Investigations on the involvement of dopamine in the gustatory responses of the fruit fly, *Drosophila melanogaster*.

By

Emily Ousterhout

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By

Emily Anne Ousterhout

Approved:

Natraj Krishnan
Assistant Professor of Biochemistry,
Molecular Biology, Entomology and Plant Pathology
(Director of Thesis)

Scott Willard
Associate Dean of CALS
(Committee)

Seth Oppenheimer
Director of Undergraduate Research
(Shackhouls Honors College Representative)

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1. INTRODUCTION

1.1 Taste

One of the most basic behaviors for all animals in order to be able to survive is finding the right kind of food and in sufficient quantity. The sense of taste, (gustation, or contact chemoreception) often considered a "primitive sense," is a vital component in this process, because for all animals, chemicals have to be detected, encoded in the central nervous system, processed, and then acted upon. At a higher level, tastes have to be recognized and remembered (learned) to prevent an animal from accidentally eating something unpleasant or even harmful. Despite its immense importance in everyday life to all animals, it is surprising that of all the known senses, the least known is that of taste. The perception of taste is a crucial behavioral function for the world's most abundant inhabitants – the insects – as well as the most successful inhabitants – the mammals – and allows for the discrimination between nutrient rich substrates and bitter toxins.

1.2 Perception of taste in insects

1.2.1 Taste organs

It may be unlikely that insects use the same categorization of taste as higher animals, but they still have to solve the same basic problems of gustatory processing faced by all animals: many different chemical stimuli at various concentrations (often in complex mixtures) need to be categorized, processed, and turned into an appropriate response. Insect taste organs were first described in the early 20th century as hair-like structures on the distal legs that induce feeding reflex reaction to sugar stimulations in butterflies (Minnich, 1921). In the beginning, the simplicity of insect taste organs innervated by only a few taste neurons

was ideal for physiological studies. Single-unit action potentials, sensitivity to taste ligands, and other physiological properties were intensively studied during the 1950s-1970s. Knowledge of insect taste has rapidly progressed with Vincent Dethier publishing his classic work "The Hungry Fly" in 1976 (Dethier, 1976). In his studies, Dethier used various flies, including the housefly *Musca domestica*, blowfly *Phormia regina*, fleshfly *Calliphora erythrocephala* and fruit fly *Drosophila melanogaster*. In the 1980s-1990s, morphological or developmental studies were also carried out in flies (Pollack and Balakrishnan, 1997; Singh, 1997). Molecular studies of insect taste receptors did not begin until around 2000. Again, breakthroughs on knowledge of molecular aspects were only possible with sequencing of the *Drosophila* genome. Searching the *Drosophila* genome has successfully led to the first discoveries of a large gustatory receptor (GR) gene family and characterization of taste receptor neurons that express divergent GRs (Clyne et al., 2000). The accumulated knowledge in flies from more than half a century of study thus describes various aspects of the insect taste receptor system. The taste system in insects is complex and not restricted to a single taste organ like the tongue (taste buds), the mammalian taste organ (Dethier, 1976; Stocker, 1994). Insect taste organs are usually distributed widely on the external surface of the body, including the labella in the proboscis, legs, wings, (**Figure 1**) and even the female ovipositors. They belong to taste *sensilla trichodea* and often referred to as "taste hairs" or "taste bristles" (Wilczek, 1967; Stocker and Schorderet, 1981; Nayak and Singh, 1983). There are some sensilla that function in tasting tastants that are not possible food sources, including male-specific sensilla that function in the detection of pheromones during courtship. However, many chemosensilla are used in the discrimination between edible and inedible compounds.

These chemosensilla are specialized, multiparous, and olfactory hair-like structures. 31 chemosensilla cover and 7 transmembrane receptors are expressed within each of the two labial palps of the fruit fly, which compose the main taste tissue of *Drosophila*. The two palps are located at the distal end of the proboscis, the fly equivalent of the human tongue. Chemosensilla are located on the antennae and maxillary palps as well, and gustatory sensilla are located on the head and within the food canal itself. Two palps close off the entrance to the pharynx, and during active feeding, these labial palps open to expose the gustatory sensilla, also known as "taste pegs," which make contact with the food as it travels through the pharynx.

Taste bristles and pegs both possess terminal pores at their tips to allow the direct exposure of food substances to the dendritic processes of the gustatory receptor neurons. Through the terminal pore, tastants diffuse into the lymph filling an internal canal to the dendritic processes of the gustatory receptor neurons (GRNs), which extend into the bristle shaft. The majority of the taste sensilla are innervated by four gustatory neurons, which extend their single and unbranched dendrites towards the terminal pore to send an axon to the central nervous system, where the processing of taste information occurs. Food quality is assessed by the fruit fly by labellar sensilla during the feeding process, where the fly comes into contact with a possible food source, receptors in the tarsus come in contact with phagostimulants, and the fly extrudes its proboscis. Ingested food then makes contact with internal taste organs.

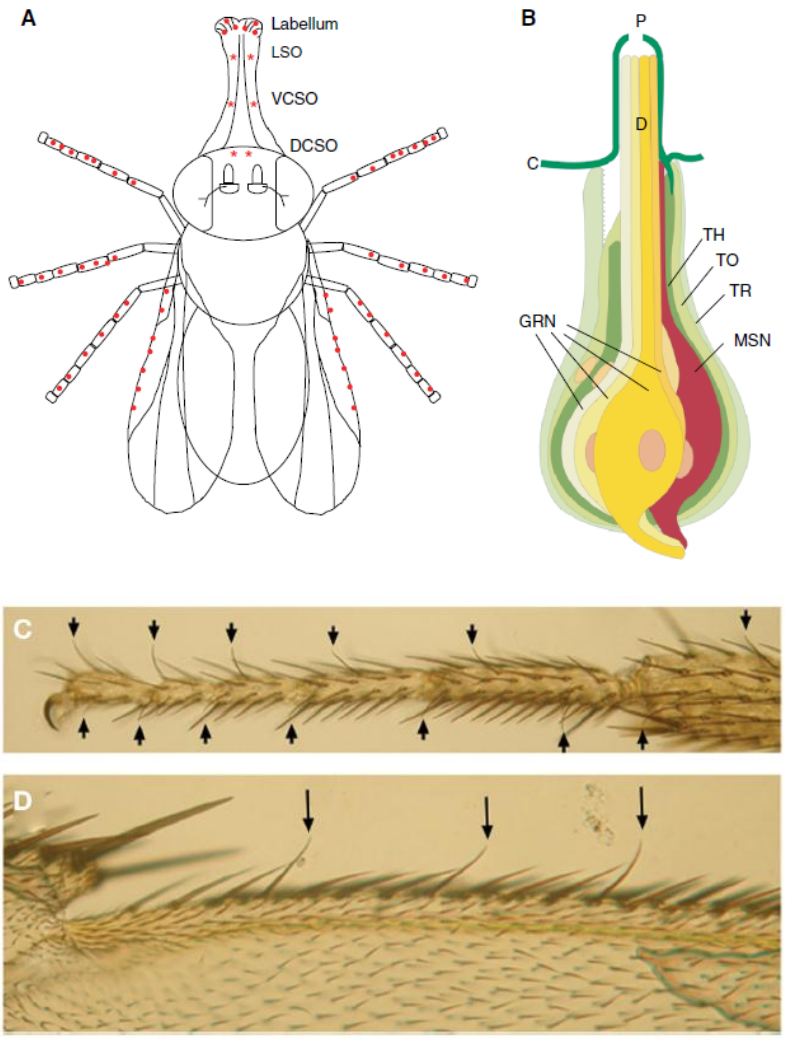

Figure 1: Organization of the *Drosophila* chemosensory system (Adapted from Amrein and Thorne, 2005; Isono and Morita, 2010).

(A) Location of Taste sensilla

Gustatory receptor neurons (GRNs) are distributed throughout the fly's body and are located at the base of taste sensilla (red dots). Most of these sensilla are bristles with sensory cells located at the base. All legs contain GRNS. Activation of GRNS is directly relayed to the CNS via their axonal extensions, which target different regions in either the subesophageal ganglion (GRNS in the labial palps and pharynx) or the thoracic ganglion.

(B) Structure of Taste Bristle

The taste bristles contain two to four GRNs, each of which extends a dendrite into the bristle. Soluble chemicals can enter the bristle shaft through the pore (P) at the tip and get in contact with the dendrite (D) and the receptors on their cell surface.

(C) and (D) Other types of taste sensilla

These are indicated by arrows along the tarsal segments of distal legs (**C**) and wing margins (**D**)

1.2.2 Taste receptors

The identification of a large family of olfactory-G-protein coupled receptor (GPCR) genes (Buck and Axel, 1991) provoked searches for taste GPCR genes using an array of molecular techniques. A novel family of candidate taste GPCR genes was found within the *Drosophila* genome by a computer algorithm to hit seven-transmembrane domains when the Drosophila genome project was nearly completed (Clyne et al., 2000). It was indicated that a total of more than forty GR genes that belong to the novel family share a signature motif with *Drosophila* odorant receptor (OR) genes expressed specifically in taste tissues. Later analysis of the whole *Drosophila* genome revealed that the fruit fly uses sixty-eight different receptors (GRs) to detect a wide variety of chemicals that it may come into contact with daily (Robertson et al., 2003). Gustatory orthologs exist in other insect species but are absent in vertebrates, yeast and bacteria. The poorly conserved nature of these sixty-eight GR genes suggests possible subjection to rapid adaptation driven by vastly different ecological niches occupied by insect species possessing these genes. The only two GRs that are expressed in gustatory receptor neurons (GRNs) that have known ligands are GR5a and GR66a, which act in the detection of trehalose and caffeine, respectively (Isono et al., 2005). The sixty-eight GR genes are expressed in the gustatory neurons in the fly's vast array of taste organs, and they encode for heptahelical GPCRs. Among *Drosophila*, GR genes, six are located on the X chromosome while thirty-eight and twenty-four are found on the second and the third *Drosophila* chromosome respectively. The GPCRs encoded by the GRs are 350-550 amino acid residues in length. The overall sequences are very divergent with homologies between two randomly chosen GRs as low as fifteen to twenty-five percent on average. GRs and ORs share a common amino acid

residue motif in the seventh transmembrane plus a C terminal domain, indicating that they have evolved from an ancestral chemoreceptor family. Genome analysis suggests that a robust expansion of GR genes has occurred only in the class *Insecta*. Future structure-function studies are necessary to understand the molecular evolution of GRs.

1.2.3 Ligands of taste receptors in insects and measuring taste behavior

There has been a failure so far in attempts to isolate taste receptor proteins biochemically from the taste organs of various animals, (i.e. bovine, rats) and there has been no success in insects, either. It is hypothesized that taste receptor proteins may be expressed in low amounts in the tissue or the affinity to taste ligands may be too low for affinity based-isolations. However, ligand profiles of taste receptors have been analyzed using *Drosophila* mutants or transformants. Based on this, the GR ligands have been classified into three groups: sugars, bitter substances, and pheromones. For many insects, including flies, butterflies, and bees, the stimulation of taste organs with a sugar solution not only induces neuronal response but also a robust feeding reflex called proboscis extension reflex response (**Figure 2**). The proboscis extension reflex (PER) response is modulated by stimuli and physiological factors such as hunger, nutrition, and arousal. Probability of PER can be used as a behavioral readout for the purposes of taste discrimination or associated behaviors as long as it quantitatively tracks a relevant tastant property like concentration. PER provides a tractable model for studying perception and plasticity.

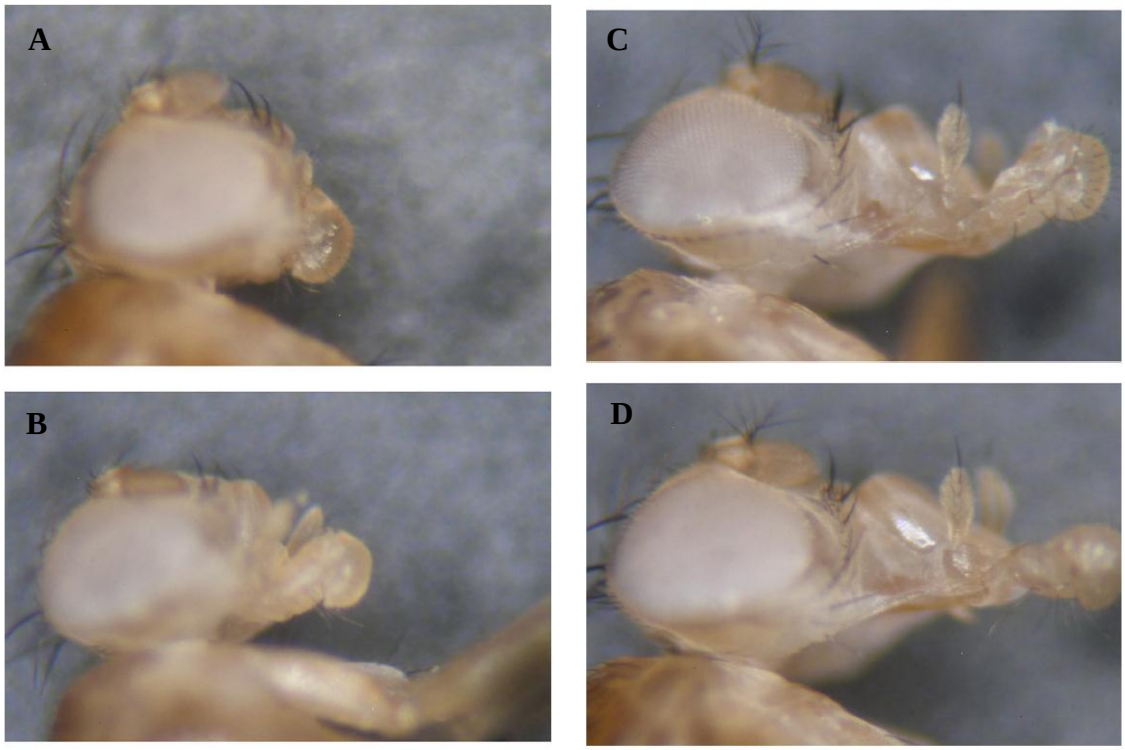

Figure 2: Increasing magnitude of proboscis extension response (**A-D**). As depicted above, the magnitude of the proboscis extension response increases from A-D, where D is the full extension of the proboscis.

1.2 A comparison of sweet and bitter taste response between insects and mammals

GRNs can be functionally characterized .by their critical role in the detection of two major substrate categories: sugars and bitter toxins. Both the fly and mammalian taste systems involve two distinct and mutually exclusive sets of taste cells that express either bitter or sweet/umami receptors. Bitter-tasting compounds are avoided by most mammals and flies, and in mammals are recognized by a single class of approximately 30 taste receptor genes (T2Rs), which are co-expressed as a single set of taste neurons in the taste buds of the tongue. Sweet and umami chemicals are detected by taste cells that express members of the /T1R family. Bitter taste cells in mice appear to express almost all T2R genes, but avoidance to bitter compounds by flies can result through any of a number of different combinations of Gr genes. Avoidance neurons in the fly labellum can express different combinations of Gr genes, which could provide the fly with neurons that exhibit distinct ligand recognition properties. The *Drosophila* taste system might be capable of discernment between various harmful compounds, enabling the animal to respond in a differentiated and ‘measured’ fashion to harmful stimuli.

1.3 Signal transduction in taste

It is not completely known how the taste ligand and receptor interact and result in neural activity. In mammals, sweet and bitter taste receptors utilize the same signaling cascade: the receptor:ligand interaction results in the activation of a G protein, thereby activating phospholipase C (PLC) and a TRP (transient receptor potential) channel, generating an action potential. *Drosophila* GRs are considered part of the family of GPCRs, and heterotrimeric G proteins could link the receptors to downstream signaling molecules (Amrein and Thorne, 2005). Although the expression of genes encoding G-alpha subunits in chemosensory organs has been reported, fly mutants for these genes are currently not available or have not been studied in regards to taste behavior or gustatory electrophysiology (Amrein and Thorne, 2005). The PLC Beta encoded by the norpA gene, which is essential for *Drosophila* visual and olfactory transduction, is expressed in neurons of taste organs, indicating that it could also be involved in taste transduction (Amrein and Thorne, 2005). Any of the fourteen *Drosophila* TRP channels could be expressed in GRNs, but only functional genetic analysis could definitively reveal any similar roles to mammalian counterparts and indicate if they are indeed involved in sweet and bitter taste transduction in *Drosophila*.

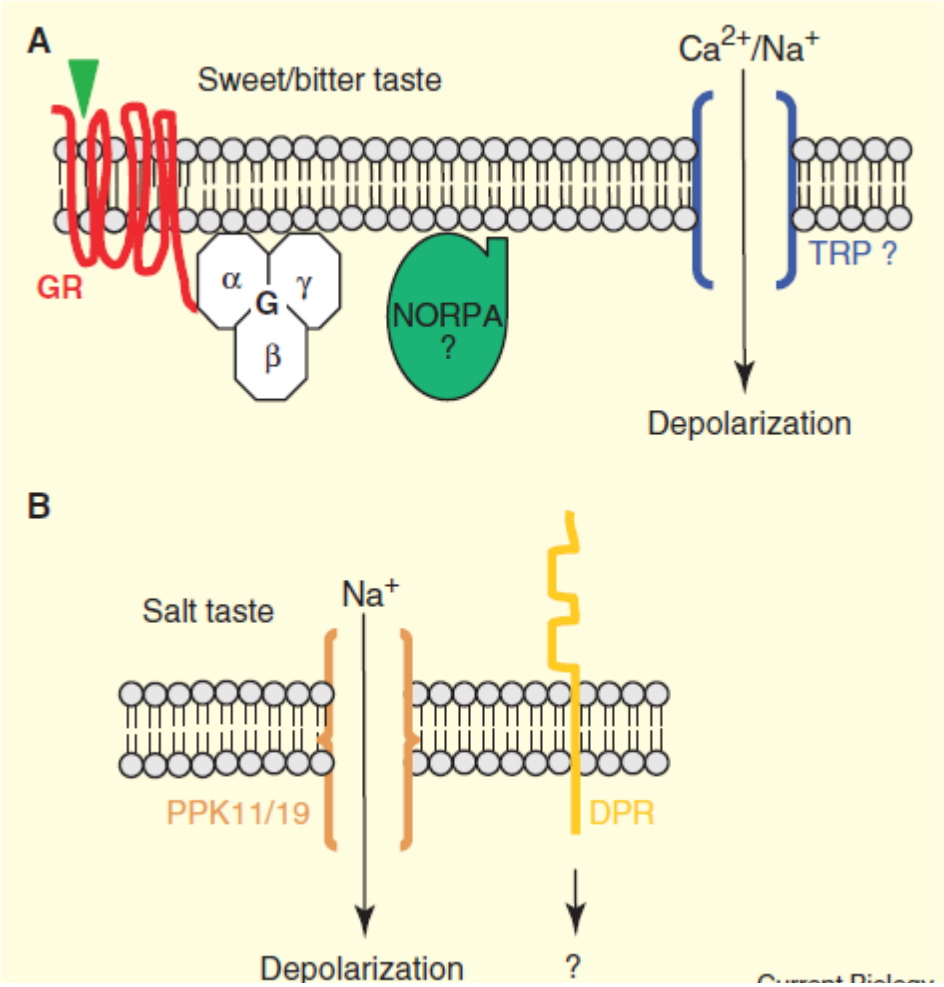

Figure 3: Taste signal transduction (Adapted from Amrein and Thorne, 2005)

(A) Detection of Sweet and Bitter Tastants

Sweet and bitter tastants aer detected by GRs, such as GR5A. Because the Grs are throught to encode GPCRs, their interaction with ligand would activate a trimeric G-protein. Phospholipase C (NORPA) and G-alpha are expressed in taste neurons, and mammalian sweet/bitter GPCR signal through a phospholipase C and TRP channel.

(B) Detection of Salt Compounds

The detection of salt is believe to be mediated through the direct influx of sodium or potassium ions into the cell through DEG/ENaC channels such as PPK11 and PPK19. Members of the DPR-Ig family of genes, such as dpr1, are involved in salt signaling, but their specific role has yet to be determined.

1.5 The role of dopamine signaling in taste behavior

Dopamine (DA) is a neurotransmitter that modulates fast neurotransmission in the central nervous systems of both vertebrates and invertebrates. In insects such as the fruit fly, DA has several roles in neural functions, from modulation of locomotor behaviors and arousal states, to appetitive and aversive learning, memory and even stress response (Restifo and White, 1990; Barron et al., 2010); Waddell, 2013; Hanna et al., 2015). Components of DA biosynthesis are highly conserved across a divergent range of animal phyla and have been well described in mammalian and Drosophila systems (Barron et al., 2010). DA synthesis requires closely regulated cooperation of two enzymatic pathways, and is highly sensitive to external cues. In *D. melanogaster*, tyrosine hydroxylase (TH), encoded by the gene *pale*, converts tyrosine to L-DOPA. Dopa decarboxylase (aromatic amino acid decarboxylase) converts L-DOPA to dopamine during catecholamine synthesis (Neckameyer and White, 1993). TH catalytic activity requires and is regulated by the co-factor, tetrahydrobiopterin (BH_4). The enzyme GTP cyclohydrolase I (GTPCH) is the initiating and limiting component of BH_4 biosynthesis and therefore also in DA production (Krishnakumar et al., 2000). Once catecholamines such as DA are produced, they can be transported by vesicular monoamine transporters (VMAT) from cytoplasm to synaptic vesicles (Greer et al., 2005). *Catecholamines up* (*Catsup*) works as a negative regulator of DA production that acts on TH and GTPCH, both of which are rate-limiting enzymes (Stathakis et al., 1999). Moreover, loss-of-function mutations in *Catsup* hyperactivate TH by a post-translational mechanism that also corresponds to increased catecholamine pool levels. A dopaminergic neuron in the SOG (supra-oesophageal ganglion), TH-VUM, is a critical modulator of taste behavior in *D. melanogaster*. Increased dopaminergic activity

has been associated with increased proboscis extension to sucrose, and decreased dopaminergic activity has been associated with the inhibition of a proboscis extension response (Marella et al., 2012). Although TH-VUM does not respond to sugars, it is believed to act over a longer time scale or in response to external cues to modulate proboscis extension to sucrose. For example, satiety state affects TH-VUM activity by promoting it during times of food deprivation, which is a time when the probability of proboscis extension is increased (Marella et al., 2012). Dopaminergic activity could thereby regulate the probability of proboscis extension in accordance with a fly's needs. Dopamine neural activity has been shown to affect proboscis extension to sucrose but not water, suggesting that dopamine regulation occurs independently of food and water intake regulation (Marella et al., 2012).

2. HYPOTHESIS AND OBJECTIVES

The fruit fly, *Drosophila melanogaster* has often been utilized as the classic model of studying many sensory systems, including those of taste and olfaction. The fly's gustatory system is, in many aspects, an ideal model for studying the perception of taste in mammals and humans. For example, carbohydrates are a major food source for both humans and the adult drosophila, and salts and acids are integral components of foods for the fruit flies. In mammals and *Drosophila* alike, the detection and appropriate intake of such chemicals is crucial for the maintenance of electrolyte homeostasis. The fruit fly is especially sensitive in its detection of such chemicals, and its detection range is comparable to that of mammals.

Although taste behaviors in the fly are relatively simple, with sugars eliciting behaviors of acceptance and bitter compounds avoidance, they are also plastic and are modified by intrinsic and extrinsic cues, including hunger and sensory stimuli. Since DA has a modulatory role in several neurobiological functions, particularly in addiction, motivation, arousal and also appetitive behavior, it was hypothesized that perturbations in DA signaling would affect taste behavioral responses. To explore the possible influence of DA signaling on gustatory responses, a well-characterized behavior (PER – Proboscis Extension Response) and the extensive genetic resources of the fruit fly was utilized. To begin to address how plasticity in this behavior is generated, the role of DA synthesis levels in regulating PER were investigated. In particular, the impact of mutations in three key genes in DA synthesis on gustatory responses in *D. melanogaster* were studied: *Catecholamines up* (*Catsup*), *pale* (*ple*), *Punch* (*Pu*), and VMAT. As mentioned previously, *Catecholamines up* is a negative regulator of DA production and acts on

tyrosine hydroxylase (TH), a rate-limiting enzyme for DA synthesis. *Pale* (*ple*) also encodes TH, and *Punch* (*Pu*) encodes GTP cyclohydrolase (GTPCH), which is important for synthesis of tetrahydrobiopterin (BH_4), which is necessary for TH activity. A mutant in the vesicular monoamine transporter (VMAT), a key transporter functioning in the transport and packaging of DA in the vesicles of the DA neurons, was also studied. Five different sugars, including fructose, dextrose, galactose, maltose, and arabinose were employed to test the responsiveness of different mutants in DA synthesis to examine and elucidate the precise role of dopamine in the gustatory responses of *Drosophila*. It is hypothesized that insights obtained from this study will help to understand the role of DA neuromodulatory neurons in gustatory sensation in flies as well as mammals because of the conserved nature of DA signaling pathway.

This study, thus, had two major objectives:

Objective 1: Standardize the specific concentration of different sugars which will elicit a response in 50% of the flies using the classic dose-response assays with wild type (w1118) flies.

Objective 2: To investigate if perturbations in DA synthesis (by elevating DA pools or reducing DA synthesis) as well as DA transport could have an impact on the PER response to EC_{50} of different sugars.

3. MATERIALS & METHODS

3.1 *Drosophila* stocks and husbandry

D. melanogaster were reared on 1% agar, 6.25% cornmeal, 6.25% molasses, and 3.5% Red Star yeast at 25 C in 12 h light:12 h dark (LD 12:12) cycles (with an average light intensity of ~2000 lux). Two different fly lines w^{1118} and Canton-S, which are wild type for the catecholaminergic pathway mutations, were used as control strains in this study.

The following mutant fly lines were utilized: *Catecholamines up* (*Catsup*), *pale* (*ple*), *Punch* (*Pu*), and VMAT. The $Catsup^{26}/CyO$ (Stathakis et al., 1999) mutation is a deletion extending 600 bp into the gene from immediately upstream of the start codon and produces no detectable protein and was derived from the mobilization of a 5´P-element insertion into $Catsup^{KO5042}$. Since *Catsup* mutant alleles are homozygous lethal, all experiments in this study were conducted using heterozygous strains. The *Pu* mutant allele utilized in this study was generated in an ethylmethane sulfonate (EMS) mutagenesis screen, and the genotype is $dp\ cn\ Pu^{Z22}\ a\ px\ sp$/SM1. Genetic characteristics of this strain are reported elsewhere (Mackay et al., 1985; Reynolds and O'Donnell, 1988). The homozygous lethal ple^2 is a loss-of-function allele recovered in an EMS screen, and the heterozygous mutant $w;\ ple^2/TM3\ Sb\ e$ was used (Neckameyer and White, 1993). For mutations in the transporter of DA, the VMAT loss of function mutant was used: $w;VMAT^{\Delta 14}/CyO$, (Romero-Calderon et al., 2008).

All behavioral studies were conducted on mutant heterozygotes crossed into the appropriate wild type background to eliminate balancers. Only male flies (2-6 day old

adults) were used in this study, since female flies have altered physiological status because of the reproductive development. Ten flies of each genotype were tested for each sugar concentration. Each fly served as an independent replicate and the experiments were repeated twice or thrice in certain cases.

3.2 Chemicals

All sugars used in this study were procured from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sugar solutions were diluted in a range of concentrations from 0 – 750 mM in nano-pure water (18.2 mΩ).

3.3 Proboscis extension reflex assay

The proboscis extension reflex (PER) does not measure feeding behavior, but rather a reflex behavior associated with feeding. After a starvation period of approximately 24 ± 1.0 h, flies were narcotized with CO_2, each fly was caught individually and placed in a pipette tip (0.5–10 µL, Laboratory Product Sales Inc., Rochester, NY) whose end was cut off. One leg of the fly protruded out of the pipette tip (**Figure 4**). The immobilized fly was then let to awaken and recover. The tarsus (the forelegs of the fly- the GRNs) was touched with a toothpick moistened with water or one of the following sugar concentrations: 0-750 mM (Galactose, Dextrose, Arabinose, Fructose, and Maltose). Each stimulus (water or sugar solution) was presented once to each individual. For each fly we recorded whether a specific stimulus concentration elicited proboscis extension (**Figure 5A**). The proboscis is usually withdrawn (**Figure 5B**), but upon stimulation of the foreleg with a feeding stimulus (for example a sugar solution), is frequently extended.

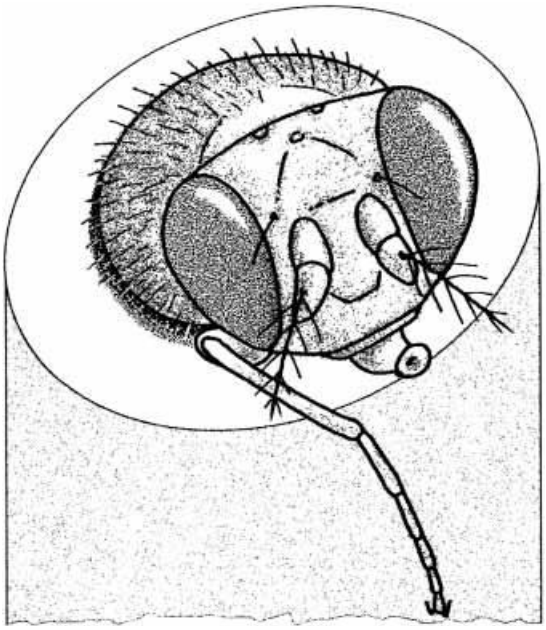

Figure 4: (Adapted from Scheiner et. al. 2014) Schematic of adult *Drosophila* mounted for measuring sugar responsiveness. Each fly was individually set in a pipette tip with a cut-off end. One leg protruded from the edge of the pipette tip. The tarsus of this leg was stimulated with a toothpick moistened with sugar solution of a certain concentration. It was noted which sugar concentrations elicit proboscis extension.

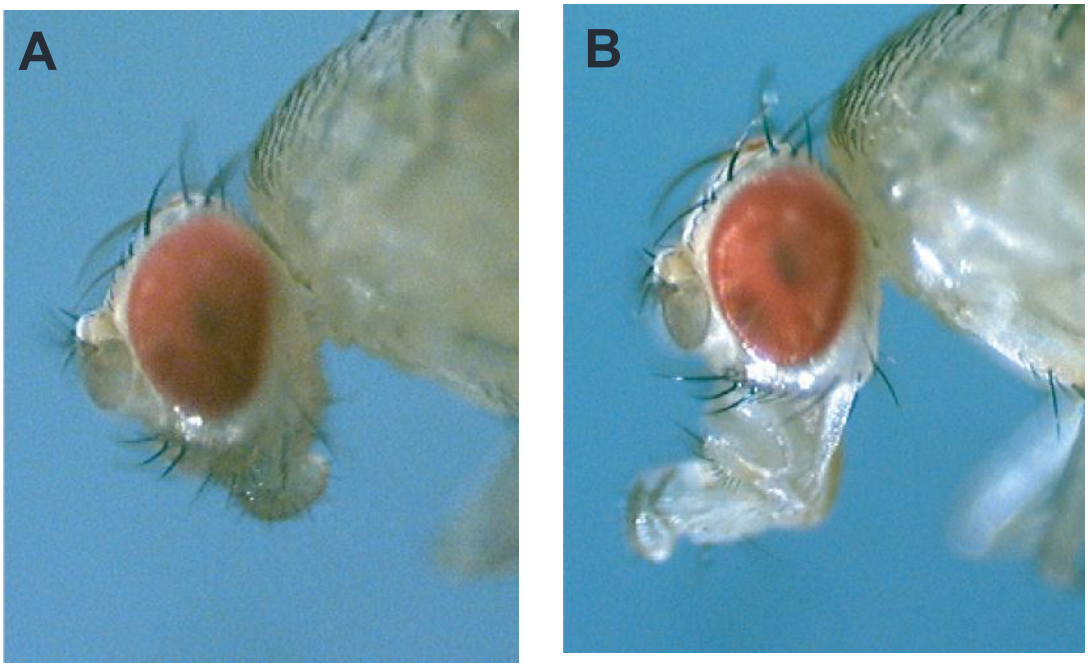

Figure 5: The proboscis extension reflex (**A**) Withdrawn (**B**) Extended (Adapted from Amrein and Thorne, 2005)

The extension of the proboscis is directly correlated with the attractiveness of the stimulus. Only flies which did not respond to stimulation with water were analyzed to prevent experimental bias by thirst.

3.3 Statistical analyses of data

3.3.1 Dose-response curves

Since the actual concentration of sugar solution being applied at the site of action (the fly tarsus) was not known, we used the term "dose". Dose-response curves were generated for various sugars tested under the first Objective using the sigmoidal dose-response curve also commonly referred to as the Hill equation, or the variable slope sigmoid equation. The EC_{50} values were calculated (GraphPad Prism v 5.01, GraphPad Software Inc. San Diego, CA):

The data were converted to % flies showing PER. Thereafter, the model

Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*Hill Slope))

was applied using Log(agonist) vs response - variable slope. EC_{50} is the concentration of agonist (sugar) that gives a response half way between Bottom and Top (However, this may or may not be the same as the response at Y=50).

Graphs were generated using GraphPad Prism v 5.01

3.3.2 One way Analysis of Variance with post-test

For analysis of results of response of various mutants to EC_{50} concentrations of various sugars, One-Way Analysis of variance with Tukey's post-hoc tests were conducted (GraphPad Instat v 3.0). Graphs were generated using GraphPad Prism v 5.01

4. RESULTS

4.1 Gustatory plasticity -Variation in responsiveness to different sugars

A distinct variation in response to different sugars was observed (**Figure 6A-E**). The least EC_{50} value for PER was observed for Galactose (7 mM) whereas for Fructose the EC_{50} value evoking PER was highest (80 mM). Thus, though fructose is the sweetest in terms of taste, yet a higher concentration of it is required to elicit the PER behavior in flies.

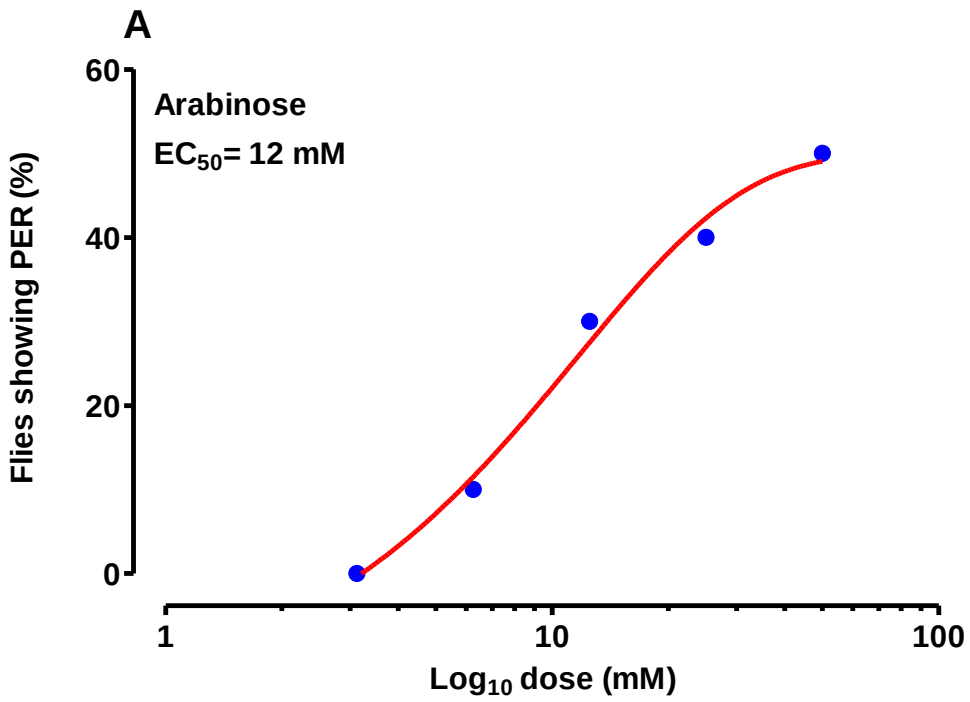

Figure 6A: Dose-response curve of Arabinose in PER

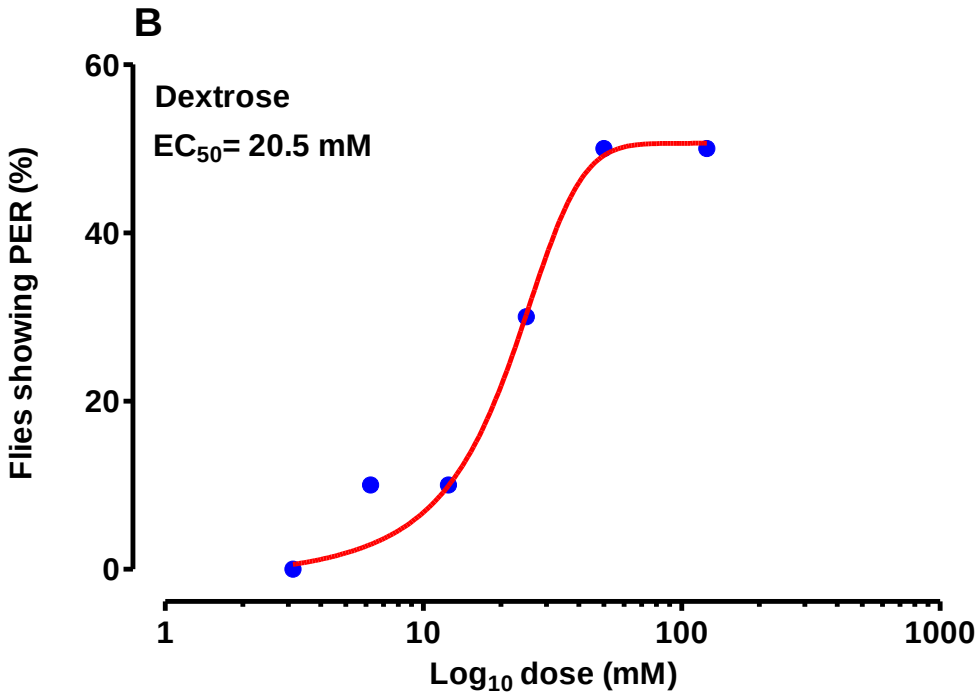

Figure 6B: Dose-response curve of Dextrose in PER

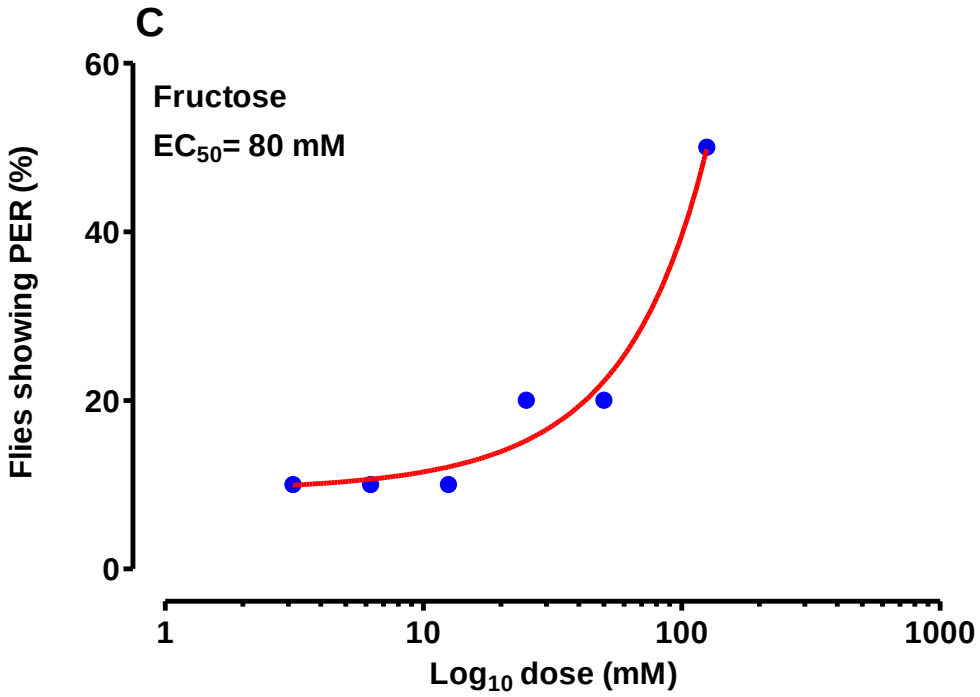

Figure 6C: Dose-response curve of Fructose in PER

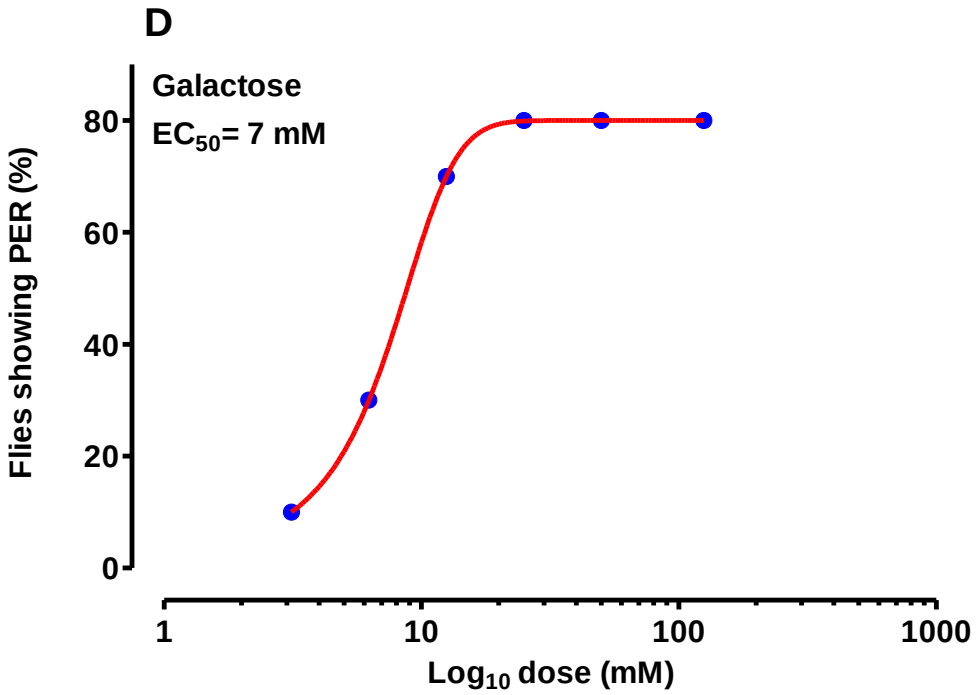

Figure 6D: Dose-response curve of Galactose in PER

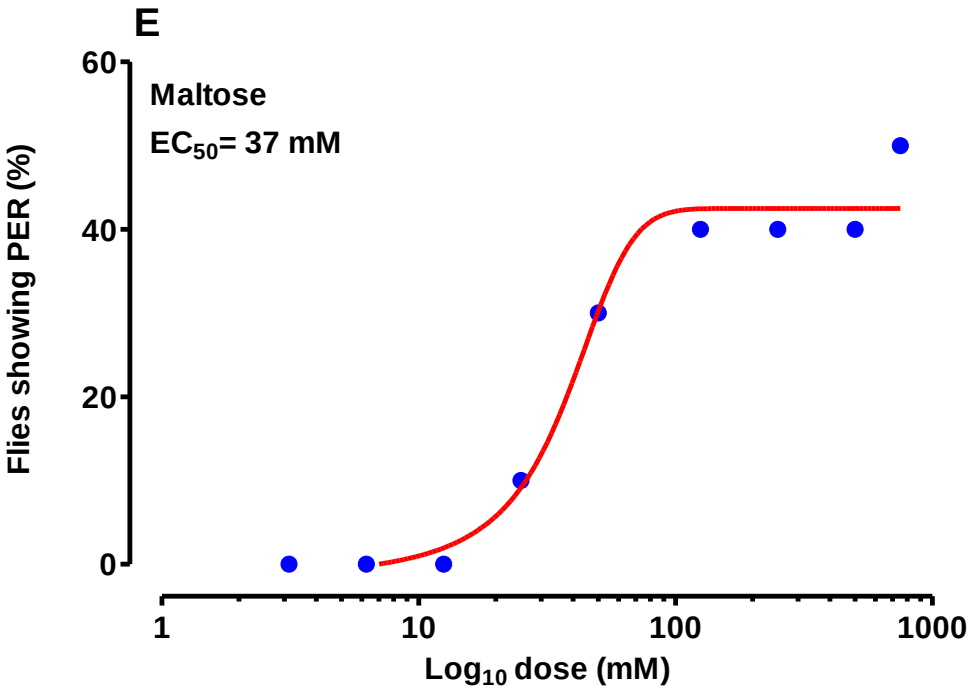

Figure 6E: Dose-response curve of Maltose in PER

4.2 Differential responsiveness to sugars following perturbations in DA synthesis or transport.

Flies with elevated DA pools (*Catsup*^{26}) or decreased synthesis of DA (*ple*^{2} or *Pu*^{Z22}) or impaired trafficking of DA (*VMAT*$^{\Delta 14}$) showed a differential responsiveness to the various sugars tested (**Figure 7 A-E**). In response to Arabinose (12 mM), there was no significant difference in PER behavior between wild-type or *Pu*^{Z22} flies (**Figure 7A**). However, *Catsup*^{26} with elevated DA pools and *VMAT*$^{\Delta 14}$ flies with impaired DA trafficking showed significantly decreased PER behavior compared to all other fly lines.

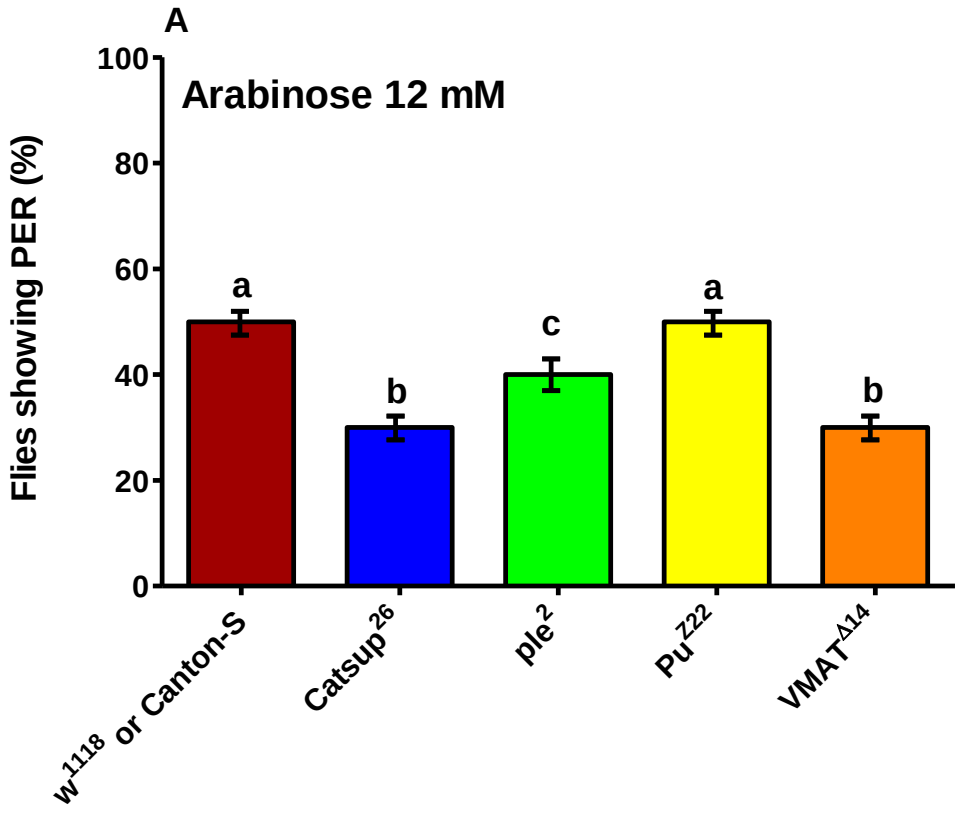

Figure 7A: PER behavior of different fly lines to Arabinose (12 mM). Data are mean ± SEM. Bars with different superscripts are significantly different at $p<0.05$.

A similar pattern was also observed in case of Dextrose (20.5 mM) (**Figure 7B**). However, in case of Fructose (80 mM), *Catsup*^{26} flies (with elevated DA levels) showed significantly higher PER behavior compared to controls or other mutants. The least PER behavior to Fructose was exhibited by *ple*^{2} flies (with decreased DA levels) (**Figure 7C**).

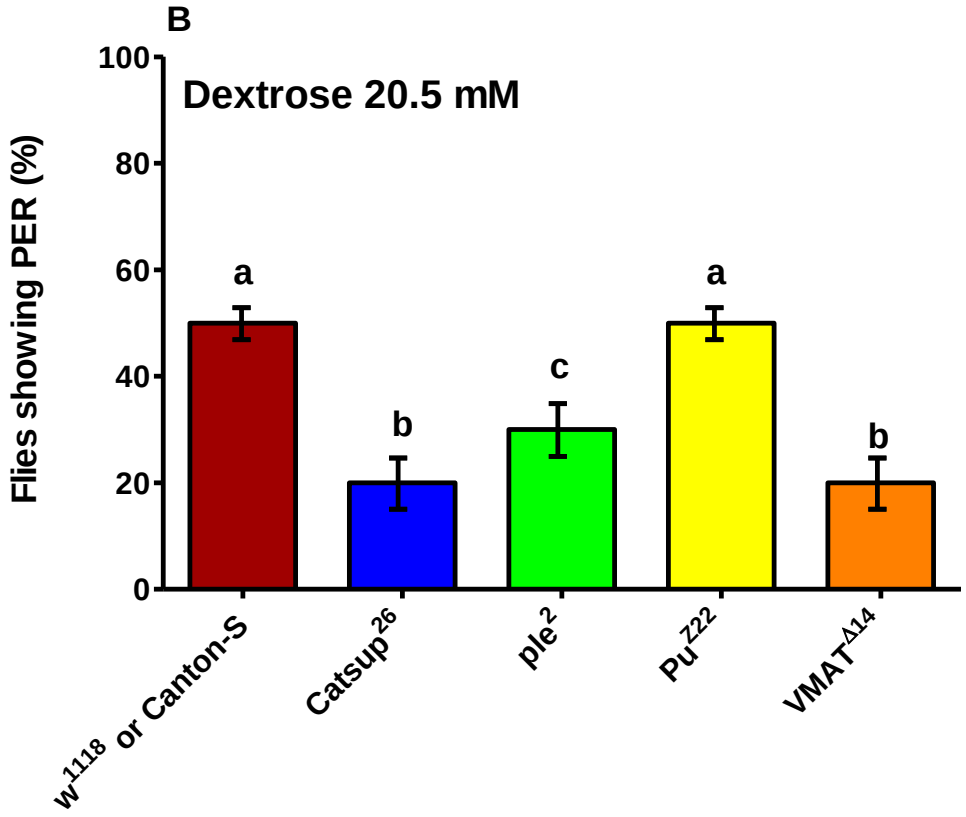

Figure 7B: PER behavior of different fly lines to Dextrose (20.5 mM). Data are mean ± SEM. Bars with different superscripts are significantly different at $p<0.05$.

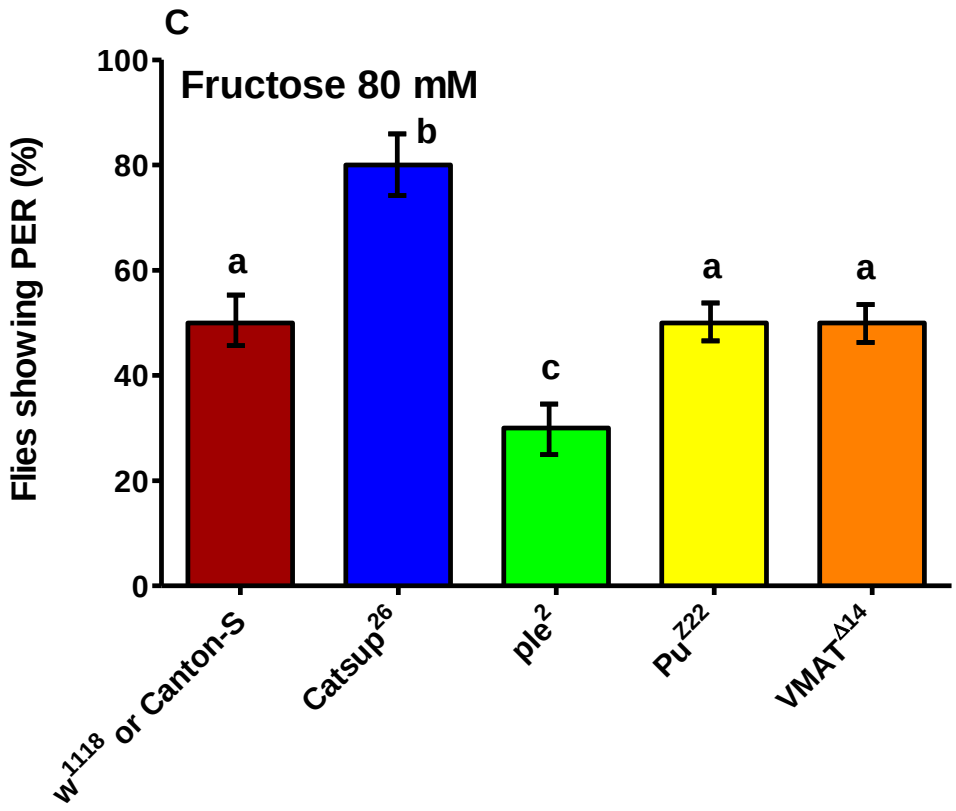

Figure 7C: PER behavior of different fly lines to Fructose (80 mM). Data are mean ± SEM. Bars with different superscripts are significantly different at $p<0.05$.

Interestingly, in case of Galactose (7 mM), both *Catsup*^{26} and *ple*^{2} flies showed significantly higher PER behavior (**Figure 7D**). Also *Pu*^{Z22} and *VMAT*$^{\Delta 14}$ flies showed significantly decreased PER behavioral response compared to either wild-type or *Catsup*^{26} and *ple*^{2} flies. Whereas, upon exposure to Maltose (37 mM), Catsup26 flies showed the least PER behavioral response which was equivalent to that exhibited by VMATΔ14 flies. No differences were recorded among wild-type flies or flies with decreased DA synthesis (**Figure 7E**).

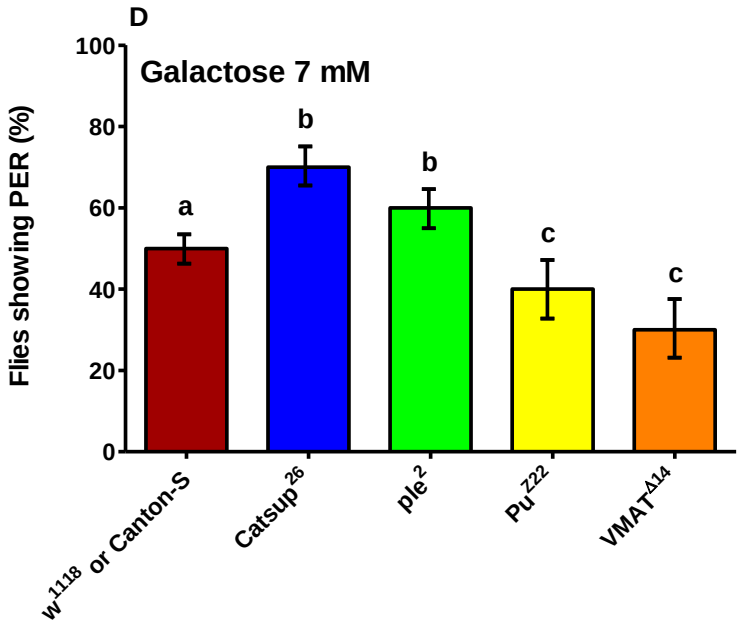

Figure 7D: PER behavior of different fly lines to Galactose (7 mM). Data are mean ± SEM. Bars with different superscripts are significantly different at $p<0.05$

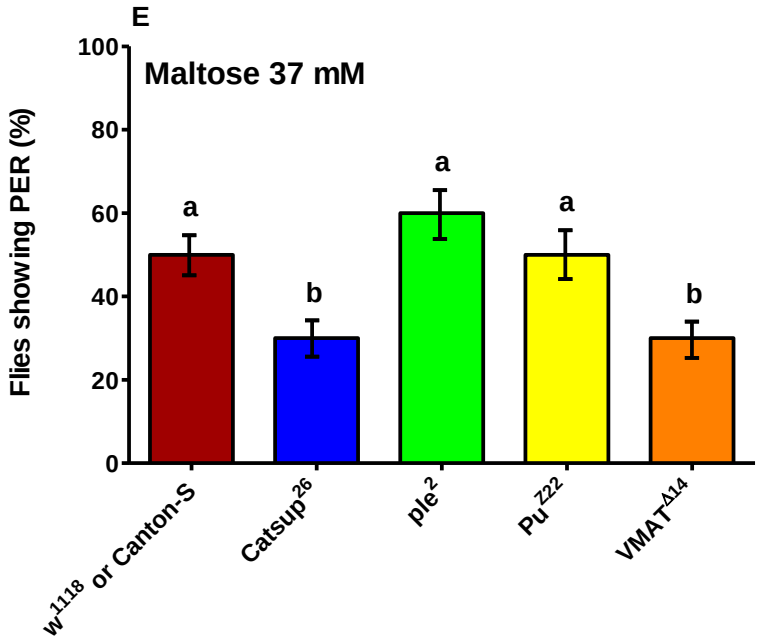

Figure 7E: PER behavior of different fly lines to Maltose (37 mM). Data are mean ± SEM. Bars with different superscripts are significantly different at $p<0.05$

When the response to different sugars was averaged out among the different fly lines, it was observed that neither elevated DA pools (*Catsup*^{26}) nor decreased DA synthesis (*ple*^{2} or *Pu*^{Z22}) had any significantly different PER behavioral response when compared to wild-type controls. However, *VMAT*$^{\Delta 14}$ flies with impaired DA trafficking did reveal a significantly decreased PER behavioral response compared to all other fly lines (**Figure 8**).

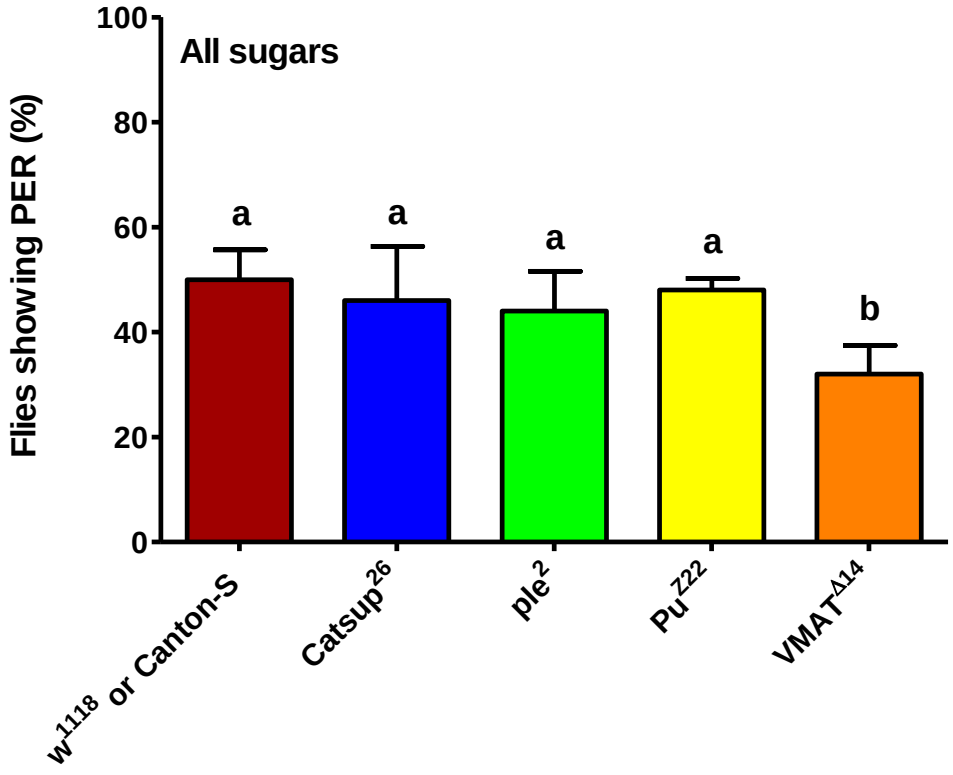

Figure 8: Average PER behavior of different fly lines to all sugars tested. Data are mean ± SEM. Bars with different superscripts are significantly different at $p<0.05$

5. DISCUSSION

This study has had a close look at the fly's taste system and utilizes a relevant behavioral assay to assess Drosophila's proboscis extension response as a manner to quantify taste perception at the organismal level and evaluate which degree of responsiveness of the mutants and wild type flies used within this study to specific concentrations of different sugars. We have emphasized the structural elements from the sensilla that transduce chemical information to the gustatory regions of the CNS. The fruit fly *Drosophila* is a complex organism that utilizes neural machinery in the detection, evaluation, and identification of the nutritional value of foods after ingestion. This study has been able to demonstrate that gustatory plasticity does exist in fruit flies and that this varies with different sugars.

In *Drosophila*, feeding begins with the PER. This very simple component of feeding behavior is very tightly regulated, with the probability of extension of proboscis depending on the nature of taste of the compound and its concentration. The response can also be modulated by hunger and satiety, such that flies which have recently fed are less likely to exhibit PER than flies which have not fed for a period of time. Though it has been reported previously that DA acts as a critical modulator of PER and that loss of DA neurons reduce PER to sucrose (Marella et al., 2012), the results of this study argue that just presence of DA either at low or elevated levels is sufficient to elicit PER in response to different sugars. Elevated DA pools do not indicate enhanced PER behavior or conversely depleted DA levels do not negate PER behavioral response. The degree of responsiveness may vary in response to different sugars (denoting plasticity). However, impairment of DA

trafficking does have an impact on the PER behavioral response as has been demonstrated in this work.

Taken together, the results of this study conclusively demonstrate that it is not the level of DA *per se* that dictates PER behavior but it is its presence and its transport from the synaptic clefts by the vesicular monoamine transporter that is important. While $VMAT^{\Delta 14}$ mutants did demonstrate PER (albeit decreased) across different sugars tested in this study, it also indicates that these monoamine transporters might be one of the major transporters of DA in gustatory signaling.

6. CONCLUSIONS AND FUTURE DIRECTIONS

This study demonstrates that DA levels do not dictate gustatory responses in Drosophila to various sugars. However DA transport does impact gustatory behavior to different sugars. There is a plasticity in the gustatory response depending on the kind of sugar and its concentration that is offered to the fruit fly.

Since knockout of DA synthesis is embryonically lethal, future work has to focus on various receptors (such as DopR, D1-like or D2R) and their conditional knockout to check gustatory behavior. Moreover, it would also be interesting to evaluate if sexually dimorphic responses to sugars occurs in fruit flies.

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