1 **From mouth to anus: functional and structural relevance of enteric neurons in**
2 **the *Drosophila melanogaster* gut**

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17 Running title: Enteric neurons in *Drosophila*

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20 **Highlights**

21 In *Drosophila*, gut innervations are observed in the proventriculus, anterior and posterior midgut, and hindgut.

22 Ilp (Insulin-like-peptide)-producing neurons sense satiety to foods and are involved in feeding behavior in
23 adults.

24 Glutamnergic motor neurons are involved in regulating the defecation cycle in larvae.

25 Some enteric neurons control neighboring organs, such as the Malpighian tubules and the trachea.

26 NP3253-GAL4-positive neurons are required for the homeostatic control of gut integrity.

27
28 **Abstract**

29 The intestinal tract is the main organ involved in host nutritional homeostasis.
30 Intestinal function in both vertebrates and invertebrates is partly controlled by enteric neurons
31 that innervate the gut. Though anatomical and functional aspects of enteric neurons are
32 relatively less characterized in *Drosophila* than in large insects, analyses of the role of the
33 enteric neurons in flies have remarkably progressed in the last few years. In this review, we first
34 provide a summary of the structure and function of the *Drosophila* intestine. We then discuss
35 recent studies of the structure and function of enteric neurons in *D. melanogaster*.

36
37 **1. Introduction**

38 Insects flourish in every environment imaginable on earth and are highly adaptable, making
39 each type unique and diverse. More than a million species of insects are known to date, and they
40 derive their energy from plants, small animals, microbes, and even fellow insects. Insects

generally grow quickly, shortening the generation time and conferring on them a relatively faster evolutionary change that supports their prosperity on our planet. One of the key organs involved in the rapid growth from any type of food is the intestinal tract. The intestinal tract, the gut, is one of the largest organs in animals, including insects, implying its importance. The intestinal tract functions to store ingested food, digest food for extraction of nutrients to be absorbed, and excrete undigested residue. Peristalsis, a radially symmetrical contraction and relaxation of muscles that propagates in a wave down the intestinal tract, is controlled by motor neurons (Furness, 2012; Olsson and Holmgren, 2001), essentially aiding digestion processes. Food ingestion and its amount in the gut is sharply recognized by sensory neurons: stretching of the gastrointestinal muscle activates the mechanosensory receptors expressed in the muscle cells, transmitting the signals to the neurons (Furness, 2012). Such information is reflected to the secretion of digestive juices, which include degrading enzymes from the gut epithelia and digestive glands.

In mammals, 70% of neurons in the body are estimated to reside in the intestine. Auerbach's plexus provides motor innervation to the muscular layer, controlling mainly peristalsis, and also has some sensory roles (Baumgarten et al., 1970; Furness, 2012), whereas Meissner's plexus provides secretomotor innervation to the mucosa nearest the intestinal lumen, governing the secretion of digestive enzymes as well as ciliary movement of the epithelia (Goyal and Hirano, 1996). The endocrine system also modulates gut functions. Incretins, gastrointestinal peptide hormones secreted by the sensing of glucose and fat in the intestine, stimulate an increase in the release of insulin from the pancreas and slow the nutrient absorption rate into the bloodstream by reducing gastric emptying, which may directly reduce food intake (Baggio and Drucker, 2007). Then, blood sugar concentration is sensed again by the intestinal cells. Low glucose levels and an empty stomach stimulate the release of the peptide hormone ghrelin from specific cells in the gastrointestinal tract. Ghrelin acts in the central nervous system to induce appetite and feeding behaviors, as well as to increase gastric acid secretion and gastrointestinal motility to prepare the body for food intake (Kojima and Kangawa, 2005; Nakazato et al., 2001). Thus, the intestinal tract plays the role of a command center for controlling energy homeostasis.

These gastrointestinal roles of the nervous and enteroendocrine systems are well studied in mammals, but relatively less is known in insects except for the control of peristalsis (Donini et al., 2002; French et al., 2014; Lange and Chan, 2008; Robertson et al., 2012) and peptide hormone secretion (Dirksen et al., 2008; McCormick and Nichols, 1993; Palmer et al., 2007; Price et al., 2002). In particular, although *D. melanogaster* is utilized as a model animal for investigating developmental processes and innate immune responses, studies of gut physiology have been less prevalent (Dow, 2003). In the last few years, however, several important findings relating to the role of enteric neurons and the function and structure of the *Drosophila* intestinal tract have been reported. In this review, we first briefly explain the structure and function of the *Drosophila* gut, and then summarize recent reports on the functions of fly enteric neurons. For more information on the endocrine control of gut function,

81 please refer to other reviews (Veenstra et al., 2008).

83 **2. Structure and function of the *Drosophila* gut**

84 The *Drosophila* intestinal tract comprises a monolayer of epithelial cells, minor
85 populations of intestinal stem cells and enteroendocrine cells, and surrounding visceral muscles
86 (Buchon et al., 2013a; Kuraishi et al., 2013). Motor neurons and sensory neurons innervate the
87 muscles and epithelia of the digestive tract (Lemaitre and Miguel-Aliaga, 2013). A
88 semipermeable membrane called the peritrophic matrix lines the lumen (Hegedus et al., 2009;
89 Lehane, 1997). The gut is divided into the foregut, midgut, and hindgut, based on the
90 developmental origin. Ingested foods are stored in the crop (only in adults), a part of the foregut,
91 and then pushed into the midgut by the pumping action of the proventriculus. The midgut is the
92 main region of digestion and absorption of the ingested foods, and is further subdivided into
93 several regions based on histological and functional differences (Buchon et al., 2013b). The
94 hindgut is responsible for the absorption of water, salts, and other beneficial substances before
95 excretion. Blind-ended ducts called Malpighian tubules, which are attached at the anterior end
96 of the hindgut, are the main organs of osmoregulation and excretion (Dow et al., 1994). Cells of
97 the intestinal tract require oxygen. Fine chitinous tubes called trachea deliver oxygen and expel
98 carbon dioxide directly to and from the tissues (Samakovlis et al., 1996). Some trachea attach to
99 the gut and branch off into smaller tubes called tracheoles, which surround the intestinal tract.

100 The peritrophic matrix is an acellular structure that forms a layer comprising chitin
101 polymers and glycoproteins such as peritrophins, protecting the midgut epithelium from
102 abrasive food and microbes (Kuraishi et al., 2013). The peritrophic matrix is thought to be
103 produced in the proventriculus in *Drosophila* adults. Though structurally different, the
104 peritrophic matrix is considered analogous to the mucous layer of the mammalian digestive
105 tract. In *Drosophila* adults, drosocrystallin (*dcy*) is a structural component of the peritrophic
106 matrix, and a loss-of-function mutation of *dcy* causes reduced thickness of the peritrophic
107 matrix and higher permeability, and is associated with higher susceptibility to ingested
108 entomopathogenic bacteria or pore-forming toxins (Kuraishi et al., 2011).

109 Digestive enzymes and transporters are required for digestion and absorption,
110 respectively, in the midgut. Bioinformatic analysis revealed the presence of many digestive
111 enzymes including carbohydrases, lipases, proteinases, and lysozymes, and transporters such as
112 glucose and ion transporters (Buchon et al., 2013b). Only a few biochemical or genetic
113 functional studies of these molecules, however, have been performed to date. The expression of
114 digestive enzymes differs between each of the midgut regions, indicating sequential processing
115 of ingested foods along the intestine, and this regionalization involves the peritrophic matrix as
116 it attaches to many proteins and is considered to be where digestive processes occur (Hegedus
117 et al., 2009). With regard to the transcriptional regulation of digestive enzymes, a recent report
118 revealed that the expression of carbohydrases and lipase is suppressed in *Drosophila* adults fed
119 a glucose-rich diet (Chng et al., 2014). High glucose levels in the hemolymph, the insect blood,
120 stimulate the secretion of transforming growth factor β (TGF- β) from the fat body, an insect

equivalent of the mammalian liver. The TGF- β activates TGF- β /Activin signaling in the epithelial cells of the midgut, resulting in the repression of digestive enzymes. Insulin signaling is well known to be involved in sugar metabolism, and this study demonstrated that TGF- β signaling also plays an important role in metabolism.

The intestinal epithelium is frequently exposed to harmful pathogens, and thus must be armed with an efficient and powerful immune system to protect itself (Buchon et al., 2013a; Kuraishi et al., 2013). Currently two resistance mechanisms against invading microbes are known: production of reactive oxygen species and secretion of antimicrobial peptides into the hemolymph. Dual oxidase, Duox, is a plasma membrane-expressing NADPH oxidase involved in the production of reactive oxygen species in the gut (Bae et al., 2010). Pathogenic bacteria such as *Erwinia carotovora carotovora 15 (Ecc15)* release uracil that activates an as-yet unidentified G protein-coupled receptor, leading to the enzymatic activation of Duox through the G protein alpha subunit q protein (Gaq) - phospholipase C β (PLC β) signaling pathway (Ha et al., 2009a; Lee et al., 2013). Upon severe infection, Duox expression is upregulated through triggering the MEKK1/MEK3/p38 pathway, which activates activating transcription factor 2 (ATF2) thereby mediating Duox transcription (Ha et al., 2009b). Antimicrobial peptides can damage and kill microbes, and their expression is under the control of the immunodeficiency (Imd) pathway (Buchon et al., 2009; Ryu et al., 2008; Tzou et al., 2000). Pattern recognition receptor peptidoglycan recognition protein (PGRP)-LE and PGRP-LC sense Gram-negative bacteria (Bosco-Drayon et al., 2012; Neyen et al., 2012), then activate the Imd pathway, which induces nuclear translocation of the NF- κ B transcriptional factor Relish that is responsible for the expression of antimicrobial peptide genes (Lemaitre and Hoffmann, 2007).

3. Innervation of neurons in the *Drosophila* intestinal tract

For simplification, in this review, we use the term “enteric neurons” to refer to neurons with neurites extending into the proventriculus, the midgut, and the hindgut. In *Drosophila* larvae, intestinal innervations are observed in the proventriculus, anterior and posterior midgut, and the hindgut (Hartenstein et al., 1994; Schoofs et al., 2014; Spiess et al., 2008). These neurites are innervated from the ventral ganglion and the proventricular ganglion, which are located near the ventral nerve cord and the proventriculus, respectively. From those ganglia, further innervations are sent to the pharynx, esophagus, the ring gland, and the central nervous system, comprising a complex stomatogastric nervous system (Hartenstein et al., 1994; Schoofs et al., 2014). Anatomical details of the stomatogastric nervous system is relatively less understood in *Drosophila* compared to large insects (Braunig, 2008; Copenhaver, 2007; Hartenstein, 1997; Rand and Ayali, 2010). Most larval enteric neurons are thought to be motor neurons that direct the movement of intestinal visceral muscles, yet, as described in the next section, there is also sensory innervation in the larval intestine, at least at the posterior part of the hindgut. Thanks to the rich library of enhancer trap GAL4 lines of *Drosophila*, enteric neurons are classified on the basis of specific GAL4-positive criteria (Table 1, Fig. 1). TRH (Tryptophan hydroxylase)-GAL4-positive serotonergic neurons innervate the proventriculus

and the anterior midgut (Schoofs et al., 2014), Ilp7 (Insulin-like-peptide 7)-GAL4-positive neurons (Miguel-Aliaga et al., 2008) and PDF (Pigment-dispersing factor)-GAL4-positive neurons innervate the hindgut (Talsma and Christov, 2012), while HGN1 (Hypogastric nerves-1)-GAL4-positive neurons innervate the posterior hindgut (Zhang et al., 2014).

In *Drosophila* adults, the proventriculus, the anterior midgut, and the hindgut apparently receive innervation from the brain, the ventral ganglion, and the proventricular ganglion (Cognigni et al., 2011). For the regulation of peristaltic movement of the intestinal tract, the majority of the innervation is likely to be efferent, although the existence of dendrites indicates sensory innervation in the gut (Cognigni et al., 2011). With respect to specific GAL4 positivity, motor neuron driver OK371-GAL4-positive neurons and sensory neuron drivers ppk1.9-GAL4 and cha7.4-GAL4-positive neurons innervate the proventriculus and the hindgut (Cognigni et al., 2011). Muscles and epithelial cells of the proventriculus and the anterior midgut receive innervation from NP3253-GAL4-positive neurons (Kenmoku et al., manuscripts in revision). Insulin-peptide-producing neurons Ilp2- and Ilp7-positive neurons innervate the proventriculus and the hindgut, while GMR51F12-GAL4 and GMR48A05-GAL4-positive neurons innervate the proventriculus and the anterior midgut (Cognigni et al., 2011; Olds and Xu, 2014). The hindgut receives innervation from HGN1-, PDF- and odd-GAL4-positive neurons (Cognigni et al., 2011; Talsma and Christov, 2012; Olds and Xu, 2014).

<i>Larvae</i>	<i>GAL4</i>	<i>Tissue</i>	<i>References</i>
	TRH-GAL4	proventriculus, anterior midgut	Schoofs et al., 2014
	Ilp7-GAL4	hindgut	Miguel-Aliaga et al., 2008
	PDF-GAL4	hindgut	Talsma and Christov, 2012
	HGN1-GAL4	posterior hindgut	Zhang et al., 2014
<i>Adult</i>	<i>GAL4</i>	<i>Tissue</i>	<i>References</i>
	OK371-GAL4	proventriculus, hindgut	Cognigni et al., 2011
	ppk1.9-GAL4	proventriculus, hindgut	Cognigni et al., 2011
	cha7.4-GAL4	proventriculus, hindgut	Cognigni et al., 2011
	NP3253-GAL4	proventriculus, anterior midgut	Kenmoku et al., manuscripts in revision
	Ilp2-GAL4	proventriculus, hindgut	Cognigni et al., 2011
	Ilp7-GAL4	proventriculus, hindgut	Cognigni et al., 2011
	GMR51F12-GAL4	proventriculus, anterior midgut	Olds and Xu, 2014
	GMR48A05-GAL4	proventriculus, anterior midgut	Olds and Xu, 2014
	HGN1-GAL4	hindgut	Cognigni et al., 2011
	odd-GAL4	hindgut	Olds and Xu, 2014
	PDF-GAL4	hindgut	Talsma and Christov, 2012

Table 1 Enteric neuron GAL4 drivers of *Drosophila melanogaster*

4. Role of enteric neurons in feeding and excretion behavior

4.1. Feeding and excretion assays

In *Drosophila* larvae, feeding and excretion are simply assessed using dye mixed

with fly food, and the behavior is evaluated by observing the amount of color in the intestine or feces (Zhang et al., 2014). In *Drosophila* adults, a capillary feeder (CAFE) assay is considered to be a standard method to analyze feeding behavior (Ja et al., 2007). The CAFE assay can be used to evaluate the amount of meal volume and the frequency of ingestion by recording the consumption of liquid-dyed food contained in a glass capillary. To analyze defecation, Cognigni et al established a quantitative and semi-automatic protocol using a lipid probe that only becomes fluorescent after exposure to the fly's digestive tract (Cognigni et al., 2011). They named this probe Fluoropoo, which was used to supplement standard fly food for the assay. The total amount of feces produced can be easily quantified by this "Fluoropoo assay". Further, the addition of the pH indicator dye bromophenol blue (BPB) to the food with Fluoropoo provides information about the size, shape, concentration, and color of the excreta. The Fluoropoo assay also predicts food intake with appropriate controls.

4.2. Feeding behavior

Ilp-producing neurons are involved in feeding behavior in *Drosophila* adults. Suppression of Ilp7-GAL4-positive neurons results in an increased food intake, revealed by the CAFE and Fluoropoo assays (Cognigni et al., 2011; Olds and Xu, 2014). In parallel, the suppression leads to a high glucose concentration in the hemolymph of adult flies. Degenerin/epithelial sodium channels (DEG/ENaCs) are evolutionally conserved mechanosensory ion channels that sense mechanical tension and generate action potentials in expressed neurons (Hwang et al., 2007; Zhong et al., 2010). Pickpocket 1 (PPK1) belongs to the DEG/ENaCs family of proteins that functions in Ilp7-GAL4-positive neurons to sense satiety to foods. PPK1 repression by RNAi-mediated knockdown or pharmacological inhibition phenocopies the suppression of Ilp7-GAL4-positive neurons, i.e., resulting in increased food intake (Olds and Xu, 2014). On the other hand, suppression of Ilp2-GAL4-positive median neurosecretory cells (mNSCs), which synapse with Ilp7-GAL4-positive neurons at the ventral ganglion and innervate the foregut, results in decreased food intake. Taken together, these findings suggest that PPK1-expressing Ilp7-GAL4-positive neurons sense the extension of the hindgut by ingested food, and transmit the information to the mNSCs, which negatively regulate the appetite of the *Drosophila* adult.

4.3. Defecation behavior

Drosophila larvae display defecation cycles of stereotypic frequency. Glutaminergic PDF-GAL4-positive and HGN1-GAL4-positive motor neurons are involved in sequential contraction of the hindgut and anal sphincter to regulate this defecation cycle (Zhang et al., 2014). These subsets of neurons form functional synapses and exhibit a periodic firing pattern that is synchronized with the hindgut contraction. The opening of the anus is accompanied by defecation. The single anal sensory neuron, the PPK (pickpocket)-GAL4-positive neuron that innervates the anal sphincter and senses the stretch when the anal slit opens, provides feedback information to the PDF- and HGN1-GAL4-positive neurons. A mechanosensory ion channel, NOMPC (no mechanoreceptor potential C), is involved in the sensing of the radial stretch of the anal sphincter in the anal sensory neuron (Zhang et al., 2014).

5. Interaction of enteric neurons with neighboring organs

5.1. Malpighian tubules

The hindgut-innervating PDF-GAL4-positive neurons are considered to derive from a neuroendocrine cell lineage that produces the peptide hormone PDF (Siga, 2003; Veenstra et al., 2008). To analyze the visceral role of PDF, Talsma et al evaluated the similarity of PDF with the mammalian peptide hormone vasoactive intestinal polypeptide (VIP) (Larsson et al., 1976). VIP is a gut-brain hormone that acts on the G-protein coupled VIP receptor to mediate the contraction of smooth muscles of the intestinal or urinary tracts. They found that *ex vivo* treatment of the adult intestinal tract with PDF does not cause peristalsis of the midgut or hindgut, but interestingly, induces contraction of the Malpighian tubules (Talsma and Christov, 2012). The Malpighian tubules are a functional equivalent of the mammalian urinary organ, and are involved in detoxification and stress sensing (Davies et al., 2014). Muscles surrounding the tubules express PDF receptors. PDF-dependent contraction of the tubule muscles *ex vivo* is dependent on PDF receptors expressed in the muscles. Artificial activation of gut-innervating PDF neurons induces contractions of the tubules *in vivo*. These findings suggest that enteric neurons control neighboring organs, the Malpighian tubules, through peptide hormone secretion (Talsma and Christov, 2012).

5.2. Trachea

Insect respiration is accomplished without lungs. Instead, oxygen is delivered directly to tissues through the tracheal system, which comprises holes called spiracles on the insect exoskeleton that allow air to enter tubes (trachea), which are invaginations of the cuticular exoskeleton that branch throughout the body, including the intestinal tract. The smallest tubes, tracheoles, penetrate cells and serve as diffusion sites for water, oxygen, and carbon dioxide. This system could be considered an alternative to mammalian blood vessels, in that the formation of capillary networks is an adaptive process mainly controlled by the metabolic needs of target tissues (Fraisl et al., 2009). Indeed, Linneweber et al found that tracheal branching, which is regulated by the proliferation of tracheal terminal cells, in the gut is sensitive to a reduction in nutrient availability (Linneweber et al., 2014). They observed that fibroblast growth factor signaling, a well-known signaling pathway that promotes tracheal branching in response to hypoxia, is not involved in nutrition-dependent tracheal plasticity. Rather, insulin signaling, a major coordinator of nutrient intake and tissue size in many animals, is responsible for regulating tracheal terminal cell growth in a cell-autonomous manner. Anatomically, posterior tracheal branches are close to hindgut innervating neurons that are Ilp7- and PDF-GAL4-positive. In fact, they showed that these neurons are activated by food intake. Furthermore, a gain-of-function experiment revealed that Transient receptor potential A1 (TrpA1)-mediated artificial activation of these cells promotes tracheal branching in the posterior hindgut. These findings indicate that enteric neurons regulate the intestinal trachea in response to the host metabolic status (Linneweber et al., 2014)

6. Relevance of enteric neurons to the peritrophic matrix and midgut structure

Kenmoku et al performed genetic screening to identify neurons important to gut integrity using GAL4 enhancer trap lines with an Inward rectifier K(+) channel Kir2.1 to block neural activity (Kenmoku et al, manuscript in revision). They identified a subset of enteric neurons, NP3253-GAL4-positive neurons, that are required for gut barrier function. Nerve fibers of NP3253-GAL4-positive neurons are observed in the brain, crop, proventriculus, and anterior midgut, and their cell bodies locate in the brain and the most anterior part of the midgut. A large number of these neurons seems to innervate the gut visceral muscles, but some innervate the epithelia. Inhibition of NP3253-GAL4-positive neurons enhances the susceptibility of flies to oral infection with *Erwinia carotovora carotovora* 15 (*Ecc15*), a Gram-negative bacterium. Oral infection with green fluorescent protein (GFP)-labeled *Ecc15* revealed led to observation of the GFP signal throughout the body of the NP3253-inhibited flies in contrast to wild-type flies that retained the GFP signal only in their abdomen.

The permeability of the peritrophic matrix is an important feature for the barrier function of the gut, which is assessed by feeding adults fluorescein isothiocyanate (FITC)-labeled particles. Particles that are not permeable to the wild-type peritrophic matrix are diffused in the gut of NP3253-inhibited flies upon feeding. Intriguingly, an altered morphology of the proventriculus and the anterior midgut, e.g., a widened lumen of the R1 region of the midgut is observed when NP3253-GAL4-positive neurons are inhibited in adults after 1-2 days post emergence. A bromophenol blue (BPB) feeding experiment showed that region-specific pH is disrupted in the NP3253-inhibited midgut. On the other hand, feeding and defecation behaviors of the NP3253-inhibited flies are normal. Artificial activation of the NP3253-GAL4-positive neurons does not induce any obvious phenotypes. The study by Kenmoku et al revealed a new role for enteric neurons in the homeostatic control of gut integrity, including the permeability of the physical barrier of the intestinal tract (Fig. 2).

Some questions remain: which subset of neurons in the NP3253-GAL4-positive cells is responsible for maintaining the structural integrity of the gut? ; which cells in the gut are innervated?; and what are the molecular mechanisms of this neural control? One possibility is that NP3253-GAL4-positive neurons control the production and secretion of the peritrophic matrix in the proventriculus. If the peritrophic matrix is impaired, epithelial cells of the midgut would be exposed to the commensal bacteria and toxic compounds in the food. This could explain the leaky midgut and susceptibility of the NP3253-inhibited flies to oral infection with pathogenic bacteria. It is also possible that NP3253-GAL4-positive neurons govern peristaltic movement of the gut, particularly that of the proventriculus. If the pumping action of the proventriculus is compromised, the secreted peritrophic matrix may not form a proper layer throughout the gut, resulting in malfunction of the gut barrier. Another possibility is that if the function of the visceral muscles is impaired, the midgut structure would be loosened as observed in NP3253-inhibited flies. Further studies are needed to assess the roles of NP3253-GAL4-positive neurons and the underlying molecular mechanisms.

7. Conclusion

In this review, we describe the involvement of enteric neurons in many aspects of gut structure and function, as well as in neighboring organs, such as the trachea and the Malpighian tubules. Compared to studies in large insects, the anatomical characterization of enteric neurons in *Drosophila* is just beginning. Further, the roles of enteric neurons in *Drosophila* intestinal physiology are relatively scanty analyzed in comparison with the roles in mammals. Little is known about how enteric neurons contribute to the secretion of digestive enzymes and the absorption of nutrients in the *Drosophila* intestine. The relationship between enteric neurons and the gut defense system in flies has not yet been analyzed (Sharkey and Savidge, 2014). Due to the wide array of available genetic techniques such the GAL4-UAS system, however, some analyses in *Drosophila* have already surpassed those in other animals. These studies were the first to demonstrate in animals the requirement for enteric neurons for maintenance of the physical gut barrier and control of the plastic vascular structure by sensing the metabolic status of the host. Future studies in *D. melanogaster* are expected to reveal the mechanisms underlying the enteric neuronal control of intestinal physiology, and to provide new concepts regarding the neuronal control of host homeostasis.

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Author contributions

T. K. and H. K. wrote the draft. H. K. drew the pictures. T. K. managed the writing. T. K., H. K., and S. K. completed the manuscript.

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Figure Legends

Figure 1 Illustration summarizing the innervation of the larval and adult intestinal tract.

In larvae, TRH-GAL4-positive neurons (yellow) innervate the proventriculus and the anterior midgut, PDF-GAL4-positive neurons (red) and Ilp7-GAL4-positive neurons (brown) innervate the hindgut, and HGN1-GAL4-positive neurons (blue) innervate the posterior hindgut. In adults, Ilp2-GAL4-positive neurons (brown) innervate the proventriculus; NP3253-GAL4-positive neurons (red) project to the proventriculus, crop, and the anterior midgut; and GMR48A05-GAL4 (yellow) and GMR51F12-GAL4 (orange) positive neurons project to the proventriculus and the anterior midgut. The hindgut receives innervations from Ilp7-, HGN1-, odd- and PDF-GAL4-positive neurons. Cell bodies are represented by circles. VNC, ventral nerve cord.

Figure 2 Presumed model of NP3253-GAL4-positive neuron-dependent gut integrity maintenance.

(A) A lateral view of the proventriculus and anterior midgut of adults. NP3253-GAL4-positive neurons (green) innervate muscles and epithelial cells of the proventriculus and the anterior midgut. (B) Inhibition of NP3253-GAL4-positive neurons (blue) with Kir2.1 leads to high peritrophic matrix permeability and abnormal gut structure. EC, enterocyte; PM, peritrophic matrix.

Fig 1

Larvae

Adult

Graphical Abstract

Brain

TRH-GAL4

To Hindgut

Foregut

Proventriculus

Midgut

Malpighian
tubules

Hindgut

Ilp7-GAL4
PDF-GAL4
HGN1-GAL4

To VNC

To VNC

NP3253-GAL4

Ilp2-GAL4

GMR51F12-GAL4

GMR48A05-GAL4

To VNC

Crop

Ilp7-GAL4
HGN1-GAL4
odd-GAL4
PDF-GAL4