Regulation of feeding behavior in *Drosophila* through the interplay of gustation, physiology and neuromodulation

Bhumika¹, Arvind Kumar Singh¹

¹Genetics Laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi 221005, India

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1. ABSTRACT

One of the most fundamental behaviors in all the organisms, in order to achieve a satiated state and internal energy homeostasis is feeding. The action of feeding in any being whether be it any vertebrate or an invertebrate involves the perception of the external environment along with the gamut of decision making processes to eat or to not eat. The feeding decision along with chemosensation through gustation and olfaction leads to intake of food with proper nutrient balance along with avoidance of bitter and toxic substances. The progressions in the understanding of the complexity of feeding behavior involving gustation, neuronal and physiological processes have been achieved through the use of unparalleled model organism *Drosophila melanogaster*. Here, in this review, we aim to discuss the studies about the taste perception of major macronutrients in *Drosophila* through gustatory receptors as well as how the involvement of neuropeptides and neuromodulators in feeding behavior modulate the plasticity in feeding decisions. This review also summarizes the involvement of insulin/insulin-like growth factor signaling pathway in nutrient sensing and how the interaction of *Drosophila* insulin-like peptides with neuromodulators regulate feeding decision process. The review provides an integrative approach towards a balanced metabolic state in *Drosophila* through the interplay of physiology, gustatory perception and neuromodulation.

2. INTRODUCTION

One of the basic tenets of any organism is the urge to survive and reproduce. This instinctive approach towards survival in all the species has given rise to an array of behavioral repertoires. The behavioral repertoires changes in response to changing environmental cues and internal state in any organism. Such plasticity in behavior allows organisms to adapt better to any kind of adverse situation. The plasticity in any behavior is achieved through the action of neuromodulators which may act in conjunction with the physiological state of an organism. Amongst the diverse behavioral repertoires, feeding is known to be the most fundamental one in both vertebrates as well as in invertebrates. Feeding process in insects comprises of sequence of events (1) which ultimately results in ingestion of food. The series of events in insects involves foraging to locate food source, chemosensory detection, meal initiation, consumption and finally termination of feeding (2). The overall purpose of the process is to achieve a satiated state along with energy homeostasis. The plasticity in feeding behavior in *Drosophila* is dictated
by the interplay of the internal metabolic demands and chemoreception of external environment either through olfaction or gustation.

*Drosophila melanogaster* has been employed extensively for studying various neuronal and molecular mechanisms due to the presence of highly conserved genome in humans and *Drosophila* (3). In addition, the availability of powerful genetic tools, unlike other model organisms, makes *Drosophila* a suitable model organism for studying developmental pathways, neurological diseases and other physiological processes (4-6). Here, in this review we aim to give an elaborate overview of feeding regulations in *Drosophila* with detailed discussion on how fly perceive major macronutrients from the environment through gustatory receptors and how the internal metabolic state of fly governs this behavior. The internal physiology of the fly is transformed into the appropriate feeding responses via the action of several neuromodulators. Hence, we also reviewed recent studies regarding neuromodulators which are involved in plasticity of the feeding process. To give an overall clear picture how the internal metabolic state is transformed into feeding decision, we also emphasized on Insulin /Insulin-like growth factor signaling pathway (IIS) which maintains internal homeostasis of nutrition and how the downstream effectors of the pathway are involved in controlling the feeding process in *Drosophila*.

### 3. PERIPHERAL PERCEPTION OF MAJOR MACRONUTRIENTS

The tendency of an organism to avoid toxic and harmful chemicals and to seek nutritious substances from its environment plays a central role in its survival and fitness. The presence or absence of environmental cues is sensed via several repertoires of chemoreceptor’s present all over the body in *Drosophila*. Gustatory perception plays a critical role in sensing and transmitting details about environmental milieu. Macronutrients, as well as micronutrients present in the environment, are sensed through multiple gustatory receptors (GRs) expressed in gustatory receptor neurons (GRN’s). These GRN’s are present in sensillum like structure which in turn present in several tissue types (7). There are four different types of GRN’s in *Drosophila* responding to four different tastants: sugar, water, low salt concentration and high salt concentration (8). The cells that respond to high salt concentration are also involved in the detection of bitter compounds and are mostly involved with aversion to feeding (9). The analysis of *Drosophila* genome revealed a total of 68 GR proteins encoded by a family of 60 Gr genes by the process of alternative splicing (10). These GR proteins of *Drosophila* are closely related to olfactory receptors and belongs to the family of G-protein coupled receptors having seven transmembrane domains comprising of 480 amino acid residues (11-12). The Gr’s are situated externally on various body parts in both males and females as well as they are located internally. Externally in adult *Drosophila*, the gustatory sensilla are sited on body parts such as legs, anterior wing margins and labellum (13-14). Other than gustatory sensilla, labellum also harbours taste pegs for gustatory perception. In females, gustatory sensilla surrounding ovipositor allows them to sense preferred nutrition site for oviposition (15). Males possess more gustatory sensilla on forelegs as compared to female which helps them in sensing pheromones, thus promoting males in the process of courtship (16). Internally in *Drosophila* some of the receptors are also expressed heterogeneously in the enteroendocrine cells of midgut for food uptake, nutrient absorption and sugar homeostasis (17).

Like mammals, *Drosophila* responds to similar stimuli such as sugars, amino acids, salt, alcohols and several other chemicals. Here in this review, we are focused primarily on the perception of major macronutrients: Sugars, protein and fatty acid.

#### 3.1. Sugars

The sweet taste of sugars provides a cue for nutritious carbohydrates. Sub-family of eight sugar receptor genes Gr5a, Gr61a and Gr64a-f expressed in ‘sweet’ neurons of each sensilla are involved in sensing the sweet taste of sugars (18-20). Gr64 cluster genes stand out to be one of the most potent sensors of sucrose, maltose and several other disaccharides and trisaccharides and various alcohols (18, 21). Dhanukar *et al* (22) through their studies provided functional evidence that Gr5a receptor is involved in sensing trehalose. Apart from external taste receptors, the internal sensing of nutrients has been suggested by the work of Fujita and Tanimura (23). Work of Miyamoto, (24) using Ca2+ imaging and behavioral assays revealed that Gr43a taste receptor in dorsal protocerebrum of the brain is involved in sensing fructose present in hemolymph thus promoting feeding in hungry flies while inhibiting feeding in satiated flies. These receptors are also known to be found in the internal taste organs.

#### 3.2. Proteins

Proteins are considered to be one of the major macronutrients in insects, required for survival in both males and females and are especially important for egg production in females. However, peripheral gustatory sensors for the amino acid in *Drosophila* adults have not been properly explored and still needs to be characterized. The internal nutritional state of *Drosophila* may aid in response to amino acids stimuli (25). Study of amino acid sensing in *Drosophila* larvae
Feeding regulation in Drosophila revealed complex responses to different amino acids. They reported IR76b, an ionotropic glutamate receptor to be involved as a co-receptor in sensing amino acids. This receptor in Drosophila adults acts like sensors for salt and several polyamines in gustatory chemoreceptors as well as in olfactory system for sensing different odors (27-29).

3.3. Fatty acids

Drosophila responds to a variety of fatty acids (FA's) ranging from short chain and long chain fatty acid to mono as well as polyunsaturated FA's (30). The FA's present in the diet elicits higher feeding rate when compared to sucrose and proteins. FA's in Drosophila are perceived by peripheral gustatory receptors involved in sensing sugars and acts independently of olfaction and pH. Unlike proteins and sugars, the sensory pathway for detection of FA's is conserved in mammals and Drosophila. The phospholipase C (PLC) signaling involved in mamals for the perception of sweet and bitter taste is responsible in Drosophila for the detection of FA's. Masek and Keene (30) utilized norpA mutants, defective in coding PLC to test responsiveness to different FA's. They found no response to FA's in norpA mutants. When PLC signaling was partially restored in norpA mutants in sweet sensing neurons the response to FA's was restored. The result suggests that PLC signaling is needed for the gustatory sensing of FA's in Drosophila and it acts via sugar sensing GRN's. The study done till date only reveals few facts about Drosophila gustatory perception of FA's. However, to gain a deeper perspective, the individual Gr's for FA perception needs to be identified.

4. MODULATION OF PLASTICITY IN FEEDING BEHAVIOR

The peripheral sensory inputs, as well as the internal physiological status, report the nutritional status to the brain which in turn regulates the feeding behavior in Drosophila and other organisms through the action of several neuropeptides. The complexity of feeding decision process is met through the crosstalk between chemoreceptor's, internal metabolic state and neuropeptides. While some sets of neuropeptides are involved in stimulating food intake, the other group of neuropeptides acts antagonistically and impedes the feeding process. Hugin, Drosophila Neuropeptide F (dNPF), structurally related neuropeptide F (sNPF), corazonin, leucokinin (LK), drosulfakinin (DSK), allatostatin A (AstA) are different neuropeptides which are involved in the modulation of feeding behavior.

Where the peripheral gustatory receptors are primarily involved in the taste detection process, the neuropeptides involved in feeding process are responsible for the feeding decision processes. One such neuropeptide involved in the decision process is Hugin. Hugin, a homologue of the mammalian encoding gene neurenomed U, is released by hugin neurons in response to nutrient signals (31, 32). The overlapping of neurites of the hugin neurons at the subesophageal ganglion (SOG) with the gustatory receptor neurons indicates some synaptic connections, thus demonstrating the role of hugin as interneuron relaying information from the gustatory receptors to target inputs resulting into behavioural outputs (33). Similarly dNPF, a human homologue of Neuropeptide Y has also the implication in stimulating food intake. The dNPF is expressed in the brain and midgut of fly and larvae (34). The expression of dNPF shows developmental plasticity and is pronounced in young early instar larvae as compared to old non-feeding larvae (35). In late non-feeding larval stages, the expression of dNPF is associated with food aversion and other social behavioral activities such as cooperative burrowing, hypermobility and food dependent clumping (35). dNPF neural network in larval Drosophila responds to sugar/sweetener gustatory stimuli in a dose-dependent manner by increasing its synaptic transmission as well as by increasing neuronal expression of dNPF (36). The results provided evidence that the chemosensory inputs are modulated by neuronal circuits thus ultimately resulting in motor output. Another example of neuropeptide responsible for stimulating food intake is sNPF. sNPF is known to be expressed in all the developmental stages in Drosophila and is also responsible for regulating the body size (37). Lee et al. (38) showed that sNPF, when overexpressed in the nervous system of mutants, augmented the food intake, while the sNPF loss of function mutants decreased the food intake. Likewise, neuropeptide Corazonin, a homologue of arthropod adipokinetic hormone (AKH) and mammalian gonadotropin hormone and expressed in salivary gland and adipocytes of the fat body also promotes food consumption (39, 40). LK, another regulator of food intake is involved with meal size and meal frequency as well as water and ion balance in Drosophila (41-44). Flies having mutation in LK neuropeptide and LK receptor genes show feeding activity with increased meal size with overall reduction frequency of food intake, thus maintaining the caloric intake similar to wild type (42).

The neuropeptide AstA released by certain neurons and endocrine cells are involved in suppression of food intake as well as in the aversion to unpalatable food without any effect on energy homeostasis (40). Chen et al. (45) suggested that AstA is not directly involved in the core feeding regulation but act like an extrinsic factor which inhibits feeding under certain uncharacterized conditions. Their work also proved Ast A to be pleiotropic, acting as both appetite suppressant as well as a sleep inducer. Another neuropeptide involved in the inhibition of food
intake is DSK. DSK is produced from Insulin-producing cells (IPC's), the same source from where Drosophila insulin-like peptides (DILPs) is released. In addition to IPCs the DSK's are also produced by several other neurons in the brain (46). DSK's are related to mammalian cholecystokinin and are responsible for inducing a state of satiety in the fly (47).

Neurotransmitters along with endocrine signals regulate the feeding behavior in response to the inner and outer nutritional state. Fat body and corpus cardiacum (CC) in Drosophila sense changes in the physiological state and account the changes to brain for proper motor response. Fat body is a multifunctional endocrine organ having a combined role of vertebrate adipose tissue and liver. It acts as an energy pool as well as nutrient sensor facilitating cross talk with different organs thus helping in growth and metabolic homeostasis (48). AKH released by CC is a functional equivalent of mammalian glucagon and is involved in maintaining triglyceride and glycogen homeostasis. Besides maintaining energy homeostasis, AKH also has a role in feeding behavior in Drosophila (49). Work of Bharucha et al. (50) revealed the expression pattern of AKH receptors which are G-protein coupled receptors, in certain GRN's in the adult SOG. The subsets of gustatory neurons which express AKHR are particularly associated with the attractive taste modality and are classified as Gr5a neurons thereby specifying its role in stimulating the feeding process.

5. ROLE OF IIS PATHWAY AND DILP'S IN NUTRIENT HOMEOSTASIS AND FEEDING

5.1. IIS signaling pathway and internal nutrient sensing

The integration of peripheral gustatory information and internal nutritional status modulates the metabolic regulation and growth in all organisms. The crosstalk between different pathways and tissues regulate energy homeostasis in mammmals, Drosophila and several other organisms. The IIS pathway acts as a sensor of individual's nutritional status and is a highly conserved pathway in both mammals and Drosophila. It is known to regulate several aspects of nutrient-dependent growth, lipid and carbohydrate metabolism, lifespan, reproduction, immunity and stress response (51, 52). In Drosophila Insulin/IGF signaling transduction is mediated by receptor tyrosine kinase called insulin like receptors (InR) which binds to DILPs. Drosophila possesses eight DILPs (DILPs1-8) which differ in their expressions both temporally and spatially. These DILPs are produced by several tissues located all over the body such as ventral nerve cord, fat body, imaginal discs, salivary glands and neurosecretory cells of the brain (53, 54). DILP 2, 3 and 5 are produced by the group of 14 neurosecretory cells organized symmetrically in two clusters in the pars intercerebralis of the brain (53, 55-56). These cells are also termed as Insulin-producing cells (IPC's). DILPs released from the IPC's are mainly involved with nutrition regulated development in Drosophila. Rulifson et al. (57) studied the role of brain IPC's, by its ablation using the cell-death promoting factor called, Reaper. They found genocopies of Drosophila InR mutants, showing phenotypes like small sized adults, reduced wing size, and increased level of glucose and trehalose in the haemolymph suggesting that ILP's produced by IPC’s plays an important role in development and energy metabolism. The IIS pathway acts in conjunction with target of rapamycin pathway (IIS/TOR) (Figure 1). Differential occurrence of nutrition in the environment causes the release of ILP’s in accordance to crosstalk with several neuropeptides and endocrine hormones.

The ILP’s on binding with InR initiates a phosphokinase signal transduction via several downstream growth regulators. The InR via phosphorylation of adaptor protein Chico, a vertebrate homologue of Insulin receptor substrate 1-4 (58) recruits and activates phosphoinositide 3-kinase (PI3K). PI3K mediates the phosphorylation of phosphatidylinositol-4, 5-bisphosphate (PIP2) at the third position to generate phosphatidylinositol-3, 4, 5-triphosphate (PIP3). PIP3 are involved in mediating a wide range of intracellular functions. PIP3 interact with several kinases through their pleckstrin homology (PH) domain. The cytoplasmic kinases Akt and PDK interact via their PH domain with PI domain of PIP3 and phosphorylates and activate themselves and further acts on downstream targets. The action of PI3K is inhibited by phosphates and tensin homolog (PTEN). The targets on which Akt mainly acts in Drosophila are Forkhead related transcription factor (dFOXO) and Tuberous Sclerosis Complex 1 and 2 (TSC1/TSC2). Akt phosphorylates dFOXO at conserved serine/threonine residues thereby reducing its transcriptional activity consequently promoting cell growth. Similarly, TSC1/TSC2 is also inhibited by Akt which in turn inhibits TOR through Rheb. Work of Puig et al. (59) provides comprehensive details about dFOXO acting as a key component of insulin signaling pathway involved in regulating downstream protein transcriptional activities as well as controlling the upstream feedback signaling of insulin signaling pathway in case of nutritional deficiency (60). . dFOXO when unphosphorylated activates a translational repressor called d4E-BP which acts as a metabolic brake in stressful environmental condition (61). 4E-BP inhibits the recruitment of translation initiation complex at 5’ end site in mRNA on binding with eukaryotic initiation factor 4F (eIF4E) thus blocking translation (62). The TOR pathway in synchronization with insulin receptor signaling controls growth in Drosophila in response to local nutrient conditions. TOR pathway specifically acts in response to the presence of amino
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5.2. Interaction of DILPs with neuropeptides involved in feeding

The DILPs in addition to regulating several physiological and developmental pathways also contributes to feeding process. The interaction of DILPs with several neuropeptides modulates feeding behavior. Wu *et al* (64) elucidated the role of dS6K, one of the effectors of IIS pathway of Dilp neurons in hunger driven behavior. They proposed the model for regulation of hunger driven behaviors in *Drosophila* larvae. According to Wu *et al* (64) two of the Dilp neurons, i.e., dilp 2 and dilp 4 are involved in down regulation of the feeding response. When the larvae are well fed, the hunger driven behaviors are suppressed through the high level action of IIS/TOR signaling. On account of this, dS6K activity is increased, thereby promoting the levels of dilp 2 and dilp 4 in dilp neurons. The DILPs negatively regulate NPF/NPFR1 dependent pathway for motivated feeding and NPF independent pathway for promoting feeding rate, hence suppressing the feeding process. When the larvae are starved, the hunger driven behaviors i.e. increase in feeding rate and acceptance of lower quality food is mediated via the down regulation of dS6K activity and henceforth through down regulation of *Dilp* signaling. The decreased *dilp* signaling causes the disinhibition of NPF/NPFR1 dependent and NPF independent pathway involved in feeding process (Figure 2).

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Figure 1. Insulin/TOR signaling pathway in *Drosophila*: The *Drosophila* insulin like peptides (DILPs 1-8) released from several sites all over the body acts on *Drosophila* insulin like receptor (dInR) and initiates a signaling cascade. The cascade involves activation of insulin receptor substrate Chico, Phosphatidylinositol 3-Kinase (PI3K) and subsequent conversion of Phosphatidylinositol 4, 5 biphosphate (PIP2) to Phosphatidylinositol (3, 4, 5) - triphosphate (PIP3) for activation of Protein Kinase B (PKB)/Akt. The phosphatase and tensin homolog (PTEN) inhibits the action of PI3K. The Akt in normal nutritional situation inhibits Forkhead Box class O (dFOXO) transcription factor thereby promoting cell growth. In case of nutritional deficiency dFOXO gets activated and in turn activate 4E- Binding Protein (4E-BP) which is a translational repressor. Akt also inhibits Tuberosus Sclerosis Complex 1 and 2 (TSC 1 and 2) and Ras homolog enriched in brain (Rheb) which negatively regulate Target of Rapamycin (TOR) pathway. The presence of high intracellular amino acids (AA) activates Rheb and finally TOR complex. dTOR stimulates protein synthesis by activating ribosomal protein S6 kinase (dS6K) and Transcription intermediary factor 1A (TIF-1A).
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The action of LK’s is also found to be associated with DILPs. The set of 26 LK producing neurons in *Drosophila* is reported to express InR and regulate water homeostasis and food intake in a coordinated fashion (44, 65). When LK neurons were inactivated, flies having phenotype bloated abdomen and decreased feeding behavior were produced. Knockdown of InR in LK neurons produced similar phenotypes with decreased feeding activity, elucidating the role of InR in LK action (65). Mushroom bodies (corpora pedunculata), a neuropil structure has a role in olfactory processing and associative learning in *Drosophila* and other insects. Zhao and Campos (66) studied the role of insulin signaling in mushroom body neurons in the context of food intake. The inhibition of insulin signaling in mushroom body neurons, disrupted starvation induced food intake, growth of foraging third instar larvae as well as reduction in number of neurons. The actual neural circuitry involved and the role of insulin signaling in the mushroom body for feeding process still needs to be unravelled. Another neuromodulator Ast A which has a role in feeding and foraging activity in *Drosophila* has been revealed to be an important coordinator of feeding behavior and nutrient homeostasis (67). DILPs and AKH are known to have an antagonistic effect on the *Drosophila* hemolymph sugar, aiming to provide energy homeostasis. Dar-2, one of the receptors of Ast A, is expressed in the IPCs and AKH producing cells and is regulated by the different sources of Ast A. Ast A are known to act on these two types of cells. Action of Ast A on AKH and IPC’s identified by Hentze *et al.* (67) proves to be an important factor in stabilization of metabolic programs in nutrient dependent manner particularly in response to carbohydrate and protein and thus ultimately effecting feeding choices. The motivation for feeding is followed by foraging in order to search for the food resources. Olfaction plays one of the major roles in locating the appropriate food source, which is followed by gustation (68). Neuropeptide sNPF involved in gustational activity is also known to be involved in olfaction in *Drosophila* and is expressed in odorant receptor neurons (69). During the period of starvation, sNPF activity in *Drosophila* promotes food searching through increased olfactory sensitivity. While in normal circumstances when presence of food is sufficient, high insulin signaling inhibits sNPFR, the receptor of sNPF (70).

6. PERSPECTIVE

The plasticity in the feeding responses of a fly is dictated at numerous levels. From the peripheral...
level of environmental perception of nutrition to intrinsic state of an organism, all control the prandial behaviour in Drosophila (Figure 3). Decoding the plasticity in feeding activities and its regulation through neuromodulators is an important step in understanding of metabolic diseases, feeding related disorders and obesity in human population. The invertebrate model organism Drosophila has proven to be an indispensable system for understanding the neural regulations of such decision making processes. We provided comprehensive details regarding studies in peripheral inputs through gustation, to modulation of decision making process as well as role of insulin signaling in sensing of nutrition and feeding process. The overall advancement in feeding studies had laid the foundation for further understanding of the full complexity of feeding process that needs to be answered. The presence of gustatory receptors in places other than taste organs allows a perfect evaluation of food without being ingested in Drosophila. These receptors are also known to convey the internal state of nutrition to the brain in flies. However, the exact nature and function of these receptors and neurotransmitters involved in the process still need to be identified. In addition, the full repertoires of receptors for identification of major macronutrients such as fatty acids, and proteins are still to be characterized. Numerous studies have been done regarding the role of neurotransmitters involved in the plasticity in the feeding process independently in Drosophila, however, there is scarcity in knowledge about the interneuron’s that mediates crosstalk between gustatory information and neurons involved in motor outputs as well as how physiology governs the decision process.

The progressions in development of assays that quantify feeding preferences and feeding rate (71-74) with the identification of mechanistic pathways involved have been able to provide us with the understanding of physiological and behavioral changes with the internal state of an organism. The studies paves further the future direction to understand the full complexity of the feeding decisions in the context of the neuronal, physiological and behavioral process.

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5) - triphosphate; PKB: Protein kinase B; PTEN: phosphatase and tensin homolog; dFoxo: Forkhead Box class O; 4E-BP: 4E- Binding Protein; TSC 1 and 2: Tuberous Sclerosis Complex 1 and 2; Rheb: Ras homolog enriched in brain; dS6K: Drosophila S6 kinase; TIF-1A: Transcription intermediary factor 1A

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**Send correspondence to:** Arvind Kumar Singh, Genetics Laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, India, Tel: 09415877997, E-mail: aksbhu23@rediffmail.com