutrient Sensor

In addition to gustatory neurons, *Gr43a* is also expressed in defined sets of

neurons of the proventricular ganglion, the brain and the uterus¹³ (Fig. 1). Ca^{2+} imaging experiments using ex-vivo preparations of brains and uterus confirmed that *Gr43a* also functions as a fructose receptor in these organs¹³ (Fig. 3).

Proventricular ganglion

The taste organs examine food chemicals before they enter the digestive system, but it is now well documented that both nutrients and potential toxins are also re-evaluated when they are in the gastrointestinal tract. For example, in mammals, sugar taste receptors expressed in the gastrointestinal tract stimulate glucagon-like peptide 1 secretion in response to sugar ingestions,²¹ and bitter taste receptors are also expressed in gut epithelial cells of both mammals and insects.^{7,8} In the mouse, activation of T2Rs in the gut leads to secretion of cholecystokinin from enteroendocrine cells, which limits absorption of dietary toxins. In addition, cholecystokinin signaling also increases expression of the ABCB1 efflux transporter, thereby actively limiting absorption of bitter-tasting toxins.^{22,23} In *Drosophila*, ingested food passes through the pharynx and the foregut and is initially deposited in the

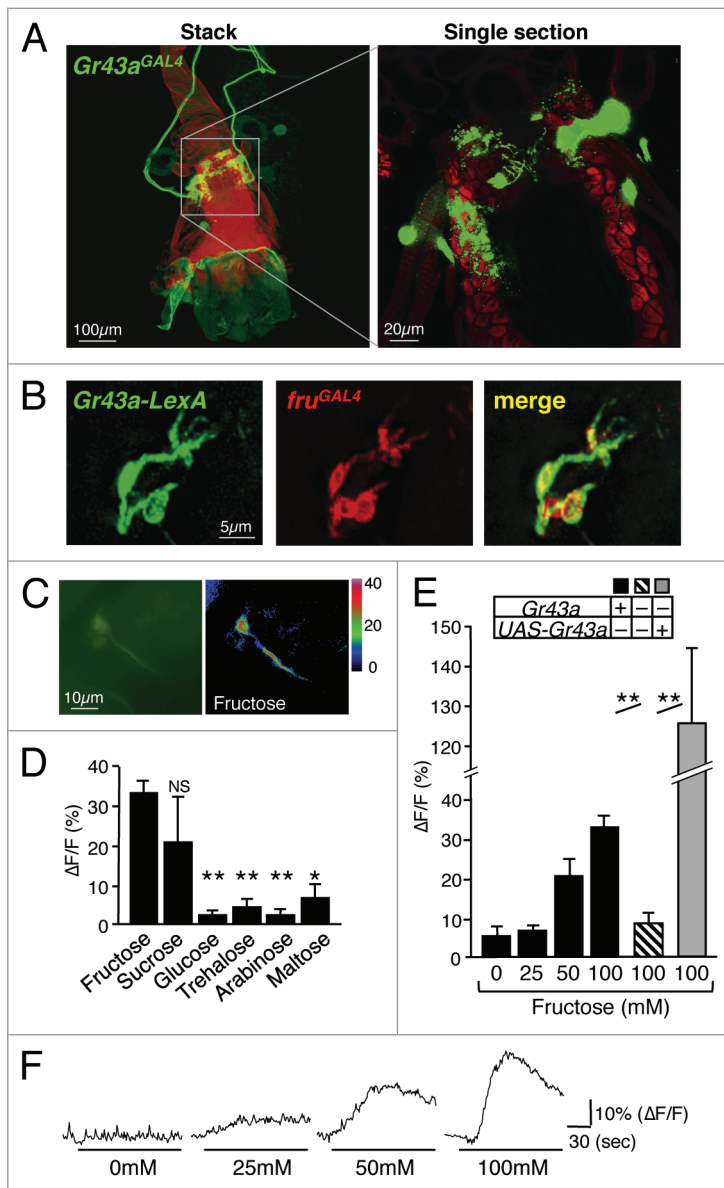


Figure 3. *Gr43a* expressing neurons in the uterus. (A) *Gr43a^{GAL4}* drives *UAS-mCD8RFP* expression in 4–6 neurons. Cell bodies are located at the middle of the uterus, sending the axons to the abdominal ganglion. The dendrites innervate the lumen of the uterus, clearly shown by the single section view. (B) *Gr43a-LexA* and SPR are co-expressed in the same neurons. *Gr43a-LexA* drives *lexAop-rCD2GFP*, *fru^{GAL4}* drives *UAS-mCD8RFP*. Note that *fru^{GAL4}* expression serves as a proxy for SPR, which are co-expressed in the uterus.^{35,36} (C) Uterus neurons expressing G-CaMP3.0 under control of *Gr43a^{GAL4}*. ΔF pseudocolor fluorescence image was taken 85 s after application of 100mM fructose (right). (D) *Gr43a^{GAL4}* neurons specifically respond to fructose. Max ΔF/F within 135 s of application is shown. All sugars are 100mM. Flies contained 2 genomic copies of *Gr43a*. ****P* < 0.0001; ANOVA. Error bars represent standard error. 5 ≤ *n* ≤ 8. (E) Response of *Gr43a^{GAL4}* neurons to fructose is *Gr43a* dependent. ****P* < 0.0001; ANOVA. Error bars represent standard error. 5 ≤ *n* ≤ 9. (F) Time-course of G-CaMP3.0 fluorescence changes in *Gr43a^{GAL4}* neurons stimulated with different concentrations of fructose. Note that ΔF/F in *Gr43a^{GAL4}/Gr43a^{GAL4};UAS-Gr43a* rescue flies is higher than in *Gr43a⁺* controls, likely due to higher expression levels of *Gr43a*.

crop. The stored food is then moved into the midgut through the proventriculus, a muscular organ that separates foregut and midgut. The proventricular ganglion is located on the dorsal side of the foregut.²⁴

This ganglion contains about 30–40 neurons, 6 of which express *Gr43a* (Fig. 2); they send dendritic terminals into the lumen of the foregut, but not into the crop duct. One group of neurons sends

axonal projections to the SOG along the esophagus through the brain, forming a nerve bundle with axons of GRNs located in the LSO and the VCSO. The other group of neurons extends axons posteriorly, where they innervate cells in the midgut.¹³ The anatomy and location of processes of these neurons suggests that fructose content of food may be monitored immediately before entering the crop and the midgut. While the neurons projecting to the SOG may serve similar roles as taste neurons (PER, food intake), the neurons projecting to the midgut may regulate food transport and/or secretion of neuropeptides/hormones in response to sugar consumption. Expression of *Gr43a* in the gastrointestinal system appears to be conserved across different insect species; the *Gr43a* orthologs in the silkworm *Bombyx mori* (*BmGr-9*) and in the cotton bollworm *Helicoverpa armigera* (*HaGR9*) are also expressed in their digestive systems.^{25,26}

Brain

Gr43a is expressed in 2–4 neurons located in the posterior superior lateral protocerebrum of each brain hemisphere.¹³ Two neurons are easily identifiable using live GFP imaging, but 2 additional neurons are characterized by lower level of expression and are only detected using antibody staining of dissected brains. We showed that the *Gr43a* expressing neurons respond to fructose, using Ca²⁺ imaging of ex vivo brain preparations at levels as low as 5 mM. Indeed, hemolymph sugar measurements have revealed that fructose levels increase to at least ~5 mM after flies feed on various nutritious carbohydrates, suggesting that these neurons are activated after ingestion of a carbohydrate rich meal.¹³ The steep increase of hemolymph fructose can be observed regardless of the type of sugar present in the meal, as long as it is metabolized, whereas non-nutritious carbohydrates (sucralose, xylose, arabinose) fail to increase hemolymph fructose. This observation suggests that a fraction of dietary, nutritious sugar is converted into fructose after ingestion, probably via the polyol pathway,²⁷ and that this conversion is used to signal to the brain that carbohydrates are consumed. We think that this signaling event is integrated with the feeding status

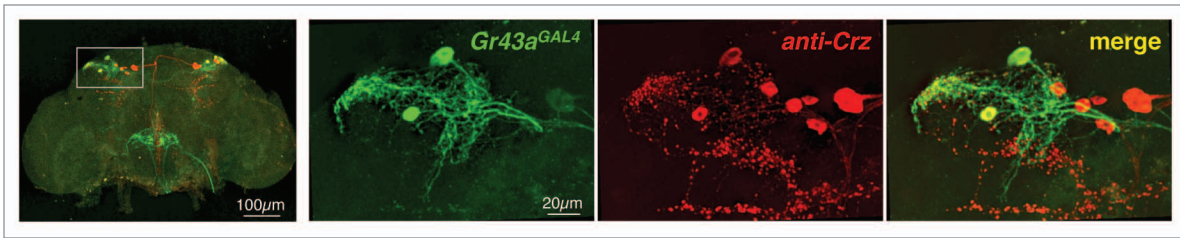


Figure 4. *Gr43a* is expressed in a subpopulation of *Crz* expressing neurons in the brain. Whole brain preparation of *Gr43a^{GAL4};UAS-mCD8GFP* fly stained with anti GFP and anti Crz antibody.⁴⁰ Crz (red) is expressed in all of the *Gr43a^{GAL4}* positive neurons (green); note the presence of 4 to 6 additional Crz expressing cells in each hemisphere that do not express the fructose receptor.

(hungry vs. satiated), thereby establishing positive or negative valence (see below).

Animals can sense the nutritional value of food and regulate their food intake through non-taste mechanisms.²⁸⁻³¹ Feeding experiments of wild type flies and flies in which *Gr43a* expression was restricted to specific cells revealed that the brain neurons function as a nutrient sensor: in hungry flies, the *Gr43a* brain neurons are necessary and sufficient to promote feeding, while in satiated flies, they function to suppress feeding.¹³ Based on these observations, we proposed that ingestion of nutritious carbohydrates rapidly increases circulating fructose, resulting in activation of *Gr43a*-expressing brain neurons. This activation is perceived positively in hungry flies and reinforces feeding, but it is perceived negatively in satiated flies, leading to termination/suppression of feeding. Evoking opposite perceptions by a single group of neurons is unusual, but not unprecedented. In mice, a small set of neurons in the piriform cortex, a higher order olfactory integration center, mediates opposite valence (attraction vs. avoidance), depending on the nature of stimuli during conditioning.³² The mechanisms by which piriform neurons in the mouse or *Gr43a* brain neurons in the fly accomplish such binary valence are unknown. In the fly, it is possible that the *Gr43a* brain neurons communicate with 2, functionally distinct, group of target neurons, a notion that is supported by distinct projection tracts of subsets of these neurons.¹³

Uterus

One role of the insect uterus is to provide a receptacle for sperm and seminal fluid during copulation. In the fly, the seminal fluid not only contains sperm to fertilize the egg, but it serves also as a

source of signals that induce numerous changes in the female's behavior. In addition, the uterus also serves as a storage space for the egg prior deposition, and as a contractible muscle for expunging the mature egg.²⁴

The uterus harbors 3 neuronal clusters, and *Gr43a* is expressed in approximately 4 out of 10 neurons in 1 of them (Fig. 3A). These neurons send dendritic and axonal projections to the uterus lumen and the abdominal ganglion, respectively. To assess and confirm ligand specificity of *Gr43a* expressing uterus neurons, we established an ex vivo preparation and performed Ca²⁺ imaging studies (Fig. 3C-F). These experiments demonstrate that fructose specifically activates these neurons, though the sensitivity and magnitude is lower than that of leg or brain neurons.

Activation of the sex peptide receptor (SPR) in neurons of the uterus is essential for females to undergo various post-mating changes, such as increase in egg production, reduction in mating activity, and a switch from a carbohydrate-rich diet to one containing more protein.^{33,34} SPR was shown to be activated by sex peptide (SP), one of several small proteins present in seminal fluid, which is transferred to the female reproductive tract during mating.³⁴ While SPR is broadly expressed throughout the nervous system, expression in the uterus neurons alone is sufficient to induce changes in post-mating behaviors.^{35,36} We note that fructose is abundantly present in the seminal fluids of mammals and some insects,^{37,38} albeit we currently do not know whether it is found in *Drosophila* seminal fluid.

Interestingly, we found that *Gr43a* and SPR are co-expressed in the uterus neurons (Fig. 3B), suggesting that these neurons may sense several cues

present in male seminal fluid. Interaction of SP with SPR leads to silencing of the neuron,^{35,36} and hence, simultaneous binding of SPR and *Gr43a* to their ligand might have counteracting (inhibitory and excitatory) effects on these cells, leading to modulation of one system by the other. However, it is also possible that hemolymph fructose is sensed by these neurons, which would imply that feeding on carbohydrate can modulate post-mating responses through counteracting SPR mediated silencing. Future studies will be necessary to elucidate the physiological role of *Gr43a* in uterus neurons for post-mating behavior.

Corazonin is the Likely Neurotransmitter in *Gr43a* Brain Neurons

Fructose and its receptor play roles in *Drosophila* nutrient sensing in multiple organ systems. A well-defined function is currently only evident in the brain, where it provides distinct valence to the experience of food intake. To dissect the mechanism of satiation-dependent valence setting, it will be crucial to define the neural circuit that is governed by *Gr43a*. A first and important step toward this goal is to identify the neurotransmitter that is released in response to *Gr43a* neural activation. We noticed a striking similarity of brain expression patterns between *Gr43a^{GAL4}* and Corazonin (Crz).^{39,40} We therefore examined potential co-expression of these 2 genes, and we indeed observed that all *Gr43a^{GAL4}*-positive neurons also express this peptide (Fig. 4). Crz, a short neuropeptide/hormone, and its receptor (crzR) are orthologs of the mammalian gonadotropin releasing hormone and its receptor, respectively. In *Drosophila*, a

role for Crz and crzR has been reported in resistance to alcohol sedation⁴¹ and the regulation of sperm and seminal fluid transfer during mating.⁴² Interestingly, the latter study provided evidence that Crz acts as a neurotransmitter, rather than a hormone. Thus, our next goal is to identify the brain neurons that express the *crzR* gene, which will provide critical information about the downstream targets in this neural circuit. We note that Crz is not expressed in other internal *Gr43a^{GAL4}* expressing neurons (uterus, proventriculus) or in taste neurons (data not shown) and, therefore, another neurotransmitter must be used to mediate *Gr43a* activity in these organ systems.

Outlook

The role of fructose as a nutrient signal is not well understood. We think that the identification of an internal fructose sensor and the observation that fructose is a regulated component of *Drosophila* hemolymph, features likely to be conserved in many other insect species, will stimulate at least 3 avenues of future studies. First, other Gr proteins known to function as taste receptors are expressed in internal organs, including the brain, and are therefore likely employed to sense internal signaling molecules (other sugars, especially glucose and trehalose, as well as amino acids). In this regard, it is noteworthy that numerous members of the *Gr28* gene family are expressed in many neuronal and non-neuronal cell populations⁴³ throughout development and in the adult, and several putative bitter and sugar receptors were found to be expressed in the gastrointestinal tract of larvae.⁸ Thus, identification of their ligands and their specific function in feeding and other behaviors will be of great interest. Second, the *Gr43a* brain neurons represent a highly tractable and relatively simple case of a brain structure that mediates opposite valence, and they therefore provide an ideal case to dissect the mechanism of choice behavior encoded in the insect brain. And third, we think that the emergence of hemolymph fructose in insects warrants efforts to explore the potential role of this sugar as a nutrient ligand in other organisms, including mammals. In humans, the increase of fructose consumption is

strongly associated with a steady increase in obesity, insulin resistance, and other metabolic syndromes.^{44,45} In addition, several studies in humans and mice suggest that fructose and glucose, the most common dietary sugars, are absorbed in distinct regions of the gastrointestinal tract and metabolized differently,^{46,47} and that fructose and glucose affect brain activity and feeding behavior in a disparate manner.^{48,49} Thus, it will be interesting to see whether this sugar—and a specific receptor for it—plays also a role in nutrient sensing in mammals.

Methods

Immunostaining

Immunostaining of whole-mount tissues was performed as described previously.⁵⁰ anti-Crz was kindly provided by Dr Park (University of Tennessee). anti-Crz and anti-ELAV were used at 1:100 dilution.

Calcium imaging

The uterus was dissected in a sugar-free ringer solution (5 mM HEPES pH 7.2, 130 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 2mM MgCl₂) and placed under agarose mounted in a glass bottom dish (MatTek corp.) such that the region containing the *Gr43a* expressing cells were viewable under the microscope. The preparation was covered with 100 ml of ringer, and 100 ml test solutions was administered through a pipette. Images for data analysis were acquired from the cell body for 30 s before and 120 s after application (1 frame/1 s). Adjacent regions were used to determine autofluorescence background. Imaging was performed with a Nikon eclipse Ti inverted microscope with 20 × water objective. The light source was a Lumen 200 lamp (Prior Scientific Inc.). Samples were excited at 488 nm (metal halide lamp), and emitted light was collected through a 515–555 nm filter. Data acquisition was performed with NIS-Elements software (Nikon). Average of 5 frames taken immediately before the application were used to define base fluorescence.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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