

## Review

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# *Drosophila* as a Model for Microbiota Studies of Neurodegeneration

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**Abstract.** Accumulating evidence show that the gut microbiota is deeply involved not only in host nutrient metabolism but also in immune function, endocrine regulation, and chronic disease. In neurodegenerative conditions such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis, the gut-brain axis, the bidirectional interaction between the brain and the gut, provides new route of pathological spread and potential therapeutic targets. Although studies of gut microbiota have been conducted mainly in mice, mammalian gut microbiota is highly diverse, complex, and sensitive to environmental changes. *Drosophila melanogaster*, a fruit fly, has many advantages as a laboratory animal: short life cycle, numerous and genetically homogenous offspring, less ethical concerns, availability of many genetic models, and low maintenance costs. *Drosophila* has a simpler gut microbiota than mammals and can be made to remain sterile or to have standardized gut microbiota by simple established methods. Research on the microbiota of *Drosophila* has revealed new molecules that regulate the brain-gut axis, and it has been shown that dysbiosis of the fly microbiota worsens lifespan, motor function, and neurodegeneration in AD and PD models. The results shown in fly studies represents a fundamental part of the immune and proteomic process involving gut-microbiota interactions that are highly conserved. Even though the fly's gut microbiota are not simple mimics of humans, flies are a valuable system to learn the molecular mechanisms of how the gut microbiota affect host health and behavior.

**Keywords:** Alzheimer's disease, cross-seeding, *Drosophila melanogaster*, gut-brain axis, gut microbiota, inflammation, neurodegeneration, Parkinson's disease

## INTRODUCTION

Trillion of microbes such as bacteria, fungi, and viruses live inside our body and on all of our surfaces, with the largest concentration found in the gut [1]. Among such symbiotic microbes, the microorganisms on the surface of the gut are called gut

microbiota. The gut microbiota has recently been shown to play an important role in the physiology and disease. Our symbiotic partners provide immune and metabolic functions by employing various molecular and genetic signaling pathways to protect the host from pathogens [2, 3]. Gut microbiota form biofilms, a layer of bacterial extracellular matrix, which protects them from attack [4]. The gut microbiota is affected by the host's genetic background, age, diet, comorbidities, and antibiotic use [5–7]. Disruption of gut microbiota is referred to dysbiosis

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and can contribute to various diseases. Recently, the involvement of gut microbiome has been reported in many areas such as aging, obesity, diabetes, fatty liver disease, cancer, and neurodegenerative diseases [5, 8–12]. Furthermore, accumulating evidence indicate that dysbiosis of the gut microbiota can cause low-grade inflammation which exacerbates chronic diseases such as atherosclerosis, asthma, and chronic kidney disease [13–16].

The brain and the gut strongly influence each other. This bidirectional relationship is called the “gut-brain axis” [17, 18]. Although much about the gut-brain axis is still unclear, it is well known that mental stress can cause diarrhea and constipation through changes in the gut microbiota. The relationship between gut microbiota and brain function has recently been analyzed in experiments using mice. Diaz Heijtz et al. compared the behavior of specific pathogen free mice with a normal gut microbiota with germ-free mice and showed that mice with microbiota gradually became accustomed to unfamiliar environments and reduce their amount of movement, whereas germ-free mice continued to move indefinitely in unfamiliar environments and tend to feel less fear. Furthermore, the gene expression of brain-derived neurotrophic factor (BDNF) was lower in germ-free mice than in mice with normal gut microbiota, suggesting that gut microbiota influence the development of BDNF-mediated neural circuits that control anxiety-related behavior [19]. Hsiao et al. reported using a mouse model of autism spectrum disorders that a metabolite called 4-ethylphenylsulfate produced by gut microbiota caused autism spectrum disorder-related behaviors, and that these phenotypes improved when *Bacteroides fragilis* was taken orally [20]. Furthermore, Sampson et al. reported that Parkinson’s disease (PD) model alpha synuclein ( $\alpha$ -syn) overexpressing mice that are germ free are protected from the disease [21]. Thus, evidence is accumulating that the gut microbiota is significantly involved in various neurologic disorders.

*Drosophila melanogaster* (henceforth *Drosophila*), one of many species of fruit fly, has classically been used as an optimal research model in the field of genetic engineering. In recent years it has been also attracting attention as an experimental animal for gut microbiota research in neurodegenerative diseases [22, 23]. *Drosophila* research has addressed the involvement of the microbiota on physical function, longevity, aging, and obesity. Furthermore, since 2018, a series of articles have been published analyzing gut microbiota in fly

models of the neurodegenerative disease [24–27]. *Drosophila* has many advantages as a laboratory animal: short life cycle, numerous and genetically homogenous offspring, less ethical concerns, availability of many genetic models, and low maintenance costs. *Drosophila* has orthologs for about 75% of human disease-related genes and the fly’s nervous system shares basic structure and functional features with humans [28]. Various neurodegenerative diseases such as Alzheimer’s disease (AD), PD, amyotrophic lateral sclerosis (ALS), Charcot-Marie-Tooth disease, and spinocerebellar atrophy adopt *Drosophila* disease model [29–33]. Moreover, *Drosophila* has a simpler gut microbiome than mice, which can be changed to any bacterial composition by experimental manipulation [34]. This makes *Drosophila* a suitable model for the study of the gut microbiome.

In this review, we discuss the history, physiology, anatomy of the gastrointestinal tract, and gut microbiota of *Drosophila*. We will then present research on *Drosophila* gut microbiota and neurodegenerative disease, and finally discuss how the findings from *Drosophila* gut microbiota can be adopted for therapeutic development of neurodegenerative diseases.

## THE HISTORY, PHYSIOLOGY, AND ANATOMY OF *DROSOPHILA*

*Drosophila* has contributed to the Nobel Prize in Physiology or Medicine at least five times [35–37]. The first case was Thomas H. Morgan in 1933, who demonstrated the chromosome theory of inheritance, which states that chromosomes are carriers of genes. By collecting mutations in *Drosophila* and conducting mating experiments among them, Morgan produced the first chromosome map. He was able to locate the genes on the chromosomes by comparing a chromosome map with the pattern of salivary gland chromosomes of *Drosophila* and proved that the genes were on the chromosomes. The second case was Hermann J. Muller in 1946, who discovered that X-ray radiation could induce gene mutations in *Drosophila*. In the 1920s he conducted an experiment in which male *Drosophila* was irradiated with X-rays, proving that the effects of radiation exposure can be transmitted to offspring and that radiation can artificially generate gene mutations. The third case was Edward B. Lewis, Christiane Nusslein-Volhard, and Eric F. Weischaus. They identified the genes involved in embryonic development using

*Drosophila* with random mutations. Once embryonic development was delayed, the mutated gene was found to be involved, thus revealing genes necessary for *Drosophila* embryonic development. Many of identified genes were found in other species, indicating that similar developmental mechanisms in *Drosophila* apply to organisms other than flies. They were awarded the Nobel Prize in Physiology or Medicine in 1995. The fourth was Bruce A. Beutler, Jules A. Hoffmann, and Ralph M. Steinman. Hoffmann discovered the role of the *Drosophila* Toll gene in the immune system. Beutler demonstrated that one of the mammalian Toll-like receptors, TLR4, is a transmembrane component of the receptor complex of the cell wall of Gram-negative bacteria. Together with Steinman's discovery of dendritic cells, the mechanism by which the innate immune system initiates the acquired immune system was identified, and these three researchers were awarded the Nobel Prize in Physiology or Medicine in 2011. The fifth case was Jeffrey C. Hall, Michael Rosbash, and Michael W. Young. They cloned a *Drosophila* clock gene (named "*period*") and found that the mRNA and proteins encoded by *period* change in response to circadian oscillations. They were awarded the Nobel Prize in Physiology or Medicine in 2017 for their elucidation of the molecular mechanisms of circadian rhythms. Thus, many important biological discoveries have been made with *Drosophila*, and it continues to demonstrate its value as an attractive model animal not only in genetic engineering but also in other fields of biology.

The life cycle of *Drosophila* is about 10 days: the first instar larvae hatch in about one day at 25°C after spawning. The first instar larvae molt into the second instar larvae after one day at 25°C, and the third instar larvae after one more day. Third instar larvae live for two days and then pupate. They spend four to four and half days as pupae. Under eutrophic conditions, adult females are ready to lay eggs two to three days after emergence. The entire developmental period of *Drosophila* is easily affected by temperature, with a twofold delay in developmental time at 18°C. Development almost completely ceases at 12°C, and it dies at temperature below at 5°C. *Drosophila* is also not tolerant to high temperatures, and will die if reared continuously above 30°C. *Drosophila* survives for two to three months in captivity, and the maximum number of eggs laid by one female is about 300 to 500 [28].

One of the most useful experimental tools in *Drosophila* is the GAL4-UAS targeted expression

system, in which GAL4 is a yeast-derived transcription factor that binds to UAS sequences and activates the expression of downstream genes. The main feature of this system is that the GAL4 strain which determines "where to express", and the UAS strain which determines "what to express", are generated independently. By analyzing the phenotypes obtained from the progeny of crosses between the targeted GAL4 and UAS strains, it is possible to efficiently perform overexpression in various combinations. Many strains that specifically express GAL4 in various tissues and cells and strains that have UAS upstream of many genes have been created and are available from stock centers around the world [38]. Furthermore, in *Drosophila*, the GAL4-UAS system can be combined with RNA interference (RNAi) to suppress specific gene expression in any tissue or cell. Transgenic lines expressing double-stranded RNAs for target mRNAs are being created and are available for most of the protein coding genes in *Drosophila* [39].

The digestive tract of *Drosophila* consists of three parts: foregut, midgut, and hindgut (Fig. 1). The foregut contains the oral cavity, esophagus, and crop (present only in adult fly), and is responsible for the physical preparation and salivary digestion of food. The midgut is the center of digestion and absorption, with over 300 digestive enzymes at work. The hindgut absorbs substances such as water and nutrients released from the Malpighian tubule, which is the equivalent of the mammalian kidney. The ingested food first enters a pouch-like structure called the crop. It then passes through the midgut, and is excreted through the hindgut. The midgut of the fly is composed of a single layer of epithelial cells, which contain the enterocytes, enteroendocrine cells, and intestinal stem cells, and is covered with muscle on the outside. On the luminal side of gut, there is a membrane composed of chitin fibrils and chitin-binding proteins called the peritrophic matrix [40, 41]. This membrane is known to allow only products degraded by digestive enzymes present on the membrane to pass through, and does not allow undigested food or bacteria to pass. Therefore, the peritrophic matrix is considered to be a physical barrier that protects the gut epithelium from pathogens. Although the role of the peritrophic matrix and its components in *Drosophila* is still unclear, the peritrophic matrix of many insects is thought to contain lysozyme and peptidoglycan degrading enzymes with antimicrobial activity, and the membrane may also serve as an essential site for gut immune responses [42].

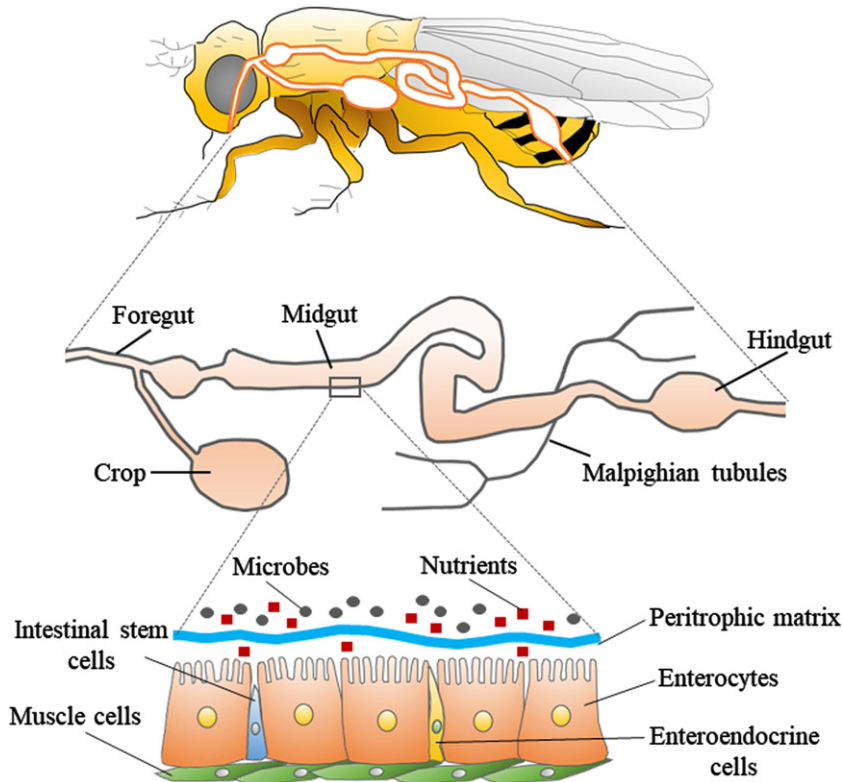


Fig. 1. Structure of the digestive tract in *Drosophila*. The upper panel shows a schematic diagram of the body and digestive tract of *Drosophila*. The middle panel shows the general parts of the digestive tract, which is divided into three parts: foregut, midgut, and hindgut. The lower panel shows the fine structure of a portion of the midgut. The layer consisting of enterocytes, enteroendocrine cells, and intestinal stem cells is covered with muscle cells. There is a peritrophic matrix between the enterocytes and the microbes, which physically separates the enterocytes from the microbes.

Flies have a much simpler gut microbiome consisting of only five to 20 microbial species, compared to humans which have about 500–1,000 microbial species in their gut [43]. Furthermore, relatively simple established methods can be used to generate germ-free flies or flies with standardized gut microbiota (such flies with standardized gut microbiota are called “gnotobiotic flies”) [34]. In detail, we can obtain axenic flies by simply soaking the eggs in sodium hypochlorite and rearing them in sterile food. If we want to give them any bacteria, we can simply mix the bacteria into sterile food. Previous research showed that even human-derived *Lactobacillus* could be colonized in germ-free flies [44]. Compared to specific-pathogen-free mice, which can only be obtained by dissecting pregnant mice, the simplicity of sterile manipulation of *Drosophila* is one of the greatest advantages of using *Drosophila* in the study of gut microbiota.

The simple and easily regulated gut microbiome of *Drosophila* helps us to understand the impact of gut

microbiota on host physiology as follows. Storelli et al. reported that rearing germ-free *Drosophila* larvae under nutrient deprivation significantly reduce the growth rate of the larvae. When *Lactobacillus plantarum* was reintroduced in the germ-free larvae, the decrease in growth rate was restored. Furthermore, they showed that *L. plantarum* genetically affected the TOR (Target of Rapamycin)-dependent nutrient sensing system [45]. Shin et al. found that rearing germ-free *Drosophila* monoassociated with pyrroloquinoline quinone-dependent alcohol dehydrogenase mutant bacteria delayed larval development and reduced body size. Such effect was restored by reintroduction of *Acetobacter pomorum*. Furthermore, they showed that this recovery effect was due to the acetic acid produced by *A. pomorum* modulating the larval insulin signaling pathway [46]. It is becoming clear that gut microbiome can influence a variety of *Drosophila* phenotypes, including aging and behavior [47–49]. Although there have been fewer studies of the gut microbiome in *Drosophila* than in mice,

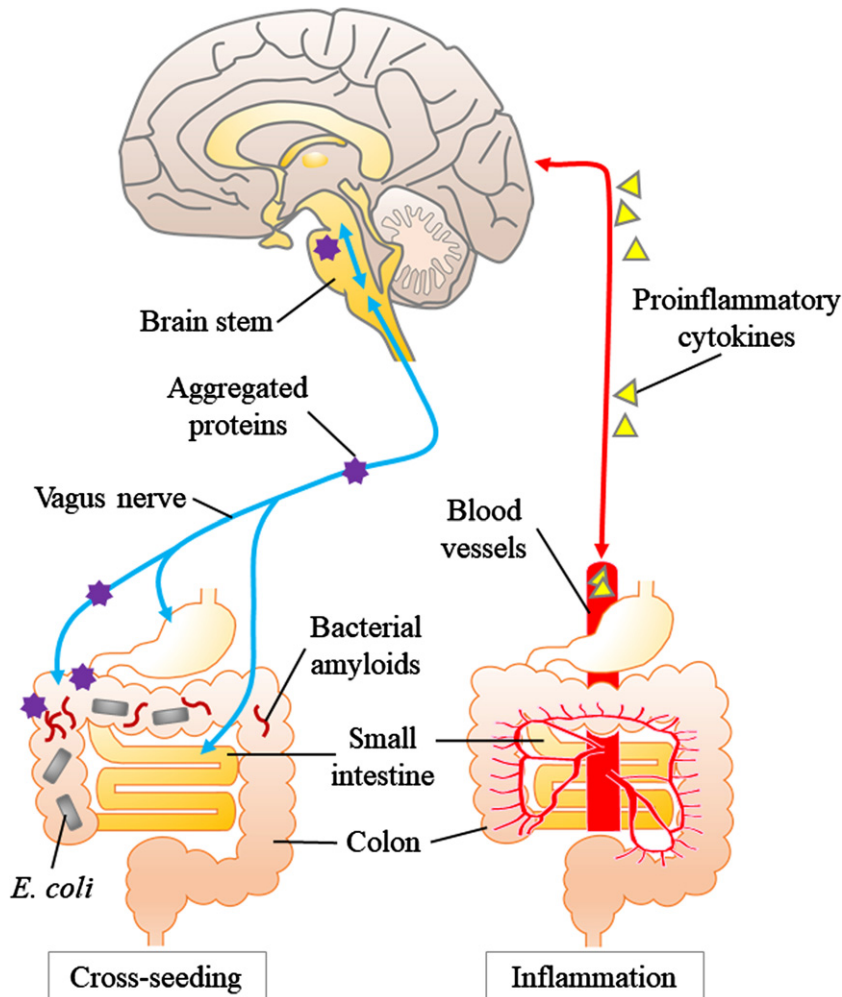


Fig. 2. Inflammation and cross-seeding: two hypotheses of abnormal aggregated protein accumulation involving the gut. The left panel shows the “cross-seeding” hypothesis. Aggregated proteins (especially  $\alpha$ -syn) produced in the gut propagate in a prion-like manner through the vagus nerve to the brainstem. Bacterial amyloid (e.g., curli) promote abnormal protein aggregation through cross-seeding. The right panel shows the “inflammation” hypothesis. Dysbiosis of gut microbiota activate the innate immune system and increase the production of proinflammatory cytokines and induce inflammation in the brain via systemic circulation.

physiological and anatomical simplicity in the use of *Drosophila* will aid the new discoveries, and help to find new pathways that will be applied to mammals.

### THE NEURODEGENERATIVE DISEASES AND GUT MICROBIOTA

The gut and brain influence each other through four pathways: the endocrine, immunological, metabolic and neural pathways. In response to change in the gut environment, gut hormones such as 5-hydroxytryptamine (5-HT) and cytokines secreted by enteroendocrine cells and immune cells, respectively, reach the brain through the bloodstream. Bacterial

metabolites and neurotransmitters such as fatty-acids,  $\gamma$ -aminobutyric acid (GABA), and precursor of 5-HT also go through the circulatory system and pass through the blood-brain barrier to signal the brain [50]. Afferent and efferent neurons of the vagus nerve connect the gut and brain stem. Recently, studies with rodents indicate that molecules such as GABA and  $\alpha$ -syn can be delivered to the brain via the vagus nerve in the trans-synaptic way [51–55]. This work suggests that the gut-brain axis plays a more important role than we had imagined.

There are two keywords for the relationship between gut microbiota and neurodegenerative diseases: inflammation and cross-seeding (Fig. 2)

[56–58]. A dysbiotic gut microbiome and damaged enteric epithelia can provoke systemic inflammation via activation of immune cells and release of cytokines [59, 60]. The hallmark of neurodegenerative diseases, such as AD and PD, is the misfolding and aggregated proteins in the brain, such as amyloid- $\beta$  (A $\beta$ ) and  $\alpha$ -syn, respectively. Minter et al. reported that A $\beta$  deposition in a mouse model of familial AD was reduced by changes in gut microbial composition due to long-term use of antibiotics, whereas soluble A $\beta$  was increased, and the same time, the expression of circulating cytokines was altered and the number of plaque-localized astrocytes and microglia was reduced [12]. Harach et al. found alteration in the gut microbiota of familial-AD model mice (amyloid-beta precursor protein, APP, transgenic mice) compared to wild-type mice. They also showed that germ-free APP transgenic mice had dramatically reduced A $\beta$  deposition in the brain compared to mice with normal gut microbiota. Interestingly, while recolonization of gut microbiota in germ-free APP transgenic mice increased brain A $\beta$  levels, microbes from standard (not germ-free) APP transgenic mice increased brain A $\beta$  levels more effectively than that from wild-type mice [61]. AD patients and AD model mice showed different gut microbial composition from healthy controls and wild-type mice, and a stress response pathway such as the MAPK signaling pathway was enhanced [62, 63]. Zhan et al. reported that lipopolysaccharide, a bioactive substance produced by Gram-negative bacilli, and *Escherichia coli* (*E. coli*) fragments colocalized in A $\beta$  plaques in the postmortem brains of AD patients, and that lipopolysaccharide was also deposited in perivascular areas of the brain, suggesting that microbial-mediated disruption of the blood-brain barrier might be involved in the neurodegeneration of AD [64]. These reports indicate that systemic inflammation and gut leakage play important roles in AD.

In the experimental setting, the abnormal assemblies of these proteins originate from the disease-specific misfolded proteins and finally adopt an amyloid polymer structure [65]. Many members of the human microbiome including *E. coli* produce extracellular fibers, which form amyloids. Curli, the first-described bacterial amyloid produced by *E. coli*, shares structural and pathological properties with human amyloids [66]. Besides, curli fibers induce high level releasing of proinflammatory cytokines such as tumor necrosis factor- $\alpha$ , interleukin-6, and interleukin-8 in human cells [67]. Moreover, the

bacterial amyloid curli activates Toll-like receptor 2, which also recognizes and targets human amyloids such as A $\beta$  and  $\alpha$ -syn [68, 69]. Altogether, dysbiosis of the gut microbiota can cause inflammation and lead to neurodegeneration.

The other keyword, “cross-seeding”, is related to direct abnormal proteinaceous changes in neurodegeneration. In many neurodegenerative diseases, experimental evidences support the concept that disease-specific proteins such as A $\beta$  and  $\alpha$ -syn acquire pathogenicity by a prion-like molecular process: the process of amyloid fibril formation proceeds after initiation by the “seed” that serves as the template [70, 71]. These pathogenic protein assemblies are also known to propagate from the periphery to the brain in a prion-like manner [65, 72]. A key question is where does the process begin? In PD constipation precedes the onset of motor symptoms many PD persons, showing that PD is closely related to the gastrointestinal tract [73]. As Braak showed in PD, Lewy bodies, which are composed of  $\alpha$ -syn, have a pathway from the dorsal nucleus of the vagus nerve upward to the substantia nigra [74]. Cersosimo showed that colonic biopsy in PD patients revealed accumulation of  $\alpha$ -syn in the submucosal plexus in 72% of PD patients, while no accumulation was observed in the control group [75, 76]. Svensson et al. showed that the incidence of PD was significantly reduced in people who underwent total vagus nerve resection for the treatment of duodenal ulcer [77]. This report supports the hypothesis that pathological changes in PD begin first in the enteric plexus and propagate through the vagus nerve to the brain. Dysbiosis of the gut microbiota can weaken the barrier function of the gut epithelium and trigger the accumulation of  $\alpha$ -syn in the enteric plexus. Thus, bacterial amyloids such as curli may initiate protein aggregation in enteric plexus. A pathway has also been proposed by Braak et al. and others [78] from the olfactory epithelium to the brain. In the roof of the nose the olfactory receptors AR processes of neurons that reside outside the central nervous system that are directly connected to the olfactory bulb as well as the entorhinal cortex. Prion-like protein misfolding may be initiated in olfactory neurons through exposure to bacterial products in the roof of the nose [79].

Is there a precedent for the gut factors to cause initiation of a protein folding disorder? This is precisely our current understanding of Creutzfeldt Jakob disease, bovine spongiform encephalopathy as well as kuru. In these conditions exposure to the prion protein in the gut through food initiates templated

Table 1  
Summary of *Drosophila* Microbiome Research on AD and PD.

Disease	Year	Flies	Summary	Ref
AD	2017	A $\beta$ <sub>42</sub> -transgenic flies	Gram-negative rod infection shortened the lifespan, impaired motor function, and increased cell death in the brain.	84
AD	2019	Flies expressing Human APP and BACE1	<i>Lactobacillus</i> administration improved phenotypes	85
AD	2020	A $\beta$ <sub>42</sub> -transgenic flies		86
AD	2020	A $\beta$ <sub>42</sub> -transgenic flies		87
PD	2019	$\alpha$ -syn transgenic flies	Phenolic acid metabolites were effective in delaying the deterioration of motor function	90
PD	2020	Endogenous PINK1 mutant flies	EGCG improved motor function and prolonged survival.	91

AD, Alzheimer's disease; PD, Parkinson's disease; APP, Amyloid-beta precursor protein; BACE1, beta-site amyloid precursor protein cleaving enzyme 1; A $\beta$ <sub>42</sub>, Amyloid beta 42;  $\alpha$ -syn, alpha-synuclein; EGCG, epigallocatechin gallate.

misfolding of the cellular form of the prion protein in the neurons of the gut which is subsequently transmitted to the brain through the autonomic nervous system, as well as the blood. The ability of functional bacterial amyloid proteins such as curli and others to initiate prion-like misfolding of neuronal proteins  $\alpha$ -synuclein and A $\beta$  have been reported in *in vitro* studies [80, 81]. Although the enteric nervous and immune systems are much simpler than those of mammals, an innervated digestive tract and a primitive immune system (including specialized hemocytes involved in phagocytosis) of *Drosophila* can serve as an appropriate model for studying gut-brain communication [82, 83].

### DROSOPHILA FOR MICROBIOTA STUDY OF ALZHEIMER'S DISEASE

Wu et al. used transgenic flies expressing human A $\beta$ <sub>42</sub> as an AD model and showed in 2017 that the gut-brain axis is a fundamental factor in neurodegeneration of AD. Gram-negative rod (Ecc15) infection shortened the lifespan of A $\beta$ <sub>42</sub>-transgenic flies, impaired motor function, and increased cell death in the brain. In these flies, phagocytes were more easily recruited to the brain as a result of enhanced migration of brain phagocytes, and these cells were more likely to produce reactive oxygen species with the elevated JNK pathway. When phagocytes were genetically knocked out, the JNK pathway was suppressed and the flies' lifespan was extended. This paper clearly showed that in the AD model *Drosophila*, changes in gut bacteria can activate immune cells to produce inflammatory cytotoxicity of central nervous system [84]. In recent years, several papers have reported on the therapeutic effects of specific bacteria and substrates (called probiotics and prebiotics, respectively) or combination (called symbiotics) that are considered to be good for health

promotion on *Drosophila* models of AD [85–87]. Among these, Tan et al. and Liu et al. used human A $\beta$ <sub>42</sub> transgenic flies as an AD model, and Westfall et al. used transgenic flies expressing human APP and  $\beta$ -secretase 1 (BACE1) enzymes as an AD model. All reports used *Lactobacillus* as probiotics and showed that *Lactobacillus* administration improved phenotypes such as disrupted compound eye formation, shortened survival period, impaired motor function, and A $\beta$  deposition. Although the number of papers is still limited, the combination of *Drosophila*'s advantages, such as the ease of regulation of gut microbiome and the homology of neural tissue with humans, will help us to study the role of gut microbiome in AD patients (Table 1).

### DROSOPHILA FOR MICROBIOTA STUDY OF PARKINSON'S DISEASE AND OTHER NEURODEGENERATIVE DISEASES

It has been shown that the PD model *Drosophila* exhibits reduced locomotion, shortened lifespan, and neurodegeneration [88, 89]. There have been two reports on the gut microbiota in the *Drosophila* PD model. Ho et al. showed that phenolic acid metabolites were effective in delaying the deterioration of motor function in human  $\alpha$ -syn transgenic flies, and that phenolic acid metabolites inhibited protofibril formation by binding  $\alpha$ -syn *in vitro* [90]. The other report is by Xu et al. [91] (Table 1). When *Drosophila* mutants with endogenous PINK1 were fed Epigallocatechin-3-gallate (EGCG), a potent polyphenol, improved motor function and prolonged survival were observed. The abundance of gut microbial composition was increased in these EGCG-treated flies, and when such abundance was reduced by antibiotic administration, the treatment effect of EGCG disappeared. By transcriptomic profiling using PINK1 mutant flies, they showed that the

expression of genes involved in fly-specific immune hormones was altered in PINK1 mutant flies, and that these gene expression changes were restored by ECGC consumption.

In ALS, another significant neurodegenerative disease, the relationship between the phenotype of ALS and gut microbiota has been investigated using mouse models. Blacher et al. showed that *Superoxide dismutase-1 (SOD1)* transgenic mice exhibit abnormalities in gut bacteria and changes in the composition of metabolites by bacteria before the onset of ALS, and that the prognosis is worse when these mice are raised in sterile conditions or after treatment with antibiotics. The authors showed that the abundance of *Akkermansia muciniphila* continued to decrease in *SOD1* transgenic mice as the disease progressed. They also showed that colonization of antibiotic-treated *SOD1* transgenic mice with *A. muciniphila* improved motor symptoms, whereas colonization with *Ruminococcus torques* or *Parabacteroides distasonis* worsened symptoms. The authors also found that the symptoms worsened when colonized with *Ruminococcus torques* or *Parabacteroides distasonis*. The authors further showed that colonization with *A. muciniphila* increased the concentration of nicotinamide in the cerebrospinal fluid of *SOD1* transgenic mice. Similar improvement (vitamin B3) in motor symptoms was observed when nicotinamide was administered [92]. Burberry et al. showed that immune cells such as neutrophils infiltrate the spinal cord and microglia are activated in the *C9orf72* mouse model. They also found that the diversity of the gut microbiota of *C9orf72* transgenic mice was reduced in pro-inflammatory environments, compared to pro-survival environments. They further showed that reducing the microbial load with broad-spectrum antibiotics or transferring microbes from mice grown in a pro-survival environment can improve the inflammation-associated phenotype and extend the lifespan of *C9orf72* transgenic mice raised in a pro-inflammatory environment [93]. The results in mice suggest that the gut microbiota changes with the onset and progression of ALS, that microbial-derived metabolites such as nicotinamide alter the progression of the disease, and that the gut microbiota may alter the motor function and lifespan of ALS model mice through immune responses. However, since gut microbiota is very sensitive to environmental changes, the results of the mouse study should be validated using other models. For now, there are no studies on gut microbiota using *Drosophila* model of ALS,

but it is likely that they will be used in the near future.

## APPLICATION AND LIMITATION OF *DROSOPHILA* GUT-BRAIN AXIS RESEARCH

We have introduced the role of the gut microbiota in neurodegenerative diseases, and the characteristics of *Drosophila* as a laboratory animal, for the study of gut microbiota in neurodegenerative diseases. A significant paper on the gut-brain axis was published in 2021 using *Drosophila*. Kim et al. have identified an amino acid sensing mechanism in *Drosophila* that leads to behavioral changes and is partially driven by the gut microbiota. In flies deficient in essential amino acids derived from the diet or produced by the microbiota, elevated expression of the neuropeptide CNMamide (CNMa) in the anterior midgut gut cells occurs via the Gcn2 and Tor pathways, which in turn modulates feeding behavior via the activity of neurons expressing CNMa receptors. This was then shown to modulate feeding behavior via the activity of neurons expressing CNMa receptors [94]. It is interesting that one of the specific molecules that directly controls the brain-gut axis has been identified.

*Drosophila* is also a useful laboratory animal to test the effects of probiotics. Westfall et al. showed that new probiotics and symbiotics prolonged survival in *Drosophila* and improved gene expression related to insulin signaling, fat metabolism, immunity, and oxidative stress. They showed that probiotics and symbiotics prolonged *Drosophila* survival and improved gene expression related to insulin signaling, fat metabolism, immunity and oxidative stress [95].

The study of gut microbiota by *Drosophila* has some limitations. Firstly, due to physiological and developmental differences, it is difficult to directly apply the results of *Drosophila* gut microbiota studies to mammals. Secondly, most invertebrates, including *Drosophila*, do not have the acquired immune system found in mammals [82]. Therefore, the inflammatory response that can be reproduced in fly disease models is likely to differ from the inflammatory response in humans. It is difficult to explain complex changes in gut bacteria in disease with only one experimental model. To decipher the complexity of gut microbiota, we need information from multiple models. The results shown in fly studies may represent a



fundamental part of the whole picture of host and gut microbiota interactions that are conserved in multiple species. Even though the fly's gut microbiota are not simple mimics of humans, flies will be a wonderful system to learn the molecular mechanisms of how the gut microbiota affect the host's health and behavior.

## CONCLUSION

*Drosophila* is a useful laboratory animal for the study of gut microbiota and neurodegenerative diseases. Studies of *Drosophila* will allow us to test the hypothesis that the gut microbiota is involved in human neurodegeneration. Disease modeling by *Drosophila* has just started using microbiota studies and is expected to be applied to a wider field in the future.

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