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Chapter

Gut Feeding the Brain: *Drosophila* Gut an Animal Model for Medicine to Understand Mechanisms Mediating Food Preferences

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Abstract

Fruit fly, *Drosophila melanogaster* is a most powerful animal model for exploring fundamental biological processes and modeling molecular and cellular aspects of human diseases. It provides the flexibility and tool box with which scientists can experimentally manipulate and study behavior as well as gene expression in specific, defined population of cells in their normal tissue contexts. The utility and increasing value of a sophisticated genetic system of flies, the tool box available for studying physiological function, functional imaging, neural circuitry from gut to brain, taste receptors expression and controlling gene expression by determining the specific cells in the intestine, makes fly gut the most useful tissue for studying the regulation of feeding behavior under changing internal state. To understand the intestine and its connectivity with the brain, *Drosophila* has proved an ideal model organism for studying gut brain axis aspects of human metabolic diseases. Various markers and fly lines are available to characterize the expression of transgenes in the intestine. The newly generated genetic tools aim to streamline the design of experiments to target specific cells in intestine for genetic manipulations based on their type and location within physiologically specialized intestinal regions. This chapter will be useful for understanding post-ingestive sensing system that mediate food preferences and to investigate fundamental biological processes and model human diseases at the level of single cells in the fly gut. Furthermore, the utility of adult fly gut can be extended to the study of dietary and environmental factors relevant to health and disease by screening for cells and micro circuits stimulated by internal state or the consumption of various nutrients.

Keywords: gut brain axis, *Drosophila*, taste receptors, enterocytes, enteroendocrine cells

1. Introduction

Mammalian central nervous system (CNS) consists of brain and spinal cord. The neuronal network extends from the brain to all over the body and various neurotransmitters help transmit the message to target cells in different tissues. The part of the nervous system located in our gut is called the enteric nervous system (ENS). In all animals, gut brain axis is a lesser known nervous system so far. Our

gut or "second brain" (ENS) chemically connects with the brain through neurons, secreted chemicals like hormones and neurotransmitters that send messages to the brain. The enteric nervous system's network of neurons and neurotransmitters extends along the entire digestive tract – it starts from the esophagus to the stomach and intestines, and down to the anus. The "gut microbiome" comprises of microorganisms living in the gut (bacteria, viruses, and fungi) that can affect the chemical messages that pass between the gut and the brain. Microorganisms residing in the gut help regulate the body's immune response. Since, the brain and the gastrointestinal (GI) system are intimately connected, therefore, they play key roles in certain diseases and to maintain our overall health to regulate cognitive and digestive behavior. The bidirectional communication between the brain and digestive system hence, are opening up avenues to think about diseases considering this angle.

Gut has recently become a subject of research in medical sciences wherein subjects with depressive symptoms, Parkinson's and Alzheimer's disease, autism, amyotrophic lateral sclerosis, multiple sclerosis, pain, anxiety and other neurodegenerative conditions are beginning to be looked to see what is going on in the gut. Effects of conditions like ulcers, constipation, and other GI problems have been a focus of research on aspects of brain functioning. The enteric microbiota impacts the gut brain axis (GBA), interacts locally with the intestinal cells and ENS as well as with the CNS through neuroendocrine and metabolic pathways. These studies suggest that GBA plays a vital role in maintaining mental health and can affect the feeding behavior when nutrient detection or absorption does not function properly as in case of metabolic conditions.

To ensure stability in the internal environment of body and drive adaptive changes, control mechanisms are key to animal's survival. Recently GI has been recognized as a major source of signals modulating feeding behaviors, food intake, metabolism, insulin secretion and energy balance. Through its interaction with microbiota, it can shape our physiology and behavior in complex and sometimes unexpected ways. A growing scientific community has exploited the genetic amenability of *Drosophila* gut in great and resourceful ways. In this chapter, we are shedding some light on a broad range of biological questions revolving around gut-brain axis, neural connectivity and its role in regulating food preferences by using inter-organ signaling and disease state, especially metabolic and neurodegenerative diseases. Despite being a relatively new research area for fly biologist, many of the mechanisms active in the intestine of flies have already been shown to be more widely applicable to gastrointestinal systems of higher system and humans, and may therefore become relevant in the context of human pathologies such as metabolic disorders, neurodegenerative diseases, gastrointestinal cancers, aging, or bowel disorders. This chapter will be summarizing our current understanding and knowledge of function of the adult *Drosophila* digestive tract with a major focus on gut-brain neural connectivity and role in digestive/absorptive functions.

2. *Drosophila* as an emerging model system for studying gut: comparison of human and fly gut

Easy genetic manipulation and effortless genetic tools make flies an insect of choice to study inter organ neuronal signaling including gut-brain axis neuronal connectivity. To understand human metabolic diseases and how a GI play a key role there is a recent focus of research. Some progressive studies also draw a link between neurodegenerative disorders and gut microbiota of humans and other insects. *Drosophila's* assistance for studying these and metabolic diseases with respect to its gut will be covered in this chapter.

Fruit flies have a simple and similar to humans- gut system (**Figure 1**). In mammals including humans, the esophagus (*Drosophila* foregut) passes the consumed food to the stomach (crop in flies), where food stores and digestion proceeds. Nutrient absorption takes place in the small intestine (anterior midgut in flies). Later nutrient, water and electrolyte absorption commences in the large intestine (fly hindgut). Finally, it reaches the rectum and anus for excretion (**Figure 1**) [1, 2]. In flies, after food passes through the middle midgut (a region of low pH, contains the iron and copper cells), it transits through the posterior midgut for further absorption and through the hindgut and rectum to exchange water and electrolytes and finally reaches the anus for excretion. Malpighian tubules (renal-like structures) are tubular excretory organs in flies connected to the midgut-hindgut junction and they absorb solutes, water and waste from the surrounding hemolymph, and release them in the gut in the form of solid nitrogenous compounds (**Figure 1**) [3]. Though the malpighian tubules are drastically different from human kidneys, similarities have been seen in function and development.

The adult *Drosophila* gut is like a tube structure lined by an epithelial monolayer comprising of four cell types: intestinal stem cells (ISCs), absorptive enterocytes (ECs), secretory enteroendocrine (EE) cells, and enteroblasts (EBs) (**Figures 1** and **2**). Ectodermally derived foregut consists of esophagus, crop, and cardia (**Figures 1** and **2**). The crop is a diverticulated structure unique to Diptera. A complex array of valves and sphincters ensure passage of intestinal matter in and

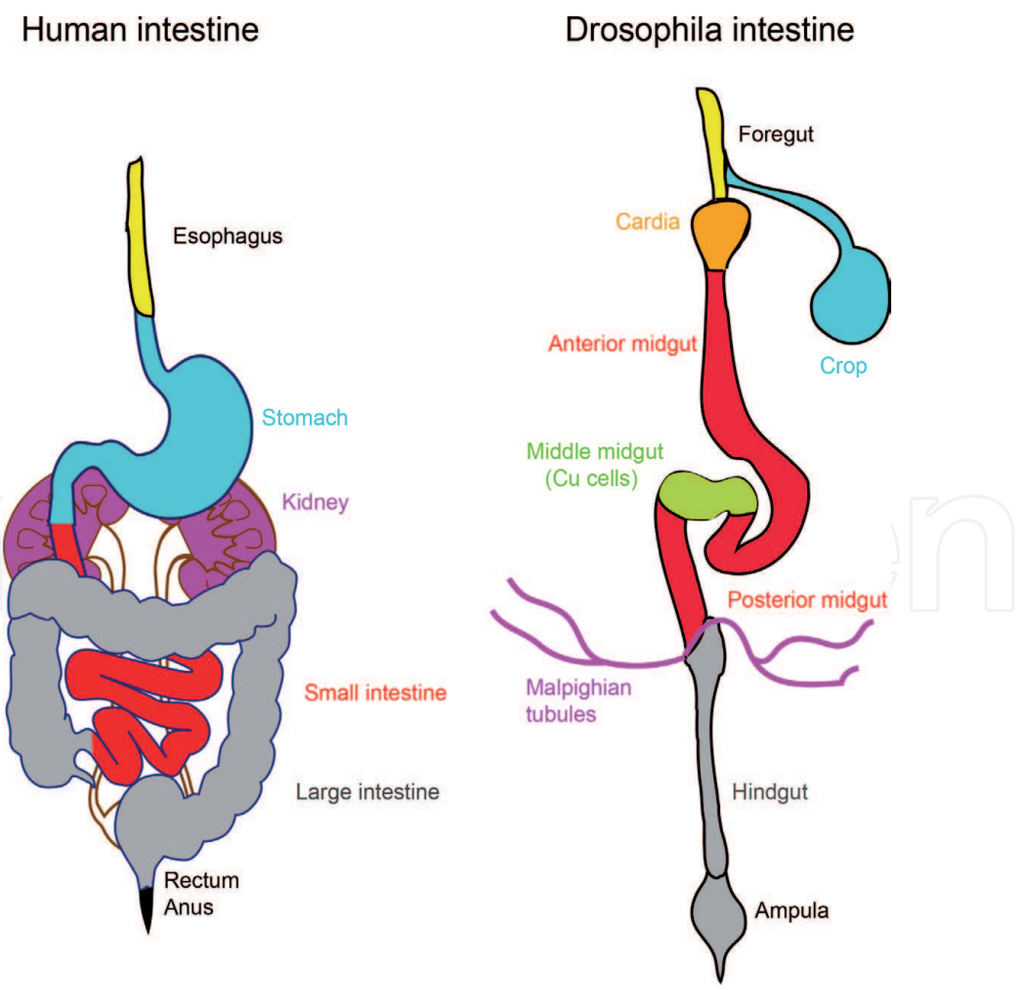

Figure 1.
Comparison between human and Drosophila gut. Organs with similar functions are coded with same colors. Drosophila contains many tissues/organs that functionally resemble to most essential human gastrointestinal system: Esophagus (foregut), midgut (small intestine) and large intestine (hindgut), stomach (crop), kidneys (malpighian tubules).

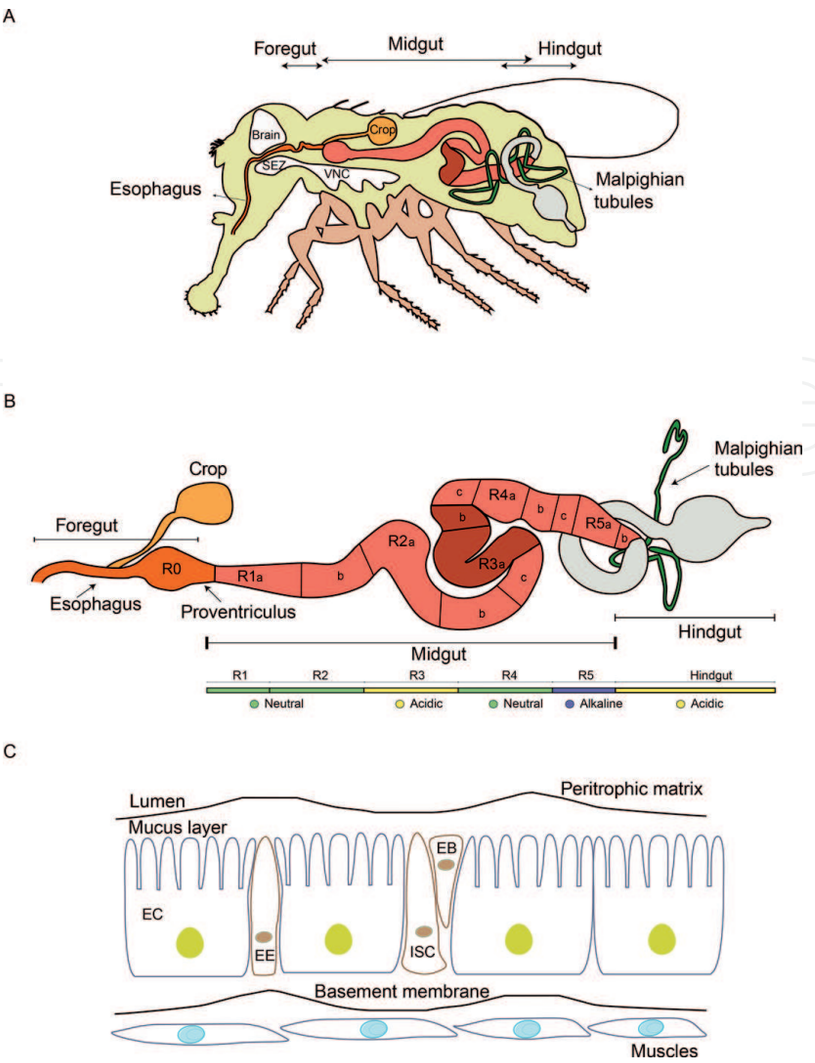

Figure 2.
Fly gut anatomy. (A) the Drosophila gut-brain axis consists of central nervous system (brain and ventral nerve cord-VNC; shown in white), gastrointestinal system (foregut, midgut and hindgut), crop and malpighian tubules. (B) the whole fly gut is divided into foregut (esophagus, crop and proventriculus), midgut (R1-R5), and hindgut (gray). pH divisions are also observed in midgut. (C) In Drosophila gut epithelia, the epithelium is protected by the peritrophic matrix and thin mucus layer apically and is covered in a basal lamina and visceral muscle cells. The fly midgut is composed of absorptive enterocytes (ECs) and secretory enteroendocrine cells (EE) that stand up from differentiation of the basally embedded intestinal stem cells (ISCs). Enteroblasts (EBs) are transient progenitors destined to differentiate into ECs.

out of the crop into the main alimentary canal. Crop function is poorly determined, its function in processes like early digestion, detoxification, microbial control, and food storage in flies has been speculated from other insects [4]. The cardia (or proventriculus) is a complex bulb-shaped structure composed of three epithelial layers. It makes the peritrophic matrix (site of antimicrobial peptide production) [5, 6] which may act as a valve, regulating the entry of ingested food into the midgut (**Figures 1, 2B** and **C**). Posterior to the cardia is endodermally derived midgut (with average length of 6 mm in adult flies), the main digestive/absorptive portion [7, 8] (**Figures 1, 2A** and **B**). It has been found that fly midgut epithelial cells have an opposite arrangement of junctions, with occluding junctions above *adherens* junctions, as in mammals [9]. The visceral muscle surrounds the epithelium. It is protected toward the lumen by secreted mucus and, posterior to the foregut, by a chitinous layer (peritrophic matrix) [10] (**Figure 2C**).

The fly midgut has been segmented into the anterior, middle and the posterior midgut (**Figures 1, 2A** and **B**). It has been further subdivided morphologically and molecularly into 10–14 regions (**Figure 2B**) [11–13]. Midgut regionalization has been

seen in the muscles, trachea and neurons that surround it [12–15]. Physical properties (*e.g.*, luminal pH), histological and cellular features (villi size, lumen width), stem cell proliferation rates, and gene expression profiles [11–13, 16, 17] have been used to characterized all midgut regions. The middle midgut (R3) contains a copper cell region in R3ab, which produces gastric acid, followed by a large flat cell region (R3c) with uncertain role (**Figure 2B**). Two boundaries flanking this region are inflection points where the midgut folds stereotypically inside the body cavity.

The Malpighian tubules release at the junction between the midgut and hindgut (**Figures 1**,**2A** and **B**). The water/ion exchange occurs in the hindgut which consist of pylorus (a second valve-like structure), ileum, and rectum [7, 8]. The muscles that surround the epithelium in flies are striated, as opposed to the smooth muscles found in mammalian intestines [18]. An outer layer of longitudinal muscles found surrounding the midgut. Circular muscles are found to be present throughout the fly tract. Physiology of the intestine is maintained and regulated by autonomic innervation and by hormones. The tracheal system forms a branched structure surrounding the gut during development [15] and may influence epithelial regeneration in the adult. Owning to similarity of flies and human gut, we will be discussing further how gut of the flies is handled and controlled to understand about neural circuitry drawing it closer to the brain and diseases related to intestinal illnesses.

2.1 Intestinal anatomy of *Drosophila* and human

Both humans and fly intestines share similar tissue, anatomy and physiological function [19, 20]. Their gut are of endothelial origin in nature [21, 22] and comprise of an epithelial monolayer of columnar or cuboidal ECs. A series of sequential depressions called the crypts of Lieberkühn, along the small and large intestine, and protruding villi along the internal surface of the small intestine in mammalian intestinal epithelium maximize its surface area [23]. Extensive folding has not been reported in the *Drosophila* intestine. Cytoplasmic extensions (microvilli) of the apical side of ECs and ISCs [24] do increase the cellular surface area facing the gut lumen in both flies and mammals. Microvilli spread parallel to each other toward the lumen to form the brush border [24–26]. A layer of mucus present above the brush border protects the host from intestinal microbes. The peritrophic matrix in *Drosophila* gut helps to sequester microbes from coming in contact with the midgut and hindgut [27, 28] (**Figure 2C**).

In both flies and mammals, the epithelial monolayer is associated on its basal side on an extracellular collagenous matrix (known as basement membrane) [29]. A checkerboard of innervated and trachea-oxygenated longitudinal and circular muscles tissue underneath the basement membrane in flies drive the peristaltic movements [30] (**Figure 2C**). Mammalian intestine has a similar organization of intestinal external musculature in the outer layers where musculature is also innervated and oxygenated by a plexus of vasculature [31, 32]. Layers including, the submucosa, (a dense layer of connective tissue containing nerves and lymphatic and blood vessels); muscularis mucosae (an additional muscle layer); and the lamina propria underling the intestinal epithelium and contains connective tissue, lymph nodes (Peyer's patches), immune cells (leukocytes, and dendritic and mast cells), vessels and myofibroblasts [33], fill the space between the outer musculature and the basement membrane in mammals.

2.2 ISCs, ECs, and EE cells of fly gut

About 65% of human-disease causing genes are shared as a functional homolog in fruit flies. This shows conservation of genes and function at an evolutionary

level. Fundamental processes such as digestion is also conserved from flies to humans. *Drosophila* intestine is composed of many cell types of heterogeneous developmental origin. Adult multipotent ISCs are present in both fly and mammalian guts [34–37]. ISCs differentiate throughout to self-renew and form new specialized cells namely absorptive type ECs and secretory type EE cells (**Figure 2C**) [38]. ECs and EE cells are found in both mammals and flies. ECs help in absorbing nutrients. EE cells release hormones for gut mobility and function. They also have antimicrobial purposes which are fulfilled by analogous cells in humans such as goblet and Paneth cells [39, 40]. *Drosophila* intestine produce both mucus and AMPs, but secretory cells (mucus-producing goblet cells) and the AMP-producing Paneth cells of mammalian gut have not been found in *Drosophila* midgut [28, 41]. Mammalian ECs and secretory cells are located at the bottom of the crypts and specifically express Lgr5 and/or Bmi1 (stem cell markers). Both of these cell types can give rise to all lineages of intestinal cells, including the transient amplifying (TA) cells that lie immediately above ISCs. TA gradually move upwards while maturing to eventually reach complete maturation close to the opening of the crypts. There is a continuous turnover of TA cells, which are either shed or become apoptotic upon maturation [23, 42].

The lineage of fly posterior midgut with only one type of mature absorptive cell and one main type of secretory cell is very simple. Although asymmetric ISC divisions in the fly midgut produce transient cells (EBs), these cells do not undergo further cell division and remain close to the ISCs before maturation. Fly midgut ISCs are situated basally and are broadly dispersed in the intestinal epithelium. The cellular composition and regeneration in *Drosophila* hindgut are interestingly similar to mammals. As in mammals, the ISCs of the hindgut are specified anteriorly and move posteriorly, as TA cells do, before their further differentiation in the posterior hindgut [37]. Nonetheless fly hindgut has not been examined as extensively as the midgut which has served as the prototype *Drosophila* tissue for the study of intestinal pathology [43–45].

2.3 The fly intestine functions

Like the regional specialization of digestive functions, the expression of digestive enzymes has also been found to confined to specific segments of the digestive tract in flies [12, 46, 47]. In addition to its roles in nutrient extraction and utilization, the digestive tract responds to the food and bacteria in its lumen. Digestion takes place in fly midgut [48] which can be further modulated by various factors like temperature, redox potential, pH, and intestinal transit [8, 48]. It has been shown that the expression and activity of digestive enzymes are tightly regulated in many insects like enzymes involved in the breakdown of sugars in flies are enriched in anterior (R1/R3) portions of the adult midgut and Peptidase genes express more posteriorly [47].

The enzymatic activity of the intestine is a key factor determining availability of certain nutrients. A substantial reduction of intestinal digestive enzyme activities (trypsin, chymotrypsin, aminopeptidase, and acetate esterase) has been reported in flies lacking EE cells [49]. Though not extensively investigated in *Drosophila,* modulation by nutrient quality and quantity, neuronal activity, and endocrine signals has been described in many insects [8, 50, 51]. Models suggesting role of ECs in integrating information about sugar uptake (sensed intrinsically in the intestine by Mondo-Bigmax) and the carbohydrate status of the fat body (relayed by TGF-β/Activin signaling) to modulate expression of the carbohydrate digestive enzymes have been proposed. Repression mechanism involving the TGF-β/Activin ligand Dawdle (Daw) which, upon refeeding with nutritious sugars

(but not non-nutritious sugars) after a period of starvation, reduces the expression of carbohydrate digestive enzymes in the adult ECs [52]. Activation of the intracellular sugar sensor complex Mondo-Bigmax promotes the expression of both Daw and the transcription factor *sugarbabe (sug)* [53]. Sug further repress the expression of amylases. Low cholesterol in the diet upregulate expression of the Hr96 nuclear receptor (homologous to the vertebrate LXR receptor involved in regulated cholesterol homeostasis) [54]. Hr96 binds cholesterol and promotes the expression of genes involved in cholesterol homeostasis and lipid breakdown including Magro (Mag) [54–56]. Mag plays a dual role in breaking down intestinal cholesterol esters to maintain cholesterol homeostasis. It also enables triacylglyceride (TAG) breakdown, required for intestinal lipid absorption and peripheral fat accumulation [56, 57]. It has been suggested that intestinal mag expression can also repressed by a sugar-rich diet in a foxo-dependent manner [58]. Such a mechanism becomes chronically active in the aging intestine due to disrupting lipid homeostasis and activation of JNK pathway affecting the metabolic homeostasis [58].

2.3.1 Role in nutrients absorption

Carbohydrates: A diverse array of transporters internalizes simple sugars into the ECs for further digestion and absorption [59] in insects like Glucose transporters, the GLUT/Slc2 family of facilitative glucose transporters and the SGLT/Slc5 family of Na^+-glucose symporters [60–64]. GLUT-like gene has been described in flies [65]. *Drosophila* genome harbors homologs of other glucose transporters including a homolog of SWEET family of sugar transporters [66, 67] a disaccharide transporter Slc45–1 [68]; trehalose transporters (Tret1–1 and Tret1–2) [69] and Slc45–1 (can transport sucrose) [68, 70]. The possible intestinal activity of many of these transporters deserves further investigation.

Proteins: A mixture of amino acids, di- and tri-peptides are products of protein break down. This chemical diversity is handled by a broad range of apical and basolateral transport systems (many are homologous to known mammalian transporter systems) [59, 71]. *Drosophila* homologs of cationic amino acid transporters [72], ion-dependent and independent amino acid transporters for neutral amino acids [73–76] and oligopeptide transporters [77, 78] are some examples. Intestinal expression of amino acid transporters Pathetic [74] has also been reported. Minidiscs [73], NAT1 and other Slc6 family members [75, 79], and the oligopeptide transporters Yin and CG2930, with enriched expression in proventriculus/hindgut and midgut [77, 78] has been shown. The nature, physiological modulation, and significance of many of these amino acid/oligopeptide transporters remains to be investigated.

Lipids and sterols: Intestinal lipid transport in *Drosophila* is still undetermined. Intestinal cells absorb free fatty acids, glycerol, mono- and diacylglycerols, and phospholipid derivatives (products of lipid digestion) along with dietary sterols. Diffusion and emulsification have been proposed for absorption [80]. Vertebrates emulsify by covering lipids with bile salts, but in insects emulsification is achieved by forming fatty acid-amino acid and glycolipid complexes, as well as fatty acids and lysophospholipid micelles [80]. In ECs, the products of lipid breakdown are used to resynthesize diacylglycerols and TAG. They get packaged together with cholesterol and fat body-derived carrier proteins to form lipoprotein particles and trafficked throughout the body [81] ensuring that the products of lipid breakdown are kept at low concentrations inside the ECs, which may facilitate diffusion. Mutants in which lipoprotein secretion from the fat body is compromised has revealed both anterior and posterior midgut regions as sites of lipid efflux [81]. The absorption of sterols is crucial to insects as they cannot synthesize sterols and require a dietary source of sterol for the synthesis of the steroid molting hormone ecdysone. In *Drosophila*

Niemann-Pick Chomologs-Npc1a and Npc2a are broadly required for intracellular sterol trafficking [82], whereas Npc1b is expressed in the midgut and is required for intestinal sterol absorption [83].

Changes in the expression of p38 kinase or the Atf3 and Foxo transcription factors cause accumulation of neutral lipid in ECs [58, 84]. It has been shown that neutral lipid increase following depletion of the EE hormone Tk [85], or in sterile female flies after mating [86]. It has been suggested that activation of intestinal lipogenesis is key to survival in diet-restricted flies. Indeed, nutrient scarcity induces expression of the sugar sensor transcription factor *sug* in the intestine which, in turn, promotes intestinal lipogenesis. Internal nutritional challenges may be equally dependent on deployment of these intestinal adaptations [86].

2.3.2 Intestinal pH

Many animals generate localized regions of low pH inside the intestinal lumen to facilitate protein breakdown, absorption of minerals and metals, and limit the survival of ingested microbes. While mammalian digestion takes place in acidic conditions, insect digestion occurs at neutral or basic pH including *Drosophila* (neutral or mildly alkaline). Luminal pH does, however, display consistent transitions along the length of the intestine and becomes strongly acidic (pH 2–4) in the copper cell region of both larvae and adults [24, 87, 88]. Posterior to this region, the midgut lumen becomes mildly alkaline again (pH 7–9), but is again acidified in the hindgut (pH 5), partly as a result of discharges from the malpighian tubules. Diet affects the acidity of rectal ampulla where final pH adjustments may take place [14]. Copper cells are specialized ECs with a highly invaginated apical membrane, similar to the mammalian gastric parietal cells [89]. During aging in adult flies, genetic interference with copper cell identity or their progressive loss are associated with loss of gut acidity [90].

The contribution of five ion transporters enriched in the acidic region have been studied [88]. These include: the potassium/chloride symporter Kazachoc (Kcc), a member the Slc12 family of electroneutral cation-chloride transporters (express in intestine) [91, 92]; the Slowpoke pore-forming subunit of a calcium-activated K^+ channel (express in neurons, muscles, tracheal cells, and two types of midgut ECs in the copper and iron cell regions) [93]; the ligand-gated chloride channel pHCL-2 which, in addition to regulating fluid secretion in malpighian tubules (express in the copper cell, iron, and large flat cell regions of the midgut) [94, 95]; the carbonic anhydrase CAH1; and the bicarbonate/chloride exchanger CG8177, belonging to the Slc4a1–3 subfamily of anion exchangers (express in a specific midgut pattern similar to that of pHCl-2) [96]. Collectively, these findings suggest that the transport of H^+, Cl^-, K^+, and $HCO3^-$ contributes to acid generation in the *Drosophila* midgut.

2.3.3 Water and osmolytes

Flies extract water from their diet to maintain hydration and ionic balance. This compensates for substantial water loss resulting from metabolic and physiological processes. Although malpighian tubules are important for this process, but intestine also contributes. Water absorption from the food occurs in the insect midgut and in rectal pads of rectum [8]. The rectal pads are also the crucial site for reabsorption of ions. Ions and water can cross the intestinal epithelium through or between cells and their transport play an important role in the maintenance of ion gradients that sustain active transport in the intestinal epithelium. The scanning ion-selective electrode technique (SIET) provides a way to probe intestinal gradients for ions such as K^+, Na^+, H^+, or Cl^- [24, 97]. K^+ and Na^+ absorption occur largely in the large flat cell and posterior regions of the midgut and, also in the anterior hindgut in the

case of Na^+ [97]. The two *Drosophila* Nha members express in intestine epithelia and their ubiquitous knockdown decrease survival, especially under Na^+ stress [12, 98–100]. Including kcc, four different genes encoding homologs of the cation-Cl^- Slc12 cotransporters express in osmoregulatory organs (gut, anal pads, and Malpighian tubules) [91, 92].

2.3.4 Metal ions

Metal ions such as copper, iron and zinc are essential micronutrients required for the correct folding and activity of a broad range of enzymes. The contribution of the intestine to metal homeostasis has not been extensively investigated but midgut regions, the Cu cell and Fe regions, are the most proposed sites of metal ion absorption. The Cu cell region turns bright luminescent orange upon Cu ingestion due to the fixation of copper by metallothionein [101, 102], and appears to be an important site of accumulation of ingested radioactively labeled Cu [101, 103]. The Fe cell region in R4a stains by Prussian blue and also accumulates exogenously administered radioactive Fe [101, 104]. Many studies have confirmed the roles for the Cu/Fe regions by exploring the molecular machinery involved in the intestinal uptake, intracellular trafficking, and efflux of metal ions.

2.3.5 Transit and excretion

Nutrient extraction and utilization may get affected by the passage of food along the alimentary canal and by its subsequent excretion. Transport of food to travel the entire length of the digestive tract takes less than 1 hour [105] in flies. As suggested, the amount of food retained in the crop is much larger in starved flies then refed flies than in flies fed *ad libitum* [2, 105]. Starvation also lowers defecation rate long before the gut is emptied [14]. Chronic food deprivation during the larval life has been shown to subsequently increase excretion in adult flies [106]. The hindgut may contribute to the pH adjustment of excreta, which may help offset the excess acid produced [14]. Changes in intestinal fluid retention are likely to involve the distal part of the hindgut (rectum and/or rectal glands), as known for its role in water reabsorption in other insects [8], and may help maximize absorption at a time of high nutritional demand. Such a mechanism is partly mediated by the sex peptide transferred by males during copulation [14, 107], affecting the HGN1 (Hindgut Neuron1) subset of hindgut-innervating neurons [14]. Further investigations are required to clarify the connections between intestinal fluid retention, absorption, peristalsis, and excretion where crop may prove to be a key organ, given that its differential peristalsis and engorgement can determine whether food is temporarily stored or released into the midgut for digestion and absorption [4]. Apart from affecting the nervous system, mutations in the *drop-dead* gene are also associated with increased crop size, reduced transfer of ingested food from the crop to the midgut, and reduced defecation [108, 109].

3. ENS and GBA

The ENS in humans, equivalent to GBA in flies is a part of the peripheral nervous system (PNS) that governs the running of the neurons which influence the GI. It is exploited nowadays in flies to understand more about how the two organs affect one another and lead to decisions regarding appetite, feeding mechanisms, taste preference and how to deal with hunger and satiety. How taste receptors detect different nutrients in the gut remains to be explored.

3.1 Gustatory receptors in gut

3.1.1 Humans

In humans, G-protein coupled receptors (GPCR) are involved in detection of five common tastes- sweet, salty, bitter, sour and umami. T1R family of taste receptors determine sweet and umami flavor. T2R family includes the bitter receptors [110]. Sweet taste receptors (T1R family) are found in intestinal tract as well as EE cells [111]. T1R1, T1R2, T1R3 and α-gustducin are expressed in the stomach, intestine and colon of humans and mice [112]. Cells of duodenal villi show co-localization of T1R1, T1R3 and α-gustuducin. Mammalian Gustducin protein is involved in bitter and sweet taste signaling and detection [113, 114]. α-subunit of gustducin is expressed in gastric cells and may play a role in nutrient detection [115–117] in rats and mice. Present in the mucosal lining of mammals and taste cells of the epithelium suggesting its possible role in taste uncovering on exposure to luminal contents [118]. Expression of T2R receptors in mouse GI tract including mT2R119 and mT2R108 has been looked into. mT2R119 expression is found in gastric and intestinal tissues, tongue and liver [115] like mT2R134 [118]. mT2R108, mT2R138 are present in the fundus, antrum, duodenum and tongue (not in liver) [119].

It has been found that sweet taste receptors stimulated in rat intestine influence and increase glucose absorption [120] through GLUT (glucose transporter) [121]. Presence of sugar in the diet galvanizes ECs into action to release hormones which in turn activate SGLT (Na^+ / glucose cotransporter) [122, 123]. Similar results are shown in sheep where sugar receptor / sensor present on the luminal membrane stimulates SGLT1 via cAMP and G-protein dependent pathway [124]. Equine T1R2 (homologous to cows and pigs) is expressed on the luminal membrane of EE cells in the small intestine. In response to increased sugars, T1R2 along with T1R3, stimulates SGLT1 and enhances the ability of gut to absorb more glucose [125]. Analysis of in vitro line of ECs suggest that T1R2 and T1R3 detect sweet taste and exposure to sucralose increases release of hormones such as GIP (Gastric Inhibitory Polypeptide) and GLP (Glucagon like peptide) which further activate glucose activation and metabolism. An inhibitor of these receptors inhibits glucose metabolism suggesting these receptors present in the gut alter feeding mechanisms and post-ingestion decisions [120, 121].

3.1.2 Flies

Gustatory system in *Drosophila* includes the taste receptors spread all over its body including proboscis, legs, wings and ovipositor. These receptors help in detecting the appropriate nutrient rich food, and avoid toxic chemicals. Because of the similarities in structure and functioning of mammalian intestine and flies' gut, the expression of gustatory receptors (Grs) in flies gut has been investigated. Using Gal4/UAS system, 15 Grs (*Gr28b.e, Gr33a, Gr36c, Gr39a.a, Gr39a.b, Gr43a, Gr64a, Gr93a, Gr28a, Gr59a, Gr28b.a, Gr28b.b, Gr28b.c, Gr28b.d, Gr58c*) are found to be expressed in gut, but only 12 of these Grs labeled EE cells in the midgut of fly [126]. Different nutrient sources to monitor the activation of EE cells in midgut have been used. With minimal sucrose, EE cells show high activation in middle midgut [127]. Other than sugar, protein cues also leads to the activation of EE cells but in posterior midgut, suggesting a role in the detection of nutrients in the diet including amino acids specifically. These cells do not get activated by carbohydrates per se and only react to proteins and amino acids. This subset of EE cells also co-expresses neuropeptides such as Diuretic hormone 31 (*DH31*) and Tachykinin (*Tk*). These brain-gut peptides get involved in feeding pathways and nutrient sensing mechanism

[85, 127, 128]. In other organisms such as rat, it has been shown that Gq-coupled calcium sensing receptor such as CaSR, expressed in EE cells is involved in amino acid sensing [129]. Taste cells or EE cells in mammals also produce several peptides which have roles in feeding, satiety, hunger as well as metabolism. They include Glucagon, Neuropeptide Y, Peptide YY and some others [130]. *Drosophila* has homologous proteins for these and other peptides which points toward obvious conservation of these peptides and their functions.

3.2 Gut-brain Neural Circuits

3.2.1 Humans

Nutrient signaling and sensing are fundamental processes that animals including humans and flies undergo [131]. Proper coordination and communication between gut and brain is necessary to regulate metabolic homeostasis and physiology in all animals. In this regard, many research groups have shown the role of enteric neurons and endocrine signals as important mediators of these processes. The way the enteric nervous system communicates to the brain via neural circuits is a multifaceted question and poorly explored. In mammals such as humans, alterations in neuropeptides and brain – gut hormone levels can derail people otherwise on the path to a healthy life. These changes can also lead to diseases such as neurodegenerative diseases, metabolic syndrome and diabetes [132].

Gluconeogenesis is a biochemical pathway by which animals make sugars from non-carbohydrate precursors and sources [133]. It is used to regulate homeostasis and a stable internal state in post – fed state [134]. Studies in rats showed that stimulation of intestinal gluconeogenesis (IGN) sends a signal from sodium – glucose co- transporters present at the intestinal mucosa to the brain, initiating a neural gut – brain axis [135–137]. Diets rich in protein [138–140] and fiber [141] promote IGN stressing on the importance of nutrient sensing for initiating several gut – brain axis [137]. It has been found that μ – opoid receptors (MOR) regulate IGN. These receptors (present in the nerves in the portal vein wall) react to neuropeptides to stimulate a gut – brain neural circuit that affects IGN, hunger and satiety mechanisms [141]. Further analysis of MOR deficient mice shows the role of MORs in regulating food intake, referred as "reward" system [142, 143]. Analyses of MOR-knockouts (MOR-KO) demonstrate how they play a role in managing satiety effects of alimentary proteins, through a neural gut-brain circuit [140].

Vagus nerve (VN; pneumogastric nerve) is the longest cranial nerve [144] in humans which runs from the medulla oblongata in brain to colon in GI [145]. It innervates other structures as well such as larynx, pharynx, heart and lungs thus affects digestive, cardiovascular and respiratory system – all at one [146]. Vagal efferent send down signals from the brain to gut, which accounts for about 10% – 20% of all the nerve fibers. Remaining 80% is accounted for by the vagal afferents carrying information from the gut to the brain [147]. Vagal sensory neurons in the GI keep an eye on stomach volume and luminal contents through different neural circuits [148]. VN contains and branches into several sensory neurons (~2300 in mouse) that further innervate and render support and supply to other internal organs. A variety of sensory neurons, one side facing the brainstem and the terminal one facing the organ such as GI [149] have been revealed. Free terminals of vagal afferents are rooted within lamina propria of intestinal villi [148]. Some mammalian models like in cat and rat, it has been shown how these sensory neurons detect different nutrients in diets with the help of unambiguous and explicit fibers [150–152]. Vagal afferent endings in the intestine express several mechanosensitive as well as chemical receptors [153]. Glucagon- like peptide 1 (GLP1) is a gut

hormone receptor that intercedes the nutrient sensing mechanism via VN [154]. GLP1R (GLP1 receptor) is present in many cells [155]. Agonists for GLP1R show how it affects brain further proving its presence in both, gut and brain [156]. Another receptor of vagal afferents, GPR65 near the intestinal villi, plays a role in nutrient detection drawing attention to how these sensory neurons are a part of the gut – brain axis [157, 158]. It detects serotonin and impact gut motility [147]. Such receptors detect several hormones present in the gut, like choleocystokinin (CKK), ghrelin and leptin which play a role in the regulation of hunger and satiety [159–161]. Because of its role in gut motility and mobility, VN and its afferent neurons present in the gut play a role in Intestinal Bowel Syndrome (IBS) [162] and new treatment plans around the same are being looked at in rat [162] and mice [158] models.

To take the findings in vagal nerves forward, nerves allowing communication of cNST (caudal nucleus of the solitary tract) with gut were focused on. Information about sugar detection to cNST via gut – brain axis is a topic of research nowadays. In live mice, it is noticed that glucose detection by cNST is robust and VN transaction silences that activation [163]. Nodose ganglion of vagus nerve when silenced prevents the sugar preference of cNST [163] suggesting the presence of a physical gut – brain axis. It has been shown that inactivation of sugar-activated cSNT prevents the mice to choose sugar from water or an artificial sweetener [163]. This study specifically calls attention to how organisms have paths for detecting nutrient signals, sensing them in the diet and also have circuitries to carry forward the signals and communicate with the rest of the body, purposely the brain.

3.2.2 Drosophila

With the help of several markers and reporter genes, it is found that *Drosophila* intestine is innervated by neurons- efferent and sensory [14]. Other studies have stated that fly's gut receives innervations from three regions – stomato-gastric nervous system [164–167]; the *corpora cardiaca*, neurosecretory structures [168]; and neurons located in the CNS extending their axons toward three different portions of the digestive tract [14, 169–172]. The expression of Ret receptor tyrosine kinase in gut innervating neurons in adult fly has recently been shown to contribute to the development of stomato- gastric ganglia in flies [173, 174]. In contrast to mammalian gastrointestinal tracts which are profusely innervated throughout their entire length, the innervation of the fly's digestive tract is restricted to only three different portions. The first is the anterior-most slice comprising the pharynx, esophagus, crop and anterior midgut. The second is midgut/hindgut junction and third is the posterior hindgut [14, 164, 167, 172]. Muscle valves present in all three regions support and regulate peristaltic regulation and intestinal transit functions of gut-innervating neurons. Most neurites terminate on the visceral muscles and some reach the underlying epithelium, particularly in the esophagus, proventriculus, pyloric valve, and rectal ampulla [14, 175] suggesting neuronal regulation of epithelial properties such as secretion or absorption. In flies, not all innervation is efferent. Gustatory neuron afferents from the pharynx send their axons to the suboesophageal zone (SEZ, the primary taste center of the fly brain), where they target a distinct domain adjacent to the projections of other (leg/labellum) gustatory receptor neurons [176–179]. Dendrites of peripheral sensory neurons can be seen in the anterior and posterior-most regions of the digestive tract [14], and appear most abundant in the esophagus and anterior midgut.

In the anterior portion of adult and larval midgut, serotonin positive neurites and various neuropeptides including Akh, Dh44, Myosuppressin, and possibly Allatostatin C and FMRFamide (or an FMRFamide-like peptide such as the NPY-like

neuropeptide short neuropeptide F [sNPF]) [131, 168, 172, 180–182] have been described suggesting chemical diversity of enteric innervation. Four serotonergic neurons are found to innervate the enteric nervous system in fly larva. These neurons project from the antennal nerve (AN) near the SEZ and extend throughout the ENs [14]. These projections end at the anterior region of the midgut and are primarily around the proventriculus region and the foregut. These innervations are considered structurally analogous to the mammalian VN because of similar projections from the brain to the different structures of the foregut. It is yet to be seen if there are functional similarities as well [172].

Pigment- dispersing factor (Pdf), Ion transport peptide, and Proctolin positive neurites have been reported in the larval and adult hindgut [169, 183–186]. All three innervated regions receive insulinergic innervation from the CNS; the *pars intercerebralis* (PI) insulin-producing cells extend axons beyond the ring gland that innervate the anterior midgut and crop in adult flies (**Figure 3**), and the insulin-like peptide 7 (Ilp7)-producing neurons of the abdominal ganglion innervate the midgut/hindgut junction and the rectal ampulla [170, 187]. Interestingly, putative dendritic termini of both kinds of insulin-producing neurons have been found in very close proximity in the CNS. This data suggests the release of different insulins to the different portions of the digestive tract may be co-regulated centrally [14].

Functional studies of insect innervation have primarily focused on the control of peristalsis and peptide hormone secretion so far. Studies of peristaltic regulation in flies have primarily concerned the effects of neuropeptides (Allatostatins, Myosuppressin, or Drosulfakinins) on *ex vivo* intestinal preparations [188–191] ascribing distinct roles for these peptides in the modulation of crop or anterior midgut contractions in adults. Both intestinal and non-intestinal roles of Pdf-expressing neurons in the regulation of muscle peristalsis for a set of hindgut-innervating neurons located in the abdominal ganglion of the CNS have been demonstrated [171, 192]. It is found that this neural source of Pdf (a neuropeptide related to mammalian vasoactive intestinal polypeptides, known for its roles in the central circadian clock) promotes peristalsis of hindgut muscles and sustains the defecation cycle in larvae [192]. Pdf can also promote contractions of the muscles of the ureters, the proximal part of the malpighian tubules [171]. Hence, the digestive tract is used by some enteric neurons as a docking site to exert their functions on other internal organs at some distance.

Recently epithelial roles for gut-innervating neurons e.g. role in the control of fluid balance have been revealed by a semi-automated analysis of defecation behavior in adult flies, providing quantitative readouts for food intake, fluid/ion balance, and intestinal transit [14, 193]. The HGN1 neurons (2–5 CNS neurons located in the posterior segments of the abdominal ganglion) innervate the hindgut and the rectum (**Figure 3**), with a subset of their neurites projecting through the visceral muscles to reach the underlying epithelium [14]. HGN1 neuronal silencing experiments resulted in increased defecation rate. These neurons are shown to be required for the post-mating changes in intestinal fluid retention due to their epithelial innervation. It has been established that HGN1 neurons and the Pdf hindgut-innervating neurons have their direct action on the hindgut and anal sphincter muscles [192]. A role for gut-innervating neurons in the maintenance of epithelial turnover has also been suggested by the finding of anatomical proximity between enteric neurites in the posterior midgut and adult somatic intestinal progenitors, and the reduced ISC to EC differentiation resulting from downregulating Hedgehog (Hh) signaling (albeit pan-neuronally) [194]. The more anterior innervation of the proventriculus may also play a role in maintaining gut permeability. This is inferred from the finding that inactivation of a relatively broad subset of neurons, including a subset of anterior midgut-innervating neurons results in an abnormal

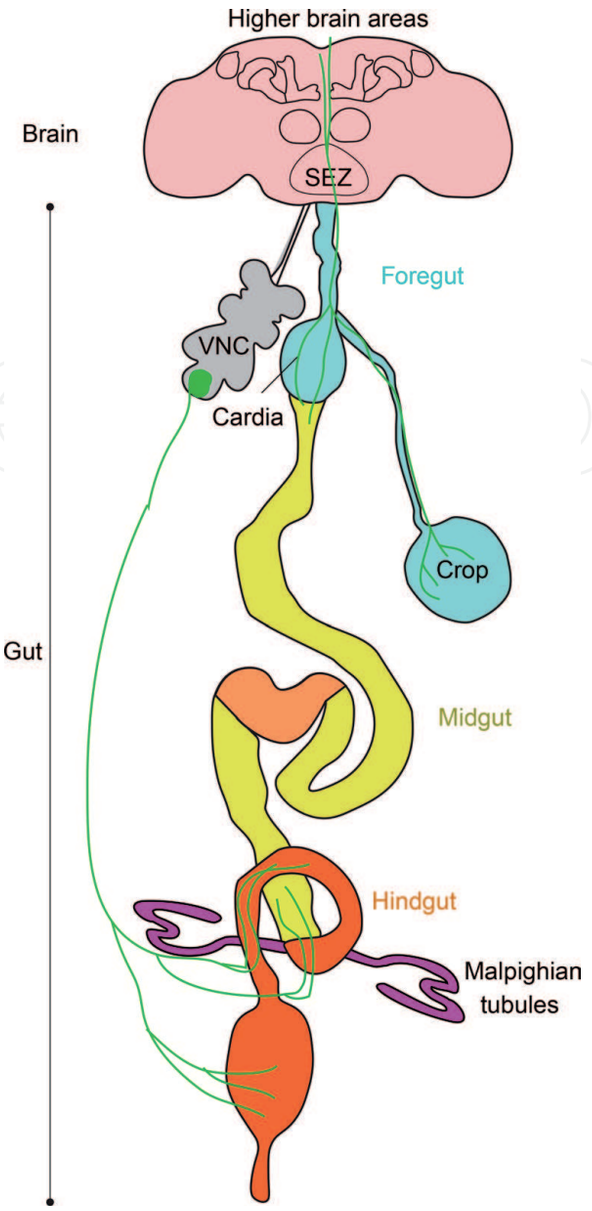

Figure 3.
Innervation of adult Drosophila intestine. Enteric innervation (shown in green) along Drosophila gut-brain axis (central nervous system and gastrointestinal system). Neurons from brain and enteric ganglia innervate anterior portion of gut. Neurons from ventral nerve cord send axons in the hindgut which also extend anteriorly along the posterior midgut. Adopted from Miguel-Aliaga et al., 2018.

proventricular structure, increased permeability of the epithelial barrier, and increased susceptibility to oral bacterial infection: all suggestive of defects in the production of peritrophic matrix [175].

In adult flies, inactivation of insulin-producing neurons results in contrasting effects on the hyperphagic response triggered by nutrient scarcity. Silencing of the insulin-producing cells of the brain PI that innervates the anterior midgut lowered this response, whereas silencing of the hindgut-innervating Ilp7 neurons increased it, and also resulted in higher circulating glucose [14, 195]. Not much is known about the importance of sparse sensory innervation of the intestine. One remarkable exception are the pharyngeal taste neurons. *Pox-neuro* (*poxn*) mutant flies lack gustatory function in the legs and labial palps but retain expression of sweet taste receptors in their pharynx and a preference for sweet compounds, highlighting the pharyngeal contribution to sugar detection [196, 197]. Further understanding of the taste circuit relaying this pharyngeal sensory signal is provided by IN1 neurons (subset of interneurons) receiving input from the pharyngeal sensory neurons.

The activity of IN1 neurons is exquisitely dependent on the amount and duration of feeding [198]. Posterior to the pharynx, in the gastrointestinal tract, the contribution of sensory innervation to nutritional homeostasis remains to be investigated.

Post-ingestive sensory feedback from the gut has been assumed to inhibit feeding based on work in other insects for example severing the recurrent nerve or the medial abdominal nerve, which transmit information from the gut to the brain, results in overconsumption in blowflies [199]. Work done in flies lends support to this idea; whereas severing the medial abdominal nerve did not disturb food consumption, severing the recurrent nerve elevated consumption of sucrose but not water or bitter tasting solutions [200]. The existence of neuronal stretch receptors on the gut that monitor the volume of ingested food is supported by both neurophysiological and anatomical data in numerous other insects [4, 80, 199, 201]. However, the existence and molecular nature of these receptors in *Drosophila* remains to be established. Interestingly, six peripheral neurons on the proventriculus have been shown to express the gustatory receptor Gr43a (function as fructose receptor), which is also expressed by some pharyngeal neurons [202–204]. These proventricular neurons extend dendritic processes into the foregut lumen, and a subset of their axons innervate the midgut, whereas another subset extends along the esophagus, forming a nerve bundle with axons of gustatory receptor neurons projecting toward SEZ. Hence, they may relay nutritional information back to central/more anterior neurons or act locally on the gut. Establishing their roles will require genetic tools able to target the enteric subset without affecting the central or peripheral Gr43a-expressing neurons.

4. Enteroendocrine hormones, neuropeptides and signals

4.1 Humans

The gut–brain transmission systems involve both circulation-based endocrine-like and neuronal communication routes [205]. Neuropeptides (transmitters or neurosecretory) act as messenger molecules of enteric, sensory, autonomic and central neurons. Several peptide families have been found in both brain and gut. They act as neuropeptides and/or gut hormones and have significantly contributed to the understanding of gut brain interaction. Central and peripheral neurons together with EE cells in the GI tract and other endocrinologically active cells produce variety of peptides [206–211] including hormones peptide YY (PYY) and pancreatic polypeptide (PP); neuropeptide Y (NPY), on the other hand [212]. These and other peptide families represented by glucagon-like peptide (GLP), ghrelin, cholecystokinin (CCK), corticotropin-releasing factor (CRF), leptin, osteocalcin and insulin (the last three are extra intestinal endocrine peptides) act on specific and genetically related groups of receptors that are expressed by distinct cells in the periphery and CNS. For their functional roles, endocrine peptides and neuropeptides are relevant for the regulation of digestion, control of food intake, metabolic homeostasis, and the impact of GI signals on sensation, emotion, affect, and cognition. Disturbances of the gut microbiota–brain axis result in changes of the expression and activity of many neuropeptides and their receptors in the CNS. Neuropeptides are therefore important secondary messengers of gut microbes in cerebral neuro circuitries that mediate the alterations in brain function and behavior that take place in response to changes in the GI microbial community [212]. Together it is emerging that neuropeptide systems such as NPY, CRF, ghrelin, and brain-derived neurotrophic factor (BDNF) play a particular role in the cerebral manifestations of gut microbiota perturbations.

In rats, choleocystokinin and glutamate neuro-epithelial circuit makes the communication between the brain and intestinal lumen possible stressing on the importance of a physical gut – brain axis [213]. It has been proposed that the physical connection between vagal nodose neurons and EE cells is present in rats which leads to regulation of gastrointestinal functions [213]. Intragastric nutrient infusion and optical readings of AgRP (Agouti-related protein) neurons in live and awake mice [214] suggest AgRP neurons affect long term homesostasis and energy balance of the body and do not get altered by minute changes in nutrient levels [215]. AgRP neurons get inhibited by high levels of satiation signals such as CCK and PYY (peptide YY). Receptors for both CCK and PYY are expressed by vagal afferent neurons' terminals that innervate GI [159] suggesting a possibility of a physical connection between the gut and brain carrying the message from one point to another [216].

4.2 *Drosophila* hormones and neuropeptides

Apart from using neurons, fly intestine can also communicate with other organs through systemic signals. Intestinal physiology is modulated by both extrinsic hormonal signals (emanating from endocrine glands, neuroendocrine structures, or organs such as the fat body) and by its own peptide hormones, produced by EE cells. In turn, gut-derived signals such as EE cell-derived peptide hormones can have long-range effects on other internal organs. EE cells accounts for 5–10% of midgut epithelial cells in flies compared to 0.4–0.6% in the mammalian small intestine [217–219]. Majority of them express peptide hormones, often more than one and with regional stereotypy [219–222]. The developmental program of EE cells shares similarities with that of neurons, probably reflecting a common phylogenetic origin [223–225]. Consistent with this idea, all known EE peptide hormones (exception insect CCHamides) [226] are also produced by the brain. Acting through these hormones, EE cells may play "neural-like" roles in regulating intestinal physiology and conveying intestinal as well as nutritional state to other cell types or organs. These roles are particularly prominent in the midgut given the relatively sparse innervation of midgut region. *Scute* mutant flies lacks all EE cells and show normal food intake and fertility, but are short-lived and display abnormal intestinal homeostasis [49].

A role for EE cells on muscle peristalsis has been suggested by the finding that ablation of Diuretic hormone 31 (Dh31)-expressing EE cells or Dh31 downregulation both reduce muscle peristalsis in the larval anterior midgut, which may function as a valve to minimize mixing of acidified and non-acidified food in the acidic region of the midgut [227]. Adult EE cells produce Bursicon. Signaling through the Bursicon/DLGR2 receptor in visceral muscle, represses the production of the visceral muscle-derived mitogen Vein and, consequently, ISC proliferation. Another study found in *scute* mutants, depletion on EE cells compromised the nutrient-dependent midgut growth that occurs post-eclosion [49] partly by the lack of EE cell-derived Tk, which normally promotes expression of the visceral muscle-derived Ilp3 insulin-like peptide shown to sustain ISC proliferation and nutrient-dependent midgut growth [49, 228]. A recent comparative fly–mouse–human study has pointed to neurotensin-like signaling from EE cells to ECs in flies, with effects on lipid metabolism and AMPK activation. Indeed, expression of mouse neurotensin from *Drosophila* EE cells (and possibly also peripheral sensory neurons) promoted lipid accumulation in both standard and high-fat diets in the midgut, fat body, and oenocytes, and also decreased gut AMPK activation [229]. The effect was dependent

on expression of the Pyrokinin 1 receptor in ECs, but did not seem to be mediated by EE cell-derived Pyrokinin 1, pointing to an involvement of a different ligand [229].

A high-sugar diet leads to increased midgut EE cell number and enhanced production of EE-derived Activin ligand (Activin-β not Daw) [230] suggesting systemic roles for EE-derived peptide hormones. Mirroring the activin-mediated fat-to-gut signaling involved in sucrose repression, midgut-derived Activin-β binds to the TGF-β receptor Baboon in fat cells which, in turn, leads to enhancement of Akh signaling it the fat body and consequent hyperglycemia [230]. CCHamides are insect hormones [231, 232] and their expression is promoted by nutrient availability and sites of expression include the gut EE cells, a subset of central neurons and, possibly, the fat body [226, 233, 234]. Their receptors are expressed in the nervous system including the insulin-producing neurons, and are absent from the gut [226, 234]. Although not strictly gut-derived, a new peptide hormone produced not by EE cells, but by an adjacent secretory gland may have provided the most compelling example to date of gut-to-brain communication. Indeed, Limostatin (Lst) peptide is produced by the *corpus cardiacum*: the Akh-producing gland which, in the adult, is found adjacent to the hypocerebral ganglion on the gastrointestinal tract, at the junction between the esophagus and anterior midgut. Lst is released in response to nutrient restriction and suppresses insulin production by the insulin-producing cells of the brain PI. *Lst* mutant flies accumulate excess fat and display phenotypes associated with insulin excess [235].

In animals with a vascular system, peptides secreted from EE cells can enter the bloodstream and reach tissues at a considerable distance, ranging from other cells in the digestive tract to brain centers regulating appetite [236]. Nutrient availability can also affect the number of EE cells; signaling through the nuclear hormone receptor Hr96, dietary lipids control EE differentiation during the first few days of adult life, providing another way to couple nutrient availability with tissue architecture and physiology [237]. Modulation of intestinal physiology by systemic signals has also been looked into [220, 221]. Control of epithelial turnover by insulin-like peptides or JH (juvenile hormone), and the coupling of dietary availability of sugars with EC digestive enzyme production via the fat body-derived Activin ligand Daw are some examples. The actions of the diuretic peptide Leucokinin (Lk), secreted into the circulation from CNS-derived nerves that terminate at the abdominal wall [14, 238, 239] is an another example. Downregulation of either this peptide or its receptor leads to abnormal excreta and extreme fluid retention that can rupture the abdominal wall [14]. Finally, a link between energy balance, intestinal permeability, and immunity has been suggested by the finding that sNPF is a target of the Crtc/CREB energy sensing pathway, and functions to maintain epithelial barrier integrity acting through its receptor in ECs [240]. Although the precise source of sNPF remains to be established, tissue-specific genetic and expression data points to roles in neurosecretory cells [240], consistent with roles as a neuroendocrine hormone or in gut-innervating neurons. Gut can also produce long-range signals to affect the physiology of other organs, for example by production of the signaling protein Hh by larval EC. Circulating Hh regulates developmental timing by controlling ecdysteroid production in the prothoracic gland, and is required for mobilization of fat body TAG stores during starvation [241].

Other functions of brain-gut peptides and hormones include detection and utilization of nutrients during hunger, stress or normal conditions. Diuretic hormone 44 (Dh44), a homolog of the mammalian corticotropin –releasing -hormone (CRH) activate by nutritive sugars. Disturbed activity of Dh44 neurons leads to fail to select nutritive sugars [131]. These neurons localized to PI in adult brain,

counterpart of mammalian hypothalamus [242] are filled with neurosecretory cells [131]. Dh44 conveys information from Dh44 neurons to Dh44 receptor R1 neurons in the brain and R2 cells in the gut suggesting requirement for nutrient selection. Artificial activation of these neurons causes rapid PER and it has been suggested that Dh44 is necessary and sufficient for gut motility and excretion in flies [131]. Both Dh44 neurons and the gut-innervating insulin-producing neurons of the PI are innervated by Hugin-producing neurons that suppress food intake and induce locomotion, providing a possible link between food-related behaviors and intestinal physiology [243]. It is seen later that Dh44+ neurons rapidly activate during amino acid feeding and are a direct sensor of dietary amino acids [244].

Fly gut peptide Dromyosuppressin [181] expresses in the number of cells in central nervous system (CNS) in adult stage, extending into the rectum, near the anus; part of the adult gut. Their immunoreactive fibers also project into the crop and show expression of Dromyosuppressin [172] and crop abundantly expresses Dromyosuppressin receptors (Dromyosuppressin receptor I) [220]. The effects of neuropeptide on neural regulation of crop motility and contractions have been shown [245]. Serotonergic neurons have also been shown to regulate insulin producing cells (IPC) located in the PI of the adult brain of the fly [246]. DILP2, 3 and 5 express particularly in midgut [187, 220, 247] and extend their axons to proventriculus, crop and corpora cardiaca [248]. DILP2 is particularly involved in carbohydrate metabolism [249]. Decreased levels of DILP2 affects stored trehalose as well [248]. IPC knockdown flies show increased glycogen storage, high levels of circulating triglycerides and extended lifespan [248].

Mammalian neuropeptide NPY (Neuropeptide – Y- precursor) has an invertebrate homologous peptide called NPF due to characteristic C- terminal F residue [250]. NPF has been shown to co- localize within midgut cells in *Drosophila* and in brain and it plays a role of co- transmitter in many neural circuits [251, 252]. Its receptors are expressed primarily in the malpighian tubules but also hindgut and midgut [253]. There are some regulatory peptides found in both gut and brain. Small neuropeptide-F (sNPF) is found in the neurons in the hypocerebral ganglion innervating the midgut as well. sNPF gene encodes four kinds of sNPFs and is predominantly found in the central nervous system suggesting it might be directly involved in several neural circuits that affect hunger and feeding mechanisms [254]. The receptor for sNPF is further identified and found to be expressed in the crop, Malpighian tubules, hindgut, and the midgut [253]. Over expression or knockdown of sNPF leads to an increase or decrease in adult feeding respectively [255]. sNPF also directly affects and alters DILPs levels in larval and adult IPCs [254]. RFAmides are a class of different neuropeptides, all containing a common C-terminal RFamide sequence [256]. Using antisera to recognize the gene products of the five genes in *Drosophila* genome encoding for RFAmides, endocrine cells in anterior, middle and posterior midgut are labeled [220], axons in the midgut and crop as well as hypocerebral ganglion. RFAmides also play a key role in food intake, sensing and feeding mechanisms pointing toward conservation in these pathways from insects to mammals [257].

Other neuropeptides include Allatostatin characterized into three kinds namely Allatostatin A/B/C [258–260]. The endocrine cells producing Allatostatin are found in the posterior midgut and is innervated by axons from thoracico-abdominal ganglion [220]. Its receptors, DAR1 and DAR2 are predominantly located in the central nervous system and gastrointestinal tract (including crop, midgut and hindgut) respectively [261, 262]. Allatostain is also present throughout midgut. Another major peptide is PDF. It is expressed in central nervous system [263] and its neurons are also found in thoracico-abdominal ganglion [184]. Axons from these neurons innervate midgut and hindgut and in the crop. These neuropeptides are closely associated to circadian rhythm [264] and locomotor activity as well.

5. *Drosophila* gut brain axis: role in health and disease

Various studies have suggested that intestinal health has a significant impact on neurodegeneration. Specifically, dysregulation of GBA cross talk has been associated with metabolic syndrome [265, 266] and psychiatric disorders. GBA is largely mediated by CNS, ENS and intestinal microbiota. With its much simpler gut microbiota (1–30 taxa) as opposed to vertebrates intestine (1–500 taxa) [267], *Drosophila* intestine provides an experimental tools where whole microbiota can be removed in its entirety to perform screens to probe the relation between gut flora and the brain disorders.

5.1 Metabolic disorders

Most genes and pathways that play crucial roles in metabolic diseases are conserved between flies and humans [268]. Diet-induced obesity in flies is associated with many of the pathophysiological consequences found in humans, including hyperglycemia, insulin resistance, cardiac arrhythmia and fibrosis, reduced longevity [269, 270] and nephrosis [271]. The gut is crucial for peripheral body fat storage and serves as a major site of dietary lipid absorption and absorbs other dietary macronutrients (sugars, proteins and fats). It also metabolizes both glucose and lipids into metabolic intermediates, which after loading into hemolymph get used in other tissues and organs. In flies, lipoprotein complexes containing apolipophorins carry sterols and diacylglycerols from the gut to other tissues [81]. Fly lipoproteins also contain Hh, a cholesterol-linked, gut-derived ligand that binds the transmembrane receptor Patched on fat body target cells to promote lipolysis during larval starvation [241, 272]. The human anti-obesity drug orlistat, a gastric lipase inhibitor, has been shown to reduce body fat accumulation in adult flies [56]. Supporting a crucial role for lipolysis, midgut lipid accumulation and global fat storage are reduced by the insulin signaling pathway inhibitor Foxo in enterocytes, via reducing the expression of *magro* as flies age [58]. Excessive lipid accumulation in the fly gut and fat body is also a feature of 'humanized' flies upon cross-species expression of the human peptide neurotensin in *Drosophila* midgut EE cells. Obesity in these flies is triggered by an evolutionarily conserved mechanism acting via the cellular energy sensor 5′ adenosine monophosphate (AMP)-activated protein kinase [229]. Additionally, the acidic pH of the gastric lumen may be important for fly obesity given that both global vacuolar-type H^+-adenosine triphosphatase (ATPase) mutants and flies treated with pharmacological inhibitors of alimentary acidity store extra fat [273]. This effect could be mediated via the gut microbiome, which both shapes and depends upon the acidity of the gut [88]. Collectively, these data emphasize the importance of gut physiology for fat homeostasis in *Drosophila* and highlight the intricate interaction between the gut epithelium and the gut microbiome.

The gut microbiota and its metabolism plays an important role in modulation of fat storage in the fly. As seen in humans, fly gut is enriched in *Lactobacillus* and *Acetobacter* species. Adult axenic flies overstore fats under various dietary conditions compared with natural gut microbiota flies [274]. *Lactobacillus* sp. abundance supports co-colonization by *Acetobacter* sp. in the adult gut, which in turn negatively correlates with the fat storage level of the fly [275]. The diet of a fly impacts the composition of the gut microbiota as a high-sugar diet shifts the gut microbiome to uracil-producing species, which promote fat storage and growth in *Drosophila* larvae [276]. The availability of dietary glucose to the adult fly depends on the microbiome because flies with commensal *Acetobacter tropicalis* eat more than axenic flies but store less TAG owing to the consumption of dietary sugar by the bacteria [277].

5.2 Neurodegenerative diseases

Alzheimer's disease (AD): AD is a progressive neurodegenerative disease characterized by senile plaques consisting of misfolded β-amyloid (Aβ) fibrils and oligomers [278]. Presence of hyper phosphorylated tau protein in the various regions of the brain including cerebral cortex, locus coeruleus, and hippocampus [279] has also been suggested. Microbial dysbiosis [280], dietary changes [281], probiotics [282], or a variety of other disease conditions [283, 284] results in involvement of the GBA in the pathophysiology of neurodegenerative diseases. Multiple studies have shown an association between gut microbiome dysbiosis and the aggregation of Aβ peptides in intestinal epithelial cells [285, 286] and the CNS [287, 288] after high-fat diet feeding. Many neurodegenerative diseases exhibit accumulation of fibrillary, misfolded proteins similar to the propagation of prionopathies in the CNS [289]. Prionopathy also involves the GBA and the local immune system, where prions accumulate in dendritic cells in the Peyer's patches and other lymphoid follicles once entering the intestinal epithelium layer [290]. Senescence-accelerated mouse model studies have identified systemic senile amyloid proteins in Peyer's patches [291]. By interacting with dendritic cells, the misfolded protein may transport to the ENS, and ultimately spread to the CNS compartment [290]. Gut bacteria can affect peripheral nerve functions through the production of neuromodulatory metabolites such as short-chain fatty acid (SCFAs) [292]. It has been suggested that Lactobacillus probiotics, given orally through feeding, showed improvement in the rough-eye phenotype, famous of the AD flies [293] and reduces *Wolbachia's* presence in the gut, which is known to be associated to neurodegenerative disorders. These studies emphasize on the relation between gut microbiota, GBA and the disorder. Pharmaceutical companies are hence targeting these probiotics and prebiotics in order to treat the disorder.

Parkinson's disease (PD): PD is a progressive brain disorder like other neurodegenerative diseases affects thinking, mobility, walking, balance and coordination. Research on PD using *Drosophila* has revealed links between gut microbiota composition and PD to one of the major genes *parkin*, which in an autosomal recessive fashion causes this disease [294, 295]. Both *Parkin* and *PINK1* genes disturb mitochondrial function and integration in PD patients. *Parkin* mutants shows steep rise in gut microbiota in comparison to control flies. Also *PINK1* mutants did not show much of a difference suggesting that gut microbiome gets affected independently in both autosomal recessive genes [294]. It is also found that *parkin* gene in ECs is required to maintain the microbial load. It has been seen that the microbial composition in *parkin* mutants is drastically different from those of wild-type flies.

Autism spectrum disorder (ASD): ASD is a developmental brain disorder characterized by impaired social behavior and disrupted communication and language. Loss of function mutants of histone demethylase KDM5 in *Drosophila* show how change in the abundance and composition of gut microbiota leads to impairment of social behavior, characteristic of ASD. Decreased levels of KDM5 cause intestinal epithelium disruption. As opposed to the control flies, the presences of gases produced by the overgrowth of bacteria cause bubble formation in the midgut in mutants [296]. It is found that KDM5 mutant flies has different composition of gut flora than controls [296]. The administered of *Lactobacillus plantarum* to KDM5 mutant flies rescued social behavior and other intestinal defects. Flies supplemented with *L. plantarum* also shows 2.3 fold increase approximately in longevity [296]. *kdm5*$^{\text{JmjC}^*}$, another kind of KDM5 mutant displayed impaired gut permeability, intestinal epithelium and microbiota. In this case as well, administration of *L. plantarum* rescued gut permeability, the

defective social interaction and communication. These studies have helped establishing a link between gut microbiota and ASD. The probiotics that rescued ASD flies are good candidates for pharmaceutical companies to be sold as therapeutic. These results can be used for drug discovery and in treatment of these otherwise, untreatable disorders.

5.3 Intestinal dysbiosis and infections

Drosophila and its microbiome has provided invaluable information in understanding intestinal dysbiosis and chronic inflammatory infections. Two pathways that are present in *Drosophila* innate immune system to act against microbiota are immune deficiency (IMD) pathway (homologous to human NFκB pathway) and dual oxidase (DUOX) pathway. IMD pathway leads to anti-microbial peptide (AMP) synthesis whereas DUOX pathway leads to the formation of reactive oxygen species (ROS). Intestinal AMP overproduction changes the microbial community structure. This change is dysbiotic in nature because it leads to cell apoptosis of host cells [297]. IMD pathway over-activation leads to an increase in dysbiotic community structure and IMD inactivation leads to over-proliferation of community members in general [44]. Inflammatory bowel disorders also involve apoptosis of intestinal cells in humans which shows a link between the intestinal pathogenesis in both mammals and flies [297]. DUOX knockdown (DUOX-KD) flies show a high mortality rate after gut infections, which indicates DUOX-dependent ROS formation. These flies show huge amounts of microbial cells in the gut as opposed to wild-type flies. DUOX-KD flies survive normally in a GF environment. Inactivation of this pathway leads to host infections [298] whereas over-activation induces oxidative stress in host [299]. Extensive research in *Drosophila* midgut shows that intestinal epithelium damage induces inflammatory signals and growth factors to lead to ISC proliferation and tumor proliferation.

5.4 Gut-cancer model

Drosophila is an important tool in medicine to explore the details of cancer. There are a wide range of cancers where *Drosophila* is taken into consideration – from brain, gut to thyroid and lung. The loss of Adenomatous polyposis coli gene (*APC,* tumor suppressor gene) in flies leads to an increase in ISC proliferation in the gut [300]. This resembles the condition seen in intestinal adenomas (benign tumor of epithelial tissue). JNK-Wg signaling controls the number of ISCs found in the gut. Injured or damaged ECs lead to an increase in JNK signaling and increase in Wg ligands in (EB). This in turn activates JAK STAT ligands - Upd2 and Upd3, which further increase ISCs' non-autonomous over proliferation [301]. Loss of *Apc* in ISCs increases JAK–STAT pathway as well, which in turn affects ISCs proliferation. This helps in establishing the conservation of pathways that regulate ISC proliferation and gut homeostasis [302]. In some studies, it is also shown that *Apc* and RAS control growth of cells in gut by interacting with one another. Non-receptor tyrosine kinase c-Src is associated to quite a few forms of cancer including colorectal cancer (CRC). In wildtype *Drosophila*, orthologous forms of c-Src act exactly how they would in mammals. Activation in c-Src leads to ISC proliferation and decrease or inactivation in c-Src inhibits further ISC proliferation [303]. These results show beyond doubt how ISC proliferation is directly involved with cancer formation and these genes and mechanisms are conserved from flies to mammals. Few studies have looked into hindgut (equivalent of mammalian colon) as a model for cancer. Oncogenes in the hindgut synergize with innate immunity system to stimulate tumor cell invasion. After bacterial infection, ISCs proliferate but less in hindgut as

opposed to midgut. RAS oncogene, RAS^{v12} induces cell invasion and dissemination of ECs into the abdominal cavity. Upon RAS^{v12}expression, hindgut shows cancer-like phenotype. It activates JNK signaling pathway which in turn increases ISC proliferation and hence, tumor growth [304].

6. Conclusion and outlook

Recently *Drosophila* has emerged as a model to study intestinal infection and pathology because of conservation between *Drosophila* and mammalian intestinal pathophysiology, regeneration, and signaling pathways that control them. Real time and other *Drosophila* assays provide the complex cellular composition of a real intestine and opportunities to assess toxicity in a whole organism at a relatively low cost compared to mammalian system. Its simpler cellular structure, flexibility to doing screening, availability of molecular markers and other genetic tools adds up to its value as an ideal model system to study intestine. Also, it has been shown that human intestinal pathogens can cause intestinal pathology in flies as well.

In the recent past, data from the intestinal mechanisms found in flies have been shown to be active in mammals, and may therefore become relevant in the context of human pathologies including diabetes, obesity, neurodegenerative diseases, gastrointestinal cancers, or aging. Parallel findings of communication between gut and brain via neuropeptides in *Drosophila* and humans highlight on the importance of GBA in maintaining health. Identification of neural circuitries is of prime importance to apprehend more about the link between the gut and brain and their link with metabolic and neurodegenerative diseases. Greater understanding of gut-brain feeding circuits, paracrine signaling of peptides and physical communication via neural circuitries will establish their functioning in several metabolic disorders, neurological syndromes, aging and cardiovascular diseases. Research using flies on specific gut regions, cell types during various developmental stages and on stem cell biology and aging have been conducted so far. Other functions including role of unidentified taste receptors in the gut, their connection with the brain mediating nutrient digestion and transport, or organs such as the crop and the proventiculus, remain poorly characterized. Deeper understanding is required on the function of gut brain neural connectivity and to investigate what may be their key role in human or insect (patho)physiology concerning feeding behavior and appetitive learning. Techniques like *in vivo* CRISPR transcriptional activation (CRISPRa) and interference (CRISPRi) approaches [288, 305], to allow tightly regulated and reversible promoter activation and blocking in fly gut can be fruitful for future studies.

Developing new techniques and behavioral assays can help us explore physiological drives: what is the gut function to maintain the overall health of the animal. It would be interesting to find out the key intestinal sensors. How the physical association between gut and brain via neural micro circuits regulate decisions regarding nutrition, hunger and satiety is under question. Better "holistic" and quantitative methods, integration of spatial and temporal information about genetic events more comprehensively are required, so that cause and effect can be uncoupled in a physiological context [306]. We anticipate and hope that fly models of intestinal pathology, in addition to uncovering newly identified genes (chemosensory and others) and basic biology mechanisms will emphasize the most conserved aspects of human intestinal biology. As a result, fly will contribute to translational research investigating drug effects, and microbial and host genetic component analyses, leading to biological findings that are broadly applicable to human health and disease.

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Conflict of interest

The authors declare no conflict of interest.

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ZS, SK and PK all substantially contributed to the conception and design of the work. Everybody participated in drafting and revising the work, made the figures, wrote the chapter and approved the final version for publication.

Abbreviations

ENS	Enteric nervous system
GI	Gastrointestinal
GBA	Gut brain axis
CNS	Central nervous system
ISCs	Intestinal stem cells
ECs	Enterocytes
EE	Enteroendocrine
EBs	Enteroblasts
TA	Transient amplifying
AMP-5′	Adenosine monophosphate
AMPK- 5′	AMP activated kinase
TAG	Triacylglyceride
Sug	*Sugarbabe*
CD36	Cluster of differentiation 36
Npc1	Niemann-Pick C1
SREBPs	Sterol regulatory element-binding proteins
Akh	Adipokinetic hormone
RNAi	RNA interference
Kcc	Kazachoc
SIET	Scanning ion-selective electrode technique
Dh44	Diuretic hormone 44
GPCR	G-protein coupled receptors
PNS	Peripheral nervous system
GLUT	Glucose transporter
SGLT	Na^+/glucose cotransporter
GIP	Gastric inhibitory polypeptide
GLP	Glucagon like peptide
TK	Tachykinin
MOR	μ – opoid receptors
IGN	Intestinal gluconeogenesis
MOR-KO	MOR-knockouts
cNST	Caudal nucleus of the solitary tract
GLP1	Glucagon- like peptide 1

sNPF	Short neuropeptide F
AN	Antennal nerve
Ilp7	Insulin-like peptide 7
VN	Vagus nerve
IBS	Intestinal bowel syndrome
HGN1	Hindgut neuron1
Hh	Hedgehog
Poxn	*Pox-neuro*
Dh31	Diuretic hormone 31
Lst	Limostatin
Lk	Leucokinin
JH	Juvenile hormone
Crtc/CREB	cAMP-regulated transcriptional co-activator/ cyclic AMP-responsive element-binding protein
SEZ	Suboesophageal zone
CRH	Corticotropin –releasing -hormone
PI	Pars intercerebralis
IPC	Insulin producing cells
DILP	*Drosophila* insulin- like- peptide
5-HT_{1A}-5	Hydroxy tryptamine /serotonin
AKH	Adipokinetic hormone
PYY	Peptide YY
AgRP	Agouti-related protein
CCK	Choleocystokinin
PP	Pancreatic polypeptide
NPY	Neuropeptide Y
PDF	Pigment- dispersing factor
CRF	Corticotropin-releasing factor
BDNF	Brain-derived neurotrophic factor
SCFAs	Short-chain fatty acid
AMP	Anti-microbial peptide
ROS	Reactive oxygen species
DUOX	Dual oxidase
IMD	Immune deficiency
APC	Adenomatous polyposis coli gene
CRC	Colorectal cancer

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