$GABA_A$ Receptor-Expressing Neurons in *Drosophila melanogaster* Promote Consumption of Appetitive and Non-Appetitive Substances

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Abstract

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Animals must regulate the amount of food consumed to ensure the appropriate intake of nutrients. This decision making process is highly plastic, heavily influenced by the internal state of the animal, external sensory signals most prominently from taste and olfactory input, and associations with past food-related experiences. Often times, these cues are competing and contribute different weights toward the feeding decision process. The neural mechanisms by which taste detection, internal states influence feeding are under heavy investigation. Here, I used *Drosophila melanogaster* as a model system to study the underlying neural mechanisms by which feeding behavior is regulated.

Previous studies indicate that four GABAergic interneurons (DSOG1) in the *Drosophila melanogaster* brain are necessary to inhibit overconsumptive behavior. This thesis examines the role of GABAergic receptors and GABAergic receptor expressing neurons in consumption behavior. Here we find Resistance to dieldrin (RDL), a $GABA_A$ receptor, is required for proper control of ingestion in Drosophila. Knockdown of *Rdl* in *VT16839-Gal4* neurons causes overconsumption of tastants, indicating that the line contains a set of neurons that regulates consumption in *Drosophila melanogaster*. These neurons are sufficient to drive consumption, as acute activation promotes consumption in flies. Complementary, acute silencing of these neurons causes decreased consumption of appetitive substances in motivated flies. Additionally, we find that there are *VT16839-Gal4* neurons in the higher brain region that are responsive to sucrose and water stimulation. Our study identifies neurons that are necessary and sufficient for *Drosophila* ingestive behavior.

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CHAPTER 1

Introduction

Regulation of Feeding Behavior

The ability to adjust feeding behaviors in different environments and contexts is essential for an animal to survive. Regulatory mechanisms for food intake are crucial for balancing caloric consumption with energy expenditure, which can influence the body weight and health of an organism. Generally, it is imperative that an adult animal's weight stay relatively the same, meaning that energetic intake from food sources is equivalent to energetic output from energy expenditure of the animal. Disruption of such balance leads to unhealthy consequences, as too little weight could indicate a lack of energy reserves, while too much body weight can compromise cardiovascular health and increases the risk of diseases like diabetes[1]. Animals have evolved physiological systems to maintain body weight by regulating feeding behavior and metabolism[2]. Feeding behavior provides fast, immediate control of energy stores.

The act of feeding consists of a decision making process and a motor output. For example, all animals need to make a decision whether to initiate feeding, what to feed on, and when to stop feeding. This decision making process is highly plastic, heavily influenced by the internal state of the animal, external sensory signals most prominently from taste and olfactory input, and associations with past food-related experiences. Often times, these cues are competing and contribute different weights toward the feeding decision process. For example, a hungry animal may disregard taste information indicating potentially contaminated food because of its immediate need for energy. In contrast, an animal that has associated tasty food with a negative experience may avoid eating innately appetizing food sources. All of this information is taken in by the brain to regulate feeding behavior.

Mammalian feeding

Genetic studies done in the mammalian system have identified major genes and molecules that influence feeding[3]. Feeding behavior across many species of animals is modulated based on the immediate nutritional needs of the animal. Neuropeptides and neurotransmitters significantly impact feeding behavior and energy expenditure by integrating hormones and nutrient signals that report internal state information. Hormones and peptides that signal internal state information such as hunger or satiety are released from peripheral tissues based on the animal's nutritional state. For example, the satiety signal leptin is released from adipose tissue[4], while the hunger signal ghrelin is released by cells in the gastrointestinal tract[5]. These signals bind to receptors in the brain to mediate feeding behaviors. The hunger signal ghrelin acts on Neuropeptide Y neurons in the hypothalamus to increase feeding, while satiety signals insulin, leptin and neuromedin U act to suppress feeding[4] [6]. The biogenic amines, dopamine and serotonin, have been implicated in playing a role in feeding regulation, with dopamine playing a large role in the reward from food, and serotonin inhibiting feeding[7] [8].

Early lesion studies implicated the arcuate nuclei in the hypothalamus of the brain (ARC) as a brain center essential for feeding behavior in rodents. More recent studies have further characterized genetically-defined neuronal populations in ARC through optogenetic, behavioral and electrophysiological approaches. The ARC contains counteracting populations of neurons

that regulate mammalian feeding behavior that have been relatively well-studied but still are under active investigation. An orexigenic population of neurons in the ARC (ARC^{AgRP}) express agouti-related peptide (AgRP), neuropeptide Y (NPY) and the neurotransmitter GABA [9]. In starved mice, ARC^{AgRP} neurons promote feeding and energy conservation, and are more active in energy deficient rodents[10] [11]. These neurons are both necessary and sufficient to drive acute consummatory behaviors in starved mice [12]. Additionally, these neurons act on downstream satiety inducing neurons through GABA and NPY signaling[13]. An opposing population of neurons (ARC^{POMC}) in the ARC promotes anorexigenic behaviors and expresses the polypeptide, proopiomelanocortin (POMC). In satiated mice, ARC^{POMC} inhibits feeding behaviors while promoting energy output and loss of body weight[12][14].

Several elements of the mammalian feeding circuit have been identified. Studies that acutely activated projections found that ARC^{AgRP} neurons drive acute consumption behavior through their projections in the anterior bed nucleus of the stria terminalis, lateral hypothalamus, and paraventricular nucleus of hypothalamus (PVH) brain areas[15]. This behavior requires functional NPY1R and $GABA_A$ receptor function in the PVH, suggesting that both signaling components are necessary for ARC^{AgRP} driven consumption[16][17].

Although it is known that ARC^{POMC} sends projections to the same brain regions as ARC^{AgRP} neurons[18], much less is known about which downstream neurons are functionally relevant to induce satiation behaviors. ARC^{POMC} neurons, however, do require melanocortin receptor expression in the brain to promote satiety[12], indicating downstream neurons express those receptors.

While a subset of hypothalamic neurons that influence feeding have been characterized, the remaining elements of the circuits underlying feeding regulation are still under heavy investigation. Although considerable progress has been made on the peptide signals involved in feeding and the hypothalamic circuitry regulating feeding, the complexity of the mammalian brain makes it challenging to determine how external sensory cues and internal states act on neural circuits and motor programs that control feeding behavior.

Drosophila feeding regulation

The fruit fly, *Drosophila melanogaster,* is an excellent model system to examine the neural circuits governing feeding behaviors because of the modern genetic tools and a million-fold fewer neurons compared with humans make it tractable to parse out the underlying basis for feeding regulation[19]. In comparison to the mammalian system, flies share some of the same biogenic amines, such as dopamine and serotonin, and have some homologues of mammalian neuropeptides implicated in the regulation of feeding. For example, similar to the mammalian homologues, the *Drosophila* homologues of neuropeptide Y, neuropeptide F and short neuropeptide F, promote feeding[20][21], while the *Drosophila* homologue of neuromedin U, hugin, also inhibits feeding[22]. The discovery of such peptides and neuromodulators provide crucial information toward understanding the regulation of feeding. However, which neurons and circuits these neuropeptides act on in the *Drosophila* central nervous to produce and regulate feeding is still under investigation.

Fly feeding behavior is a complex behavior consisting of multiple subprograms that allow dynamic regulation of food intake[23]. Feeding begins with the search for potential food sources[24]. Once a candidate food source is found, flies will initiate feeding by extending their proboscis to appetizing and potentially nutritious substances or rejecting bitter, potentially toxic compounds. Flies continue ingestion if appetizing substances are detected in the pharynx and will cease ingestion once the fly is satiated[25]. All of these feeding subprograms are dynamically regulated by multiple factors including the detection of tastants by gustatory sensory neuron, and the nutritional state of the fly which is influenced by neuropeptides and modulatory interneurons.

Influence of the gustatory detection on feeding

The detection of tastants can heavily influence feeding behaviors by allowing animals to detect and reject potential toxic compounds and accept potential nutritious compounds for ingestion. The process of tastant detection begins at distinct primary taste neurons housed in taste sensilla located on the proboscis, mouthpart, legs, and wings[26]. Each taste sensillum contain 2-4 distinct gustatory taste neurons (GRN) detecting sweet, bitter, water, or pheromone. The ability for GRNs to detect tastants is mediated through gustatory receptors (GRs) and pickpocket ion channels (PPKs)[27,28]. Sweet GRNs express several gustatory receptors including GR5A and GR64F, while bitter GRNs express a different group of Grs, including GR66A [29,30]. The remaining classes of GRNs detect contact sensory cues through Ppk channels, with water GRNs expressing Ppk28 and pheromone GRNs expressing PPK23[31,32]. Each class of GRN sends their projections to non-overlapping regions in a brain region called the Subesophageal Zone (SEZ), providing evidence for a labeled line system at the first synapse[30,33]. In support of a labeled line organization, recent whole brain calcium imaging studies have reported that taste detection activates central neurons that are modality-specific, with the exception of a few neurons that respond to both sugar and water[34]. Although the activity map of tastant-responsive neurons in the fly brain is useful, an understanding of the principles underlying taste processing is far from complete. One aim of the field is to identify elements of these taste-responsive pathways and examine them at a closer level, including probing what information these neurons receive and how their activity is dynamically regulated by nutritional state information.

A few studies have identified neurons responsive to taste stimulation including second-order neurons, interneurons, and motor neurons necessary for feeding subprograms. Second-order sPGN neurons that send their projections to the antennal mechanosensory and motor center (AMMC) have been found to be sucrose responsive and trigger sucrose acceptance[35]. In addition, classes of neurons that promote sucrose consumption have been identified. In behavioral studies, feeding neurons (FDG) trigger the feeding motor program and respond to sucrose information from the proboscis in energy replete flies[36]. Another class of interneurons, ingestion neurons (IN1) is active when flies consume sucrose, sensing sucrose from the internal mouthparts and triggering ingestion[37]. Finally, motor neurons have been identified that are essential for fly feeding behavior. Taste-responsive E49 motor neurons are essential and sufficient for feeding initiation[38], while motor neurons MN11 and MN12 are essential for consumption[39].

Regulation of feeding by internal state

The regulation of feeding is not only influenced by incoming sensory stimuli and the palatability of the food source but it is also dependent on the satiety state of the fly: fed flies are much less likely to extend their proboscis to palatable substances compared to food-deprived flies. This behavioral plasticity requires nutritional state information to act on the central nervous system to modulate feeding behaviors. Two major areas of research are under heavy investigation: How is internal state detected and reported? Where in the feeding circuit does internal state information modulate feeding behavior?

Several mechanisms have been proposed to explain how feeding behaviors are modulated based on the internal state of the fly[25]. One model proposed that adaptation to sensory stimuli over time leads to cessation of feeding. However, this model of sensory adaptation was largely disproved by early studies support the idea that there are other mechanisms in play to end feeding behavior other than taste receptor adaptation[40]. More recent studies have found evidence for more centralized regulatory mechanisms that support the notion that there are nutrient sensors that detect internal state.

Evidence of internal sugar sensing came from studies determining that flies have the ability to distinguish nutritive and non-nutritive sugars post ingestion. Taste-independent modulation of feeding was found in flies lacking external taste receptors[41]. Flies are able to detect tasteless sugars[42,43] and distinguish between nutritious and non-nutritive sugar[41], further indicating there are mechanisms to detect internal sugar levels

Several classes of neurons directly sensing internal state have been identified in *Drosophila*. Interestingly, these neurons reside across several brain regions. In the ellipsoid body, 10-13 pairs of R4 neurons require the sodium/solute like co-transporter, SLC5A11 (cupcake), for starved flies to prefer nutritive sugars over non-nutritive sugars[44]. Cupcake modulates potassium channel activity to read out hunger state in these R4 neurons[45]. Neurons in the superior protocerebrum express gustatory receptor GR43A to detect low fructose levels in the blood hemolymph, indicative of starvation state[46], and in turn modulate feeding behaviors accordingly. Neurosecretory neurons in the pars intercerebralis (PI) expressing Diuretic hormone 44(DH44), a homologue of mammalian cortotropin, are critical for detecting and selecting nutritive sugars[47]. Although a few internal sensors have been identified, further investigation is necessary to determine the relationship between these neurons and their actions on feeding circuits.

Additionally, internal state is not only directly sensed by neurons in the central nervous system, but also reported through endocrine signals secreted by tissues in the periphery. These systemic signals then act on neurons in the central nervous system to modulate feeding. Secreted signals act on neurons in the central nervous system to regulate feeding behavior. Unpaired2, a homologue of the mammalian leptin, is secreted by the fat body organ in response to high carbohydrate, fat and amino acid levels[48]. Unpaired2 acts on insulin secreting medial neurosecretory neurons in the PI to modulate feeding behavior[49]. During starvation, the signal Adipokinetic hormone (AKH), a homologue of the mammalian Glucagon, is secreted by the corpus cardiacum to modulate feeding behaviors and metabolism[50,51]. Interestingly, AKH also act on the Interoceptive neurons (ISNs) to promote sucrose consumption[52].

Many studies indicate the importance of other neuromodulatory peptides in the regulation of feeding. Short neuropeptide F and corazonin neurons increase feeding behaviors, as overexpression of sNPF or activation of corazonin neurons increases consumption[21,53]. There is also evidence of neuropeptides that report satiety and act on anorexigenic pathways. For example, Drosofulkinin (DSK) a homologue to the satiety signal, cholecystokinin, modulates feeding. Reducing DSK expression in insulin-producing medial neurosecretory neurons in the PI increases food consumption[54]. AllostatinA neurons decrease feeding behaviors, including feeding initiation and consumption[53]. Myoinhibitory peptide (MIP) and MIP neurons decrease feeding and influence body weight. Silencing MIP neurons or mip mutants resulted in increased feeding and body weight[55,56]. Although several classes of neurons influencing feeding behavior have been identified and characterized, downstream targets remain to be investigated.

Not only do hungry flies accept sweet substances at a lower concentration than fed flies, but hungry flies are more likely to accept food sources that are contaminated with bitter compounds compared to fed flies. Possible models of how taste-processing pathways could be modulated by nutritional state of the fly include: hunger increases sugar sensitivity and that indirectly suppresses the bitter pathway[57]. Other possible models include starvation-dependent interneurons that directly modulate both sugar and bitter pathways to increase acceptance behavior toward sugar and bitter mixes, or a model where the sugar and bitter pathways are modulated independently[58]. There is evidence that the sugar and bitter sensing pathways can be directly modulated by satiety state. Evidence of the latter model was found in several studies. One study found that bitter responses on bitter GRNs are directly modulated through the octopaminergic receptor Oct-Tyr[59]. This starvation-dependent modulation is mediated through octopaminergic interneurons OA-VL1 whose firing rate decreases as starvation time increases. The bitter pathway can be separately modulated by hunger and sugar pathways through different neuropeptides[58].

Direct evidence of a starvation-dependent modulation of the sugar pathway comes from studies of examining changes at the GRN level. In starved flies, dopamine increases synaptic release from the sugar GRN through the dopaminergic receptor, DopEcR[60]. In addition to acting directly on sugar GRNS, dopamine can also act at the interneuron level. A single dopaminergic neuron, TH-VUM, modulates feeding initiation based on the hunger state of the fly[61]. The hunger-state dependent activity of TH-VUM was demonstrated through electrophysiological recordings, finding that starved flies had higher firing rate compared to fed flies. The hunger-dependent promotion of feeding by TH-VUM was found to act through the downstream dopaminergic receptor, dopamine-2 like receptor (D2R). Further studies will be necessary to investigate which D2R neurons influence feeding initiation to determine how TH-VUM influences feeding initiation.

In addition to the influence of feeding behavior by hunger state, thirst state can also influence feeding behavior. Interoceptive neurons (ISNs) located in the SEZ detect osmolarity in the blood hemolymph and antagonistically regulate thirst and hunger[52]. Higher activity in ISNs promotes sucrose consumption at the expense of water consumption while lower activity in the ISNs promotes water consumption and inhibits sucrose consumption.

Studies indicating starvation or satiety dependent modulation of feeding have provided evidence for several mechanisms for both detection of internal states and starvation-dependent modulation of feeding circuits. Studies have implicated direct nutrient-sensing neurons as detecting carbohydrate levels in the blood hemolymph. In addition, there have been numerous studies showing evidence of systemic signals reporting nutritional state of the fly acting on neurons in the central nervous system to regulate feeding behavior. Future studies investigating the interaction and dependence of these pathways will be critical to our understanding of the neural circuitry of feeding.

GABAergic inhibition of Consumption

One major aspect of feeding is the regulation of consumption. As with other aspects of feeding behavior, consumption requires inhibitory feedback signals to stop food intake behaviors. Overeating can lead to undesirable health defects such as cardiovascular dysfunction and diabetes, while non-selective consumption can result in the consumption of noxious/deleterious/toxic substances. Animals have mechanisms to regulate consumption and prevent these undesired consequences. Circulating hormones and neurons signaling satiety are active once an animal has consumed enough food to account for its energy needs to stop feeding. Potentially toxic substances can be identified through taste circuits to prevent ingestion. Additionally, feeding may be inhibited where other critical behaviors take precedence over feeding.

There are multiples classes of neurons that inhibit various aspects of feeding behavior in flies. For example, early studies found that lesioning the recurrent nerve in blow flies, *Phormia regina* (Meigen), resulted in overconsumption of sugars until flies were bloated[62]. These studies suggested that inhibitory signals regulating the volume of consumption originate from the recurrent nerve. More recent studies have identified and characterized a class of four GABAergic neurons in *Drosophila melanogaster* named descending subesophageal ganglion (DSOG1), whose function is critical for the proper regulation of consumption[63]. Suppressing the activity of DSOG1 neurons results in massive overconsumption of both appetitive and non-appetitive substances, indicating that their function is essential to regulate the quantity of consumption and the intake of appropriate food sources. Flies with non-functional DSOG1 neurons overconsume independent of taste quality (they eat non-appetitive bitter substances in addition to appetitive substances) and independent of satiety state (they eat when fed or starved). DSOG1 neurons most likely act as a stop signal of general consumptive behavior because they have constant basal activity and were not found to be modulated by nutritional state or taste information. Finally, calcium imaging studies identified that DSOG1 neurons act upstream of motor neurons that are essential for feeding initiation and ingestion. The role that DSOG1 neurons play in regulating consumption is unique as prior studies in blow flies report hyperphagic behavior of appetitive food sources when the recurrent nerve is severed, while in mammals hyperphagic behavior has been reported when hypothalamic neurons are manipulated through the use of optogenetics[13,15,16]. Manipulating DSOG1 neurons is unusual in that their activity prevents an indiscriminate, hyperphagic phenotype.

How do these neurons act as a stop signal for consumption? What does DSOG1 act on? Are neurons that drive this consumption directly downstream of DSOG1? Although some

interneurons triggering consumption of appetitive substances have been identified and characterized[36,37], a class of interneurons that are critical and sufficient to trigger consumption of both appetitive and non-appetitive substances has not been identified. In this thesis, I will describe a subset of neurons whose role is essential and sufficient for consumption of appetitive and non-appetitive substances.

CHAPTER 2

$GABA_A$ receptor-Expressing Neurons in *Drosophila melanogaster* Promote Consumption of Appetitive and Non-Appetitive Substances

Summary

In this study, we investigated neurons crucial for triggering consumption. We used molecular genetic, anatomical, and behavioral studies to identify GABAergic-receptor-expressing neurons that are necessary and sufficient to promote ingestion of both appetitive and non-appetitive substances. Knock down of *Resistance to dieldrin* (*Rdl*) in these neurons caused an indiscriminate overconsumption phenotype. Activation of these neurons caused an increase in consumption of appetitive and non-appetitive substances, while silencing these neurons caused a decrease in consumption of these substances. Taken together, these studies identified RDL neurons that play a major role in the circuit for consumption.

Introduction

The ability to adjust feeding behaviors in different environments and contexts is essential for an animal to survive. Regulatory mechanisms for food intake are crucial for survival by balancing caloric consumption with energy expenditure, which may influence the health, fitness, and body weight of an organism[1]. Feeding regulation entails making the decision whether or not to initiate feeding, what to feed on, and when to stop feeding[23]. This regulation requires the integration of internal state information, external sensory signals, and associations with past food-related experiences.

The fruit fly, *Drosophila melanogaster,* is an excellent model system to examine the neural circuits underlying the regulation of feeding behaviors because powerful genetic tools available allow the manipulation of specific neurons, and because the fly brain contains a million fold fewer neurons compared to the human brain (100,000 vs 100,000,000,000)[19]. These features make the fly brain a tractable system for uncovering the neural circuitry behind feeding regulation.

The fly has stereotypical feeding behavior involving a series of feeding subprograms. Feeding behavior initially involves detection of a potential food source with the legs or proboscis, which contain taste receptors neurons that allow the fly to make an evaluation before attempting consumption[26]. Detection of sweet food sources will drive feeding initiation, while detection of bitter compounds will promote rejection. Once a palatable food source is detected, a fly initiates feeding by using the proboscis extension response (PER), an acceptance behavior indicated by an extension of their proboscis. As the fly begins to consume, the fly then detects those appetitive substances with taste neurons in their pharynx or internal mouthparts[26,64]. The fly continues to ingest until internal cues signal that the fly is satiated.

A few taste-responsive neural components of these feeding subprograms have been uncovered. Distinct primary sensory gustatory neurons can detect either water, sugar, bitter or pheromones[30–32,65]. Gustatory neurons on the legs, proboscis labellum and mouthparts send projections to non-overlapping regions in the subesophageal ganglion (SEZ), the major taste-processing center in the brain[30,33]. Many higher order components of these feeding circuits reside in the SEZ. Two sucrose-responsive classes of interneurons that play a role in sucrose consumption have been identified: feeding neurons (FDG) respond to sucrose detection on the proboscis to promote initial consumption of sucrose[36], while ingestion neurons (IN1) neurons, respond to sucrose detection in the pharynx to promote sustained sucrose consumption in hungry flies[37]. In addition, sPGN neurons send their projections to the antennal mechanosensory and motor center (AMMC) have been found to be sucrose responsive second-order neurons that trigger sucrose acceptance[35]. Finally, the motor neurons that are necessary to for feeding initiation[38] and the motor neurons that control the pumping mechanism for consumption have been identified[39].

Several additional classes of modulatory interneurons have been identified that play a role in regulating feeding initiation and consumption but are not directly taste-responsive[52,61,66]. A set of four interneurons, named DSOG1, play an essential role in regulating consumption through GABAergic signaling[63]. These neurons send wide arborizations throughout the SEZ and their activity suppresses feeding initiation and consumption. Although DSOG1 neurons do not receive taste information or the nutritional state information of the fly, they have chronic activity that suppress indiscriminate consumption of both appetitive and non-appetitive substances. While the DSOG neurons play a critical role in suppressing consumption, the

identification of neurons that directly trigger consumption remains under investigation and remains critical for our understanding of how feeding is dynamically regulated.

Results

RDL Regulates Feeding Behavior.

Four GABAergic interneurons (named DSOG1) were previously identified to inhibit multiple aspects of feeding behavior in *Drosophila*[23], including proboscis extension and consumption. While these neurons play a key role in inhibiting consumption, the neural circuits that trigger non-selective consumption have not been identified. Therefore, we sought to identify neurons that are sufficient to induce consumptive behavior by characterizing the neurons downstream of the previously characterized DSOG1 neurons, known to suppress consumptive behaviors of all substances[63]. Since DSOG1 neurons require GABAergic signaling to inhibit consumption, we reasoned that DSOG1 neurons may inhibit GABA-receptor expressing neurons that are sufficient to drive ingestive behaviors.

There are five GABAergic receptors that exist in *Drosophila*: a heteromultimeric cationic channel consisting of a GABA and glycine-like receptor of *Drosophila (*GRD) and ligand-gated chloride channel homolog 3 (LCCH3)[67], a $GABA_A$ type receptor: Resistance to Dieldron (RDL)[68], and 3 types of $GABA_b$ receptors ($GABA_b$ R1, $GABA_b$ R2, $GABA_b$ R3) [69]; putative downstream neurons of DSOG1 may express one or more of these receptors. To determine which GABAergic receptor(s) regulate consumption time, we knocked down expression of the existing GABAergic receptors using RNA interference and measured its effects on the duration of water and bitter substance consumption. We utilized the GAL4/UAS system[70], to express RNA to interfere with expression levels of GABAergic receptors. These RNAi lines express 300-400 bp gene fragments in inverted repeats under the control of UAS to silence expression of genes[71]. Using the *nSyb-Gal4* line to drive the expression of RNA interference, we knocked down the expression of each of the GABAergic receptors pan-neuronally and used the temporal consumption assay to measure the duration of time that individual, satiated flies spent consuming water or the bitter substance, denatonium (Figure 1A).

Hydrated, fed, wildtype flies will not drink water or denatonium, whereas flies with inactivated DSOG1 neurons consume vast amounts of water or denatonium. As expected, control flies did not consume water or denatonium, when they were hydrated and fed. In contrast, flies with *Rdl* knocked down significantly overconsumed both water and denatonium (Figure 1B), 101.6 s more denatonium and 27.7 seconds more of water compared to the control flies, similar to the DSOG1 phenotype. Overconsumption of denatonium indicates that the drive to consume in these flies seems to override normal bitter taste inhibition of feeding. In contrast, knockdown of other GABAergic receptors resulted in little or no consumption of water or denatonium. This suggests that the RDL receptor is the only GABAergic receptor required for wildtype consumptive behavior of water and bitter substances.

Although RDL plays an important role in regulating consumption, it is broadly expressed in the nervous system and is not specific for neural circuits that mediate consumption. To screen for candidate consumptive neurons, we knocked down *Rdl* in only small subsets of neurons within the central nervous system by using existing Gal4 lines from the Clandinin, Flylight and Vienna collections. These Gal4 drivers express Gal4 in subsets of neurons in the central nervous system by either having an enhancer segment driving Gal4 expression or an enhancer trap expression system where Gal4 is inserted into the fly genome[72–74]. Out of 183 Gal4 lines, we only identified two Gal4 lines, *VT16839-Gal4* and *VT27941-Gal4* that showed robust and

reproducible overconsumption of both denatonium and water compared to all other lines in this screen (Figures 1C-D, Figure S1). The *VT27941-Gal4* enhancer contains an intronic region of the *Rdl* gene, providing further evidence of *Rdl*'s role in consumption.

To determine which neurons are contained in the Gal4 lines, we crossed them to *UAS-mCD8::GFP* reporter flies and examined GFP expression in the brain and ventral nerve cord. Expression of Gal4-driven mCD8::GFP in *VT27941-Gal4* was broad throughout the central nervous system, therefore we mainly focused on the Gal4 line with the sparsest labeling, *VT16839-Gal4. VT16839 Gal4* labels ~50 neurons in the brain and ~50 neurons in the ventral nerve cord (Figure 2A).

16839 Neurons Regulate Consumption.

The phenotype of DSOG1-mediated overconsumption is independent of taste quality or nutritional state. To test whether knockdown of *Rdl* in VT16839 neurons also causes overconsumption of nutritive and non-nutritive substances, we used RNA interference and assayed consumption using TCA. Knocking down *Rdl* in VT16839-Gal4 neurons resulted in flies overconsuming denatonium, water, and sucrose compared to control flies (Figure 2B). These results suggested levels of *Rdl* are critical for limiting ingestion of non-appetitive bitter substances and the appetitive tastants sucrose and water. Since RDL in VT16839 neurons is essential to reduce consumption of nutritive and non-nutritive taste compounds, we wondered if it was also necessary to prevent consumption of compounds not detected by the gustatory system. We tested if the tasteless compound, 20% PEG was sufficient to drive overconsumption. 20% PEG is tasteless because it occurs at an osmolarity that is not detected water-sensing neurons[31], and also lacks both bitter and sweet substances. Flies with *Rdl* knocked down in 16839 neurons overconsumed 20% PEG (Figure 2B), suggesting that taste input from sugar, bitter, or water taste neurons are not required for the overconsumption phenotype. Interestingly, overconsumption of 20% PEG was comparable to overconsumption of water, denatonium and sucrose (Figure 2B). These results demonstrate that flies overconsume independent of taste quality when *Rdl* is knocked down in *VT16839-Gal4* neurons.

16839 Neurons Are Necessary and Sufficient for Consumptive Behavior.

While VT16839 neurons regulate feeding behavior, VT16839 neurons may simply be modulatory neurons with RDL playing a role outside the taste circuit. Another possibility is that 16839 neurons may be a causal neuron for consumption behavior. We sought to test the latter possibility by acutely activating these neurons. Acute, ectopic activation allows us to test the sufficiency of these neuron(s) to trigger consumption on a short timescale. We activated these neurons using *UAS-ReaChR*, the optically-gated non-specific cation channel[75]. We tested if these neurons could trigger consumption of tastants by shining 635nm light on individual flies for 1-2 min prior and during the temporal consumption assay. Activation with 635 nm light, resulted in overconsumption of not only the appetitive substances, sucrose and water, but also denatonium (Figure 2C) in *VT16839-Gal4; UAS-ReaChR* flies. In contrast, the control flies that did not express *UAS-ReaChR* and *VT16839-Gal4*, did not consume much of any tastant.

If VT16839 neurons are causal neurons and not modulatory, these neurons should also be essential for normal consumption. We tested whether these neurons are necessary for wild type

consumption by acutely silencing these neurons under conditions where the fly had a strong drive to consume. Since starved flies will consume sucrose, if VT16839 neurons are necessary for consumption, we should expect to see a reduction in consumption when 16839 neurons are silenced. We acutely silenced VT16839 neurons using the allele encoding the temperature sensitive dynamin, *shibire*^{ts}[76] in starved flies and tested their consumption times. At the restrictive temperature of 33°C, vesicles should no longer be recycled, therefore depleting neurons of neurotransmitter-filled vesicles and preventing synaptic transmission. At the restrictive temperature, there was a significant reduction in consumption of 500 mM sucrose compared to room temperature controls (Figure 2D). Taken together, these results suggest that VT16839 neurons are necessary and sufficient for consumptive behavior.

16839 Neurons are Sufficient to Suppress DSOG1 Mediated Overconsumption.

DSOG1 neurons are GABAergic interneurons that inhibit consumption. VT16839 neurons are GABA-receptor expressing neurons that trigger consumption and are necessary for normal consumption. One hypothesis is that GABA release from DSOG1 binds to RDL on VT16839 neurons to inhibit their activity and decrease consumption. To test whether 16839 neurons are upstream of DSOG1 we performed an epistasis experiment by co-silencing the two populations of neurons. As expected, silencing DSOG1 neurons with *shibire*^{ts} results in overconsumption of both appetitive and aversive substances ([63], Figure 3a). Since VT16839 neurons might be downstream of DSOG1, we tested whether blocking synaptic transmission in VT16839 neurons would suppress the overconsumption phenotype. We co-silenced DSOG1 neurons and 16839 using *UAS-shibire*^{ts} and measured consumption through TCA. Flies with DSOG1 and 16839 neurons co-silenced resulted in significantly less sucrose consumed compared to DSOG1 silenced alone (Figure 3A). In addition, co-silencing the both populations of neurons also resulted in suppression of the DSOG1-mediated consumption of denatonium. These results demonstrate that 16839 can override overconsumption caused by inhibition of DSOG1 neuron. The data are consistent with the notion that VT16839 neurons may act downstream of DSOG1 neurons (Figure 3B), although they do not rule out other models such as parallel pathways.

A Subpopulation of 16839 Neurons Regulate and Trigger Consumption.

We sought to narrow down the causal neurons in *VT16839-Gal4* that trigger consumption by using intersectional approaches to limit expression of VT16839 neurons. Because knocking down *Rdl* in either *VT16839-Gal4* or *VT27941-Gal4* resulted in overconsumption of water and denatonium, we decided to only look for neurons present in both Gal4 lines. We made split-Gal4 driver lines where a complementary half of Gal4 is expressed in each of the driver lines. Only neurons that have both halves of the Gal4 will have a functional Gal4 to drive expression of effector proteins. Therefore, this approach limits functional Gal4 expression to the neurons common to both driver lines[77]. As a result, functional Gal4 expression of mCD8::GFP was limited to ~10 neurons in the brain and ~16 in the VNC (Figure 4A). Distinct populations of neurons were apparent in the split-Gal4 driver line. In the brain, there was a class of neurons strongly labeled in higher brain whose projections span near the pars intercerebralis along with several dimly labeled neurons in the subesophageal ganglion. Another two populations were labeled in the abdominal segment of the VNC.

We tested whether the neurons that trigger consumption are labeled by the split-Gal4 driver lines by knocking down expression levels of *Rdl* using RNAi. Knockdown of *Rdl* in the split-Gal4 resulted in overconsumption of both appetitive and aversive stimuli, indicating the causal neurons are labeled by the split-Gal4 (Figure 4B).

We tested whether the neurons labeled by the split-Gal4 can trigger consumption through acute activation experiments. We activated the neurons by using the optically-gated cation channel, csChrimson[78] and shining red light on each fly as we measured consumption time. Activation of the split-Gal4 line csChrimson resulted in the majority of flies constitutively pumping (45 of 51 flies) in the presence of red light without any stimulant. These flies may be exhibiting constitutive pumping behavior because csChrimson is strongly activating the neurons to the point that they no longer need any stimulus to attempt to consume.

In these csChrimson experiments, consumption was masked by constitutive pumping, making it difficult to reliably measure consumption time in the temporal consumption assay. As a result, we decided to quantify consumption by measuring volume ingested instead of time spent drinking. We estimated volumes of ingestion by using capillary tubes and measuring the volumes before and after individual flies drink. When VT16839 split neurons were activated using csChrimson, flies consumed more appetitive and aversive tastants in our volumetric consumption assay (Figure 4C) compared to control flies. This indicated that VT16839 split-Gal4 neurons can trigger consumption of both appetitive and non-appetitive substances.

VT16839 Neuron(s) are Sucrose and Water Responsive

It is unlikely that all *VT16839-Gal4* neurons trigger consumption. In order to narrow down candidates that might regulate and elicit consumption, we sought to identify potential neurons causal for consumption through functional studies. Using calcium imaging, we decided to look for neurons that respond strongly to stimuli that trigger consumption strongly. We tested *VT16839-Gal4* neurons for calcium responses to sucrose in starved flies using nls-GCaMP6s. One population of neurons in the higher brain region consistently responded to sucrose stimulation on the legs (Figure 5A). Out of 6 flies, 5 flies had 1-3 neurons responsive to leg sucrose stimulation. Interestingly, 4 of 9 sugar-responsive neurons were also responsive to leg water stimulation (Figure 5B).

Conclusion

The dissection of neural circuits that underlie consumption remains an important challenge toward understanding the regulation of feeding behavior. Our study identifies neurons that regulate the consumption of non-appetitive and appetitive substances, and depend on the expression of RDL receptor for proper regulation of consumption. These RDL receptor-expressing neurons are able to orchestrate consumptive behaviors regardless of taste inputs, as knockdown of *Rdl* expression within these neurons not only causes overconsumption of sugar, bitter, and water substances, but tasteless substances as well. Taste input is not necessary for consumption through this pathway, suggesting that mechanosensation may be a driver in consumptive behavior.

Our data demonstrate that a subset of RDL-expressing neurons not only regulate consumption, but are also necessary and sufficient for consumption behavior. Acute activation of these neurons caused overconsumption of sweet, bitter and water substances, indicating that activity in these neurons can promote consumptive behavior. The overconsumption of bitter substances in fed flies indicates that optogenetic activation overrides any regulation of feeding by taste quality. In contrast, blocking neurotransmission of these neurons results in decreased sucrose consumption in starved flies, demonstrating its critical role in sucrose consumption. These RDL-expressing neurons may also play a critical role in other types of consumption as well, since blocking activity in these neurons suppresses the non-selective consumption mediated by silencing DSOG1 neurons. The suppression of consumption in this epistasis experiment is consistent with the notion that these neurons act downstream of DSOG1 neurons but does not exclude the possibility that these neurons act in a parallel pathway.

Our data suggest that these neurons may signal consumption of generally appetitive substances, as there are 1-3 neurons (~ 20%) within the higher brain region of *VT16839-Gal4* that normally physiologically respond to both sucrose and water stimulation, positive substances in starved flies. When a subset of *VT16839-Gal4* neurons is activated optogenetically, activity of these neurons is being manipulated under non-physiological circumstances, therefore forcing the fly to consume non-selectively. The same is true when levels of *Rdl* are knocked down using RNA interference; activity within RDL neurons is increased due to less inhibition of the neurons, creating a situation where the fly is more likely to consume. It may be possible that the neurons within *VT16839-Gal4* that are sucrose and water responsive may be entirely separate population from the RDL-expressing neurons, future studies may address this possibility. In the case that these neurons are the same population, future studies could address the possibility that these neurons could gain bitter responsiveness with Rdl levels knocked down. Additionally, it would be interesting to test if water responses would increase in thirsty flies.

Previous studies in *Drosophila* have identified classes of neurons that are responsive to sucrose and play a role in specifically regulating its consumption[36,37]. Studies using whole brain calcium imaging have revealed an overall labeled-line model where most taste-responsive neurons respond to only one taste modality and few neurons respond to both water and sucrose, possibility representing a positive valence signal[34]. Our data identifies a Gal4 line that labels neurons whose activation is sufficient to trigger consumption of both appetitive and aversive substances. Moreover, we find that there are individual neurons within this Gal4 line that

respond to both sugar and water. Since RDL-expressing neurons can trigger consumption, these data suggest that the activity in these neurons may trigger the fly to consume by detecting positive valence cues. In one model, there may be distinct circuits for sweet, water, and bitter food sources that all converge on these neurons, or neurons upstream of them. The summation of these taste inputs, represented by activity in these *VT16839-Gal4* neurons, would determine whether or not to consume. Future studies will be necessary to address how the sucrose and water responsive *VT16839-Gal4* intersect with other identified components of taste circuits, such as DSOG1.

Together, our study demonstrates that *Rdl* function in a subset of neurons is critical for the regulation of consumption of all substances, regardless of taste modality. In addition, the existence of neurons that respond to generally appetitive substances rather than selectivity to one taste modality suggests that there may exist neurons that represent generally appetitive food sources, signaling the fly to consume. Further studies characterizing these neurons and where these neurons lie within feeding circuits would add value to our understanding of the neural basis underlying feeding decisions.

Materials and Methods

Experimental Animals

The Gal4 lines used for the behavioral screens were from the following collections: the Janelia Flylight collection[72], Vienna VT collection[74] and the Clandinin Gal4 collection[73]. GABAergic receptor RNAi lines were from the TRiP and VDRC collection[71]. Additional fly lines used include: *nSyb-Gal4*[79], *UAS-ReachR* (II)[75], *UAS-csChrimson* (X)[78], *UAS-Shibire^{ts}*[76]; *UAS-nls-GCaMP6s*[80].

The following RNAi lines were used for the GABAergic receptor screen:

Name in Figure1B	Stock #
UAS-empty attp2	Bloomington stock # 36303
UAS-empty attp40	Bloomington stock # 36304
UAS-GABA-B-R1	Bloomington stock # 28353
UAS-GABA-B-R2	Bloomington stock # 27699
UAS-GABA-B-R3	Bloomington stock # 42752
UAS-GABA-B-R3(2)	Bloomington stock # 26729
UAS-Rdl(1)	Bloomington stock # 31286
UAS-Rdl(2)	Bloomington stock # 31662
UAS-Rdl-RNAi (3)	Vienna# 41103
UAS-Rdl-RNAi (4)	Vienna 100429
UAS-LCCH3	Bloomington stock # 32019
UAS-GRD-shRNA	Bloomington stock # 38384
UAS-GRD	Bloomington stock # 29589

For VT16839 Split-Gal4: *VT16839-Gal4* and *VT27941-Gal4* enhancer segments were amplified using IsoD1 flies. *VT16839-Gal4* and *VT27941-Gal4* enhancer segments were cloned into pBPZpGAL4DBDUw (Addgene Plasmid #26233) plasmid and pBPp65ADZpUw (Addgene Plasmid #26234) plasmid, respectively. Cloned plasmids were sent to BestGene Inc. and injected into attp2 (*VT16839-Gal4-DBD*) and vk00027 sites (*VT27941-Gal4-AD*).

Behavioral experiments

Female flies were tested for behavior 5-10 days after eclosion. All flies with the exception for *shibire^{ts}* flies (20°C) were grown at 25°C and flipped onto fresh food 2 days prior to the experiment. All starved experimental flies were flipped into vials with wet kimwipes for specific starvation durations prior to the experiment. Flies were glued on slides with nail polish and then were placed in a humidified chamber for 2-4 hours. Temporal consumption assays were performed as previously described[63]. Time spent consuming was recorded until flies rejected

the substance ten times, consecutively. For RNAi experiments, *UAS-Dicer2* (III) was used to boost efficiency of the knockdown. For activation experiments using ReaChR and csChrimson, experimental flies were kept on 0.4 mM all-trans retinal (Sigma), 2-4 days prior to the experiment. Activation experiments were done under 635 nm light (LaserGlow) and assayed one fly at a time. Flies were habituated under the laser for 1-2 minutes prior to assay. For *shibire*^{ts} experiments, mounted flies were incubated at 30°C -32°C on a peltier device and given water for 10-15 minutes prior to experiment. For volumetric consumption assays, Drummond Wiretrol 3 µl capillaries (CAT #5-000-1003) were filled with 5 µl of tastant mixed with blue dye (0.25mg/mL blue dye (Erioglaucine, Sigma)) and attached to tubing connected to a 1 ml syringe. One edge of the capillary was painted with vasoline to prevent spilling of tastant. Before and after pictures were taken in the presence of a standard ruler. The difference in pixels before versus after behavioral testing was measured using Adobe Photoshop software. Volume was calculated by first converting the change in pixels into the change in mm.

Immunohistochemistry

Antibody fixation and staining was performed as previously described[63].The following antibodies were used: rabbit anti-GFP (Invitrogen, 1:1000 dilution); mouse anti-nc82 (Hybriodoma bank 1:500 dilution). The following secondary antibodies (Invitrogen, 1:100 dilution) were used: 488 anti-rabbit, 568 anti-mouse. All images were taken using a Zeiss confocal microscope. Brightness and contrast was adjusted for images using ImageJ.

Calcium Imaging and analysis

***16839-Gal4* imaging:**

The imaging preparation was carried out as previously described[34]. Flies were anesthetized with CO_2 and mounted into a chamber using nail polish and melted wax. The proboscis was waxed out for proboscis stimulations. Brains were dissected in cold artificial hemolymph[30]. Antenna, cuticle, and air sacs were removed and the esophagus was cut using fine forceps. For leg stimulation, the proboscis was removed and legs were stimulated using an electrode with an agar block and 10 µl tastant pipetted onto the 2% agar block attached to an electrode holder. Stimulations were visually guided with a pen camera aimed at the fly and stimulation times were noted. Flies were stimulated with water, 1 M sucrose and/or a bitter mix of 100 mM caffeine and 10 mM denatonium to the legs, 2-3 times per imaging session.

The imaging was carried out as previously described[34]. Calcium transients were recorded using a fixed-stage 3i spinning disk confocal microscope with a piezo drive and a 20x water objective at 1.6 zoom. 10-23 z sections at ~ 0.5 Hz, were taken using the piezo drive in a given imaging session. Calcium signal was imaged using a 488 nm laser.

Images were aligned using scripts in ImageJ. After realignment, a flat stack of the max projection of 5-10 z planes was made and used for analysis. Hand drawn ROIs were chosen based on baseline images of anything resembling nuclei (nls-GCaMP6). A background ROI was chosen for subtraction to correct for changes in fluorescence due to movement.

Figure legends

Figure 1. *Rdl* Modulates Consumption Time

(A) Diagram illustrating the temporal consumption assay. The time individual flies consumed various substances was recorded.
(B) GABAergic receptors were tested for their role in water and bitter consumption using RNAi to knock down expression pan-neuronally with *nSyb-Gal4; UAS-Dcr2* flies. Box plots show median consumption time for water consumption and 1 mM denatonium. Knockdown of *Rdl* resulted in flies overconsuming water and bitter. Kruskal wallis test, Dunn's post-hoc with Bonferroni correction; ***p<0.0001; n=10-33/genotype.
(C) Screen for water overconsumption candidates (mean ± SEM); Gal4 lines were tested for their role in water consumption through RNAi of RDL. Bars show consumption times (mean ± SEM) for water. n=10-22/genotype.
(D) Screen for bitter overconsumption candidates; Gal4 lines were tested for their role in bitter consumption through RNAi of *Rdl*. Bars show consumption times (mean ± SEM) for 1 mM denatonium. The majority of Gal4 lines did not show consumption of denatonium. n=10-22/genotype.

Figure 2. *VT16839-Gal4* Neurons Trigger and Are Critical in Consumption Behavior

(A) *VT16839-Gal4* drives expression of *UAS-mCD8::GFP* in the brain and VNC (scale bar = 100 μm)
(B) RNA interference knockdown of *Rdl* in 16839 neurons increased consumption time of water, 1 mM denatonium, 500 mM sucrose, and 20% PEG. Box plot; student's t-test; ***p<0.0001; n=22-72/genotype
(C) Activation of *VT16839-Gal4* neurons using ReaChR caused increased consumption time of appetitive (500 mM sucrose or water) and non-appetitive (1 mM denatonium) substances. Box plot shows *VT16839-Gal4; UAS-ReaChR* flies consume more appetitive and non-appetitive substances; Kruskal wallis test, Dunn's post-hoc; ***p<0.0001; n=46-49/genotype
(D) Silencing of *VT16839-Gal4* neurons in 24 hour starved flies using *shibire^{ts} (shi^{ts})* caused decreased consumption of sucrose (500 mM) in starved flies. Box plot shows 24 hour starved *W1118(wildtype); UAS-shi^{ts}* spent more time consuming 500 mM sucrose compared to starved *16839-Gal4* /*UAS-shi^{ts}* flies; Kruskal wallis test, Dunn's post-hoc; ***p<0.0001; NS=not significant; n=50-55/genotype

Figure 3. *VT16839-Gal4* Overrides DSOG1-mediated Overconsumption

(A) Epistasis experiment. Co-silencing DSOG1 neurons and *VT16839-Gal4* neurons decreased consumption time. Box plot shows *98-Gal4; UAS-shi^{ts}/16839-Gal4* spent less time consuming 1 mM denatonium and 500 mM sucrose compared to *98-Gal4; UAS-shi^{ts}*, DSOG1 neurons silenced alone. student's t-test; ***p<0.0001; n=19-47/genotype
(B) Model. Silencing *VT16839-Gal4* overrides the overconsumption phenotype caused from silencing DSOG1 neurons.

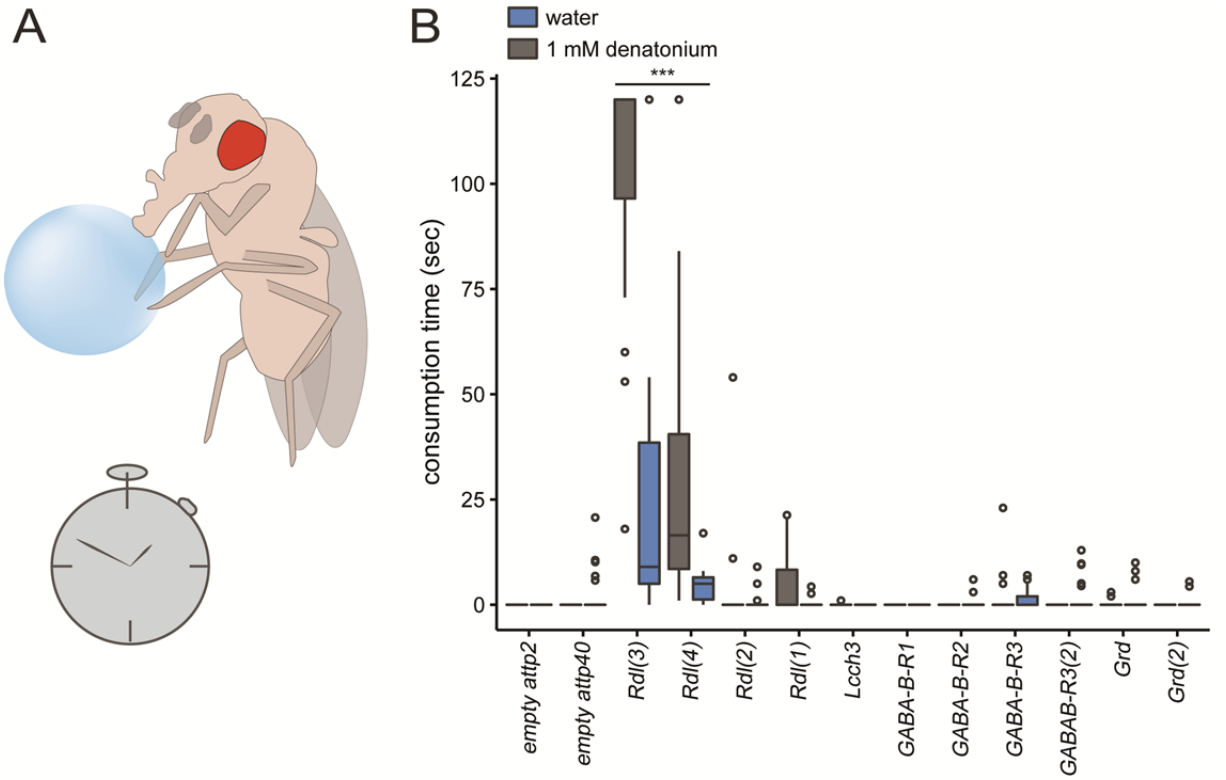
Figure 4. A Subset of *VT16839-Gal4* Neurons Trigger Consumption

(A) *VT16839-Gal4 -DBD, VT27941-Gal4-AD* flies drives expression of mCD8::GFP in the brain and VNC (scale bar =100 μm)
(B) Knockdown of *Rdl* in 16839-split neurons resulted in increased consumption. Kruskal wallis test, Dunn's post-hoc; ***p<0.0001; n=64-70/genotype
(C) Activation of *VT16839-Gal4* neurons using csChrimson increased consumption volume of appetitive and non-appetitive substances. Kruskal wallis test, Dunn's post-hoc; ***p<0.0001; n=21-63/genotype

Figure 5. Sucrose and Water Responsive Neurons in the Higher Brain Region of *VT16839-Gal4* Neurons

(A) Left, schematic of the brain region imaged for sucrose and water responses. Right, example deltaF/F images of the higher brain region shows sucrose responsive *VT16839-Gal4* neurons (scale bar =50 μm). Fly 1 had water and sucrose responding neurons, while fly 2 had sucrose responsive neurons.
(B) Example deltaF/F traces (2 flies of 6 total) of sucrose and water responses showing for higher brain ROIs in two different flies. Fly 1 had one water and sucrose responding neurons (out of eight total), while fly 2 had one sucrose responsive neuron and one sucrose and water responding neuron(out of seven total). Each plot shows all higher brain ROIs for an individual fly with a color representing a specific ROI. Dotted line represents the time when the fly was stimulated.

Figure 1.


A
B
water
1 mM denatonium
***
consumption time (sec)
125
100
75
50
25
0
empty attp2
empty attp40
Rdl(3)
Rdl(4)
Rdl(2)
Rdl(1)
Lcch3
GABA-B-R1
GABA-B-R2
GABA-B-R3
GABAB-R3(2)
Grd
Grd(2)

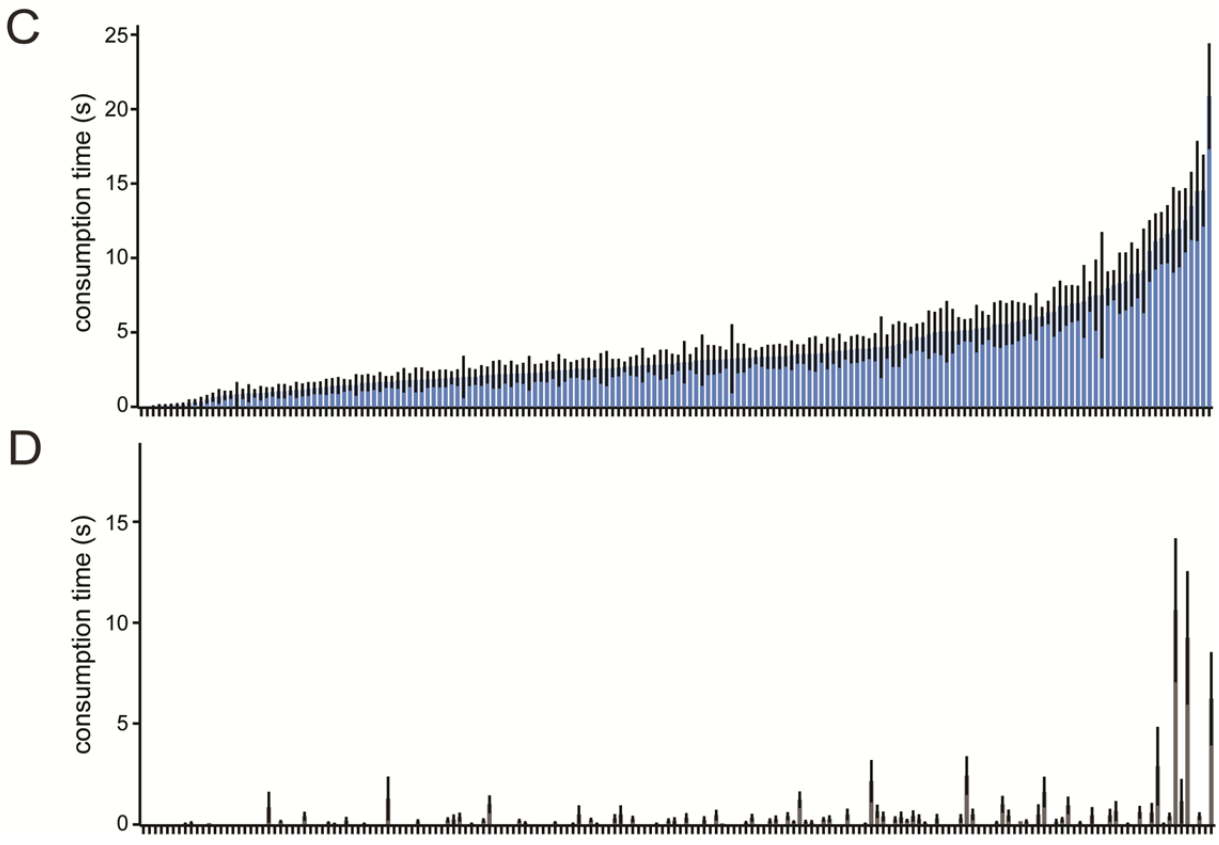

C
consumption time (s)
25
20
15
10
5
0
D
consumption time (s)
15
10
5
0

Figure 2.

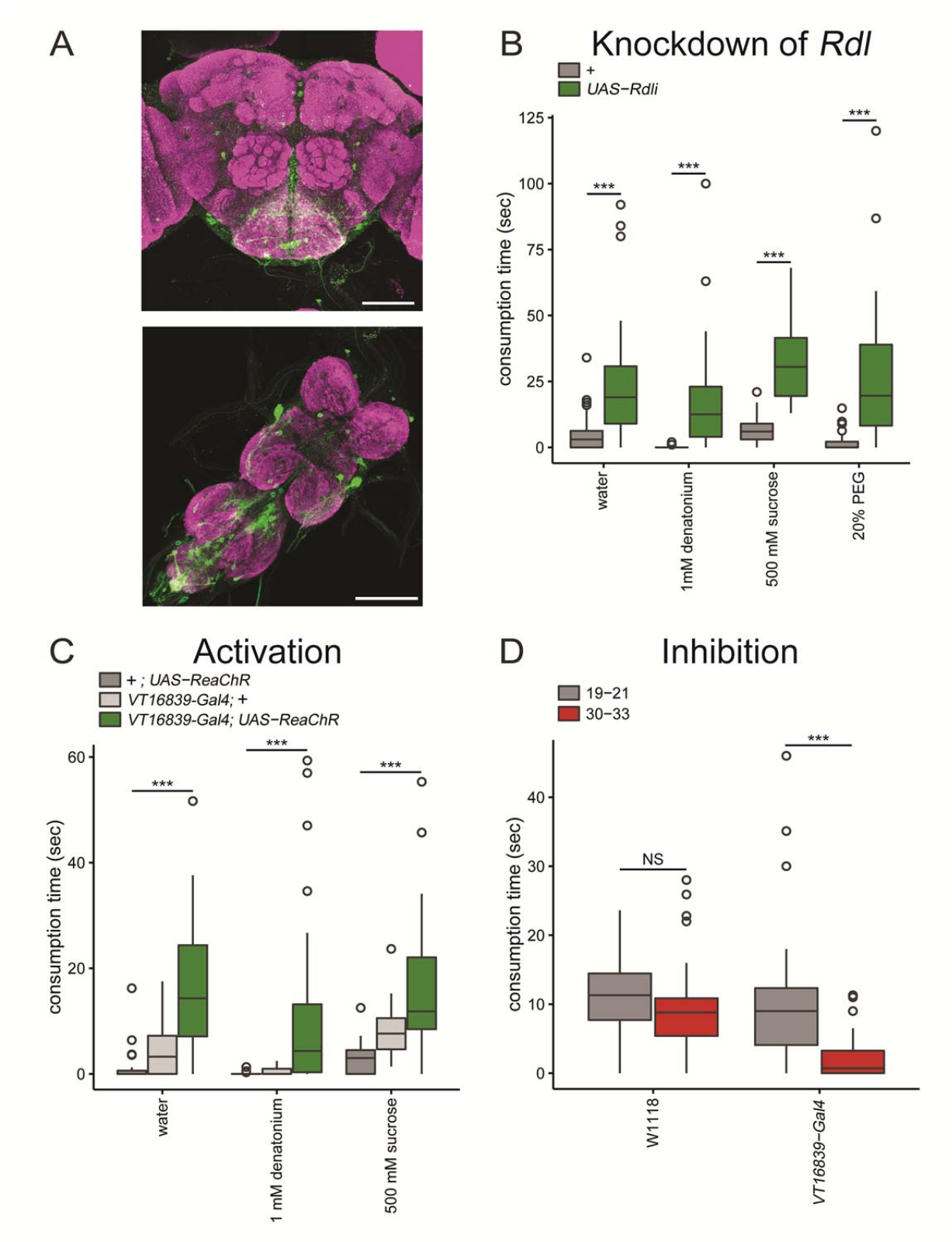

A
B
Knockdown of Rdl
+
UAS−Rdli
consumption time (sec)
125
100
75
50
25
0
***
water
1mM denatonium
500 mM sucrose
20% PEG
C
Activation
+ ; UAS−ReaChR
VT16839-Gal4; +
VT16839-Gal4; UAS−ReaChR
60
40
20
0
water
1 mM denatonium
500 mM sucrose
D
Inhibition
19−21
30−33
NS
W1118
VT16839−Gal4

Figure 3.

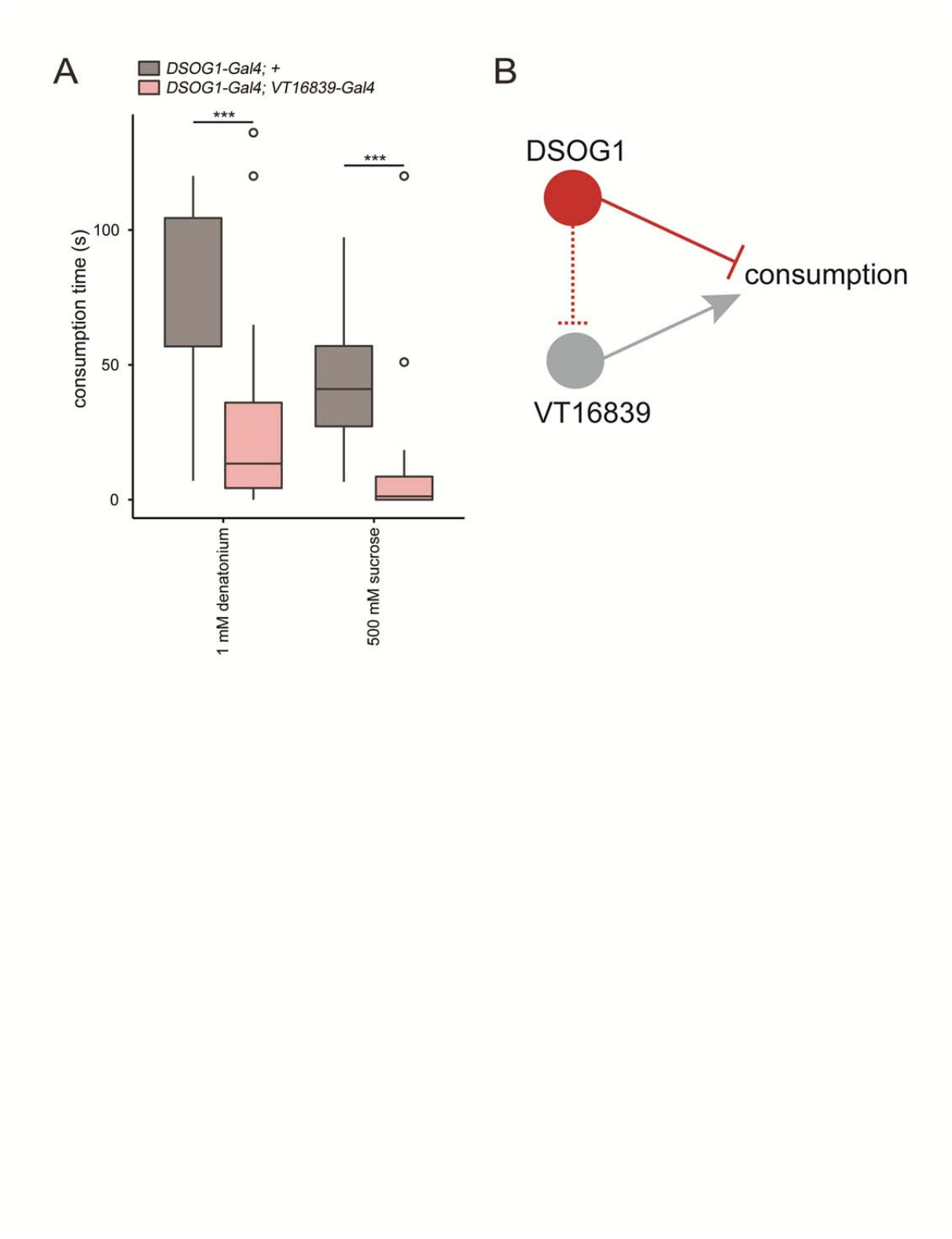

A
DSOG1-Gal4; +
DSOG1-Gal4; VT16839-Gal4
***
***
consumption time (s)
100
50
0
1 mM denatonium
500 mM sucrose
B
DSOG1
consumption
VT16839

Figure 4.

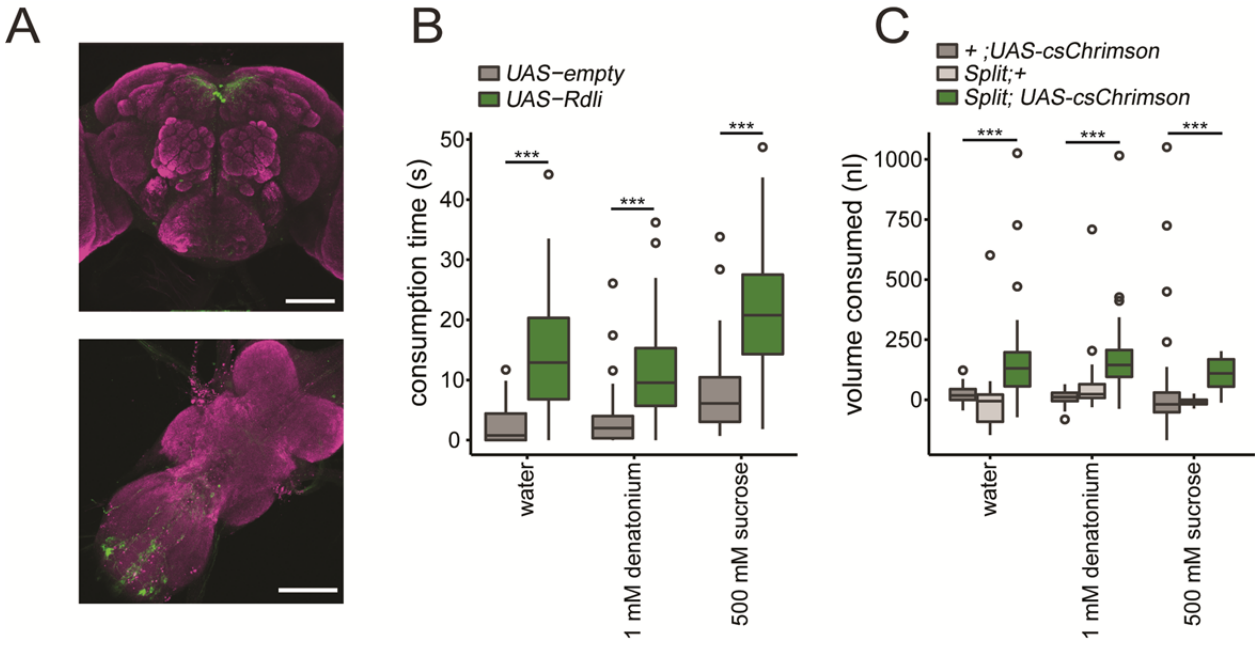
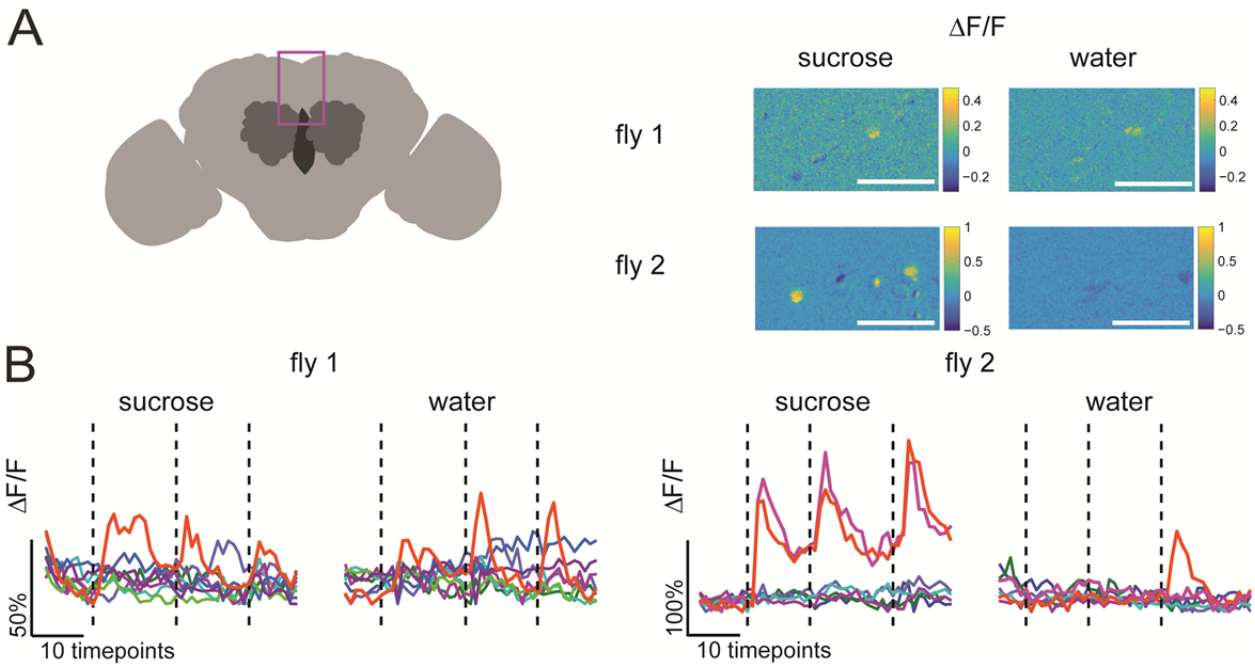

A
B
UAS−empty
UAS−Rdli
consumption time (s)
50
40
30
20
10
0
***
***
***
water
1 mM denatonium
500 mM sucrose
C
+ ;UAS-csChrimson
Split;+
Split; UAS-csChrimson
volume consumed (nl)
1000
750
500
250
0
***
***
***
water
1 mM denatonium
500 mM sucrose

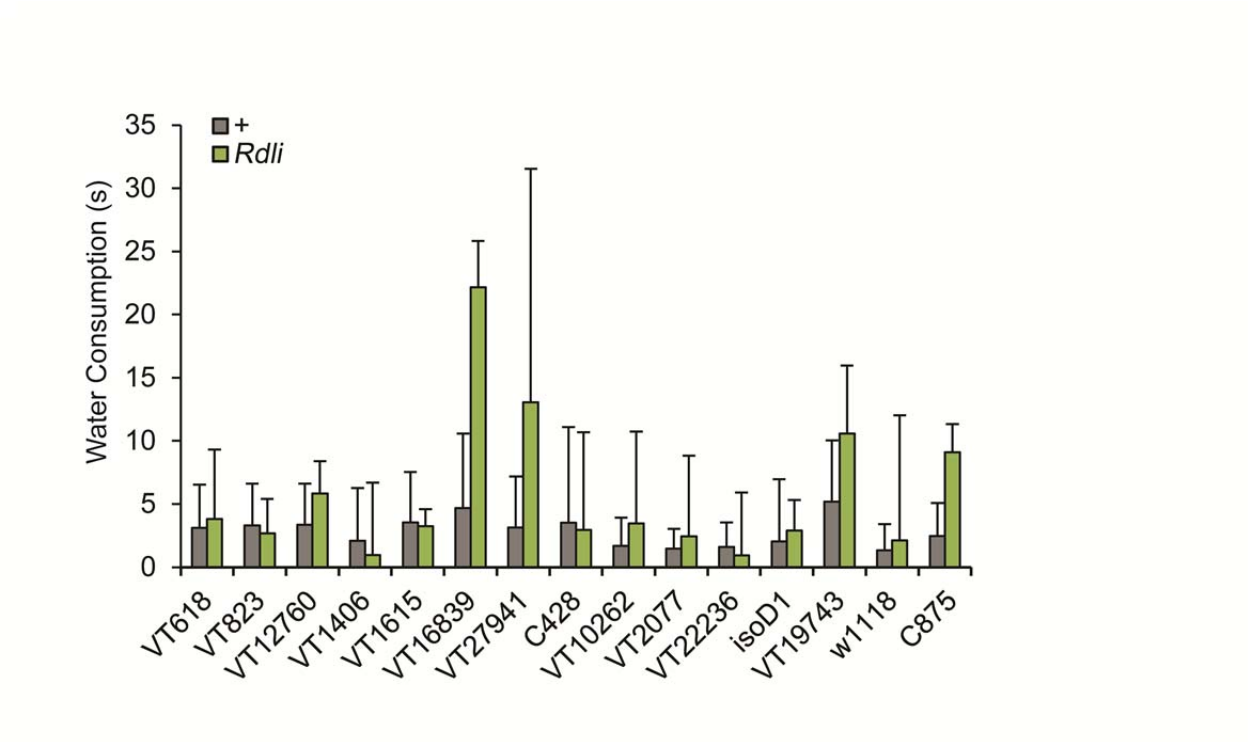
Figure 5.


A
ΔF/F
sucrose
water
fly 1
fly 2
0.4
0.2
0
−0.2
1
0.5
0
−0.5
B
fly 1
fly 2
sucrose
water
ΔF/F
50%
100%
10 timepoints

Supplemental Figure 1. *VT16839-Gal4* and *VT27941-Gal4* lines show increased water consumption with *Rdl* knockdown.

(A) Knockdown of RDL in candidate Gal4 lines. Bar graph showing time consumption of water (mean ± SEM). Candidate Gal4 lines were tested against sibling flies for reproducibility of overconsumption phenotype for water. Both *VT16839-Gal4* and *VT27941-Gal4* showed robust and reproducibility of overconsumption. n=11-35/genotype

Supplemental Figure 1.



CHAPTER 3

Discussion

In chapter 3, I showed that the GABAergic receptor RDL plays a critical role in regulating the non-selective consumption of appetitive and non-appetitive substances. Since RDL-expressing neurons can strongly drive consumption behavior, I investigated what factors directly drive the activity of these neurons. Using calcium-imaging, I identified taste-responsive neurons in the Gal4 line, with highest responsiveness to sucrose stimulation in food-deprived flies. These sugar-responsive neurons reside in the higher brain region, suggesting that these neurons may be driven by sucrose to promote consumption behavior in a state of hunger.

Inhibition of non-selective consumption

Our behavioral data shows that sugar, water, bitter and even mechanosensory stimuli are enough to drive consumption when *Rdl* levels are reduced (Figure 2B). Flies with *Rdl* knocked down do not constitutively consume and require a stimulus to elicit consumption behaviors. This suggests that mechanosensory or bitter input is capable of triggering consumption only when RDL is not present at functional levels. In contrast, in wild type flies, only the appetitive substances, sugar and water, can elicit calcium transients large enough to trigger activity in these neurons. Likewise, ectopic activation of these neurons forces consumption of non-appetitive substances. In sum, a model for these data is that these neurons respond to sugar and water, but when inhibition is lowered or activity is increased (due to knockdown of *Rdl* or ectopic activation of these neurons) these neurons may promote consumption of even non-appetitive substances. Further studies are needed to test whether these neurons are not responsive to bitter or mechanical compounds in flies with wild type levels of *Rdl* but become responsive upon knockdown of *Rdl*. Taken together, these RDL-expressing neurons may act as command neurons to elicit consumption, whose activity can influence the probability of eliciting consumption behavior regardless of taste information.

RDL neurons in the taste circuit

Previous studies have identified two different classes of neurons that trigger sucrose consumption; FDG neurons are located in the SEZ and respond to sugar stimulation on the proboscis[36] and the cholinergic IN1 neurons respond to sugar stimulation of the internal mouthparts[37]. These two classes of neurons respond selectively to sucrose, suggesting that there is a pathway selective for regulating the consumption of sucrose. Similarly, ectopic activation of these neurons lead to increased consumption of sucrose but not water or bitter. These studies indicate that consumption of sucrose is regulated independently of consumption of water or bitter and argue for distinct circuits mediating consumption for each class of tastant.

The RDL-expressing neurons differ from previously identified consumption neurons because either knock down of *Rdl* or optogenetic activation of these neurons elicits consumption of not only appetitive substances, but non-appetitive substances as well. Our calcium-imaging data identified neurons in the *VT16839-Gal4* line that are responsive to multiple taste

compounds, sugar and water, suggesting that these neurons receive taste information from both sugar and water circuits.

Where do these RDL neurons lie in the context of previously identified components of the feeding circuit? Do FDG and/or IN1 neurons send signals from sugar GRNs to these RDL neurons? Similarly, lesioning of the recurrent nerve in *Drosophila* led to a selective increased consumption of sucrose, not bitter or water[63]. Does input from the recurrent nerve inhibit activity of RDL neurons to inhibit consumption of sucrose selectively? It would be critical to address the question of whether FDG, IN1, and input from the recurrent nerve send signals to RDL neurons to control consumption of sucrose to better understand how elements of the feeding circuit interact to influence feeding behavior.

Nutritional state modulation of consumption behavior

The regulation of selective consumption is important to maintain energy homeostasis by promoting the ingestion of nutritious and appetitive substances while avoiding the consumption of potentially toxic substances. However, in more desperate conditions when an animal is starving, consumption becomes less selective and more risk-taking towards any potential food sources. Since RDL-expressing neurons can powerfully drive non-selective consumptive behaviors, RDL-expressing neurons might be differentially modulated based on the severity of the starvation state to promote non-selective consumption for survival in conditions of sparse resources.

Prior studies have identified hunger-dependent modulation of sugar responses in sugar GRNs[60] and hunger-dependent modulation of bitter responses in the bitter GRNs[59]. In addition to directly modulating GRNs, hunger-dependent modulation acts on interneurons that modulate feeding initiation behavior[59]n the RDL neurons, it remains possible that the RDL neurons and DSOG1 influence parallel pathways. Further characterization of the RDL neurons that promote consumption and the DSOG1 neurons that inhibit consumption will enable us to distinguish these models. Understanding the interactions between the different neurons that regulate feeding will provide insight into the temporal dynamics and plasticity in feeding decisions.

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