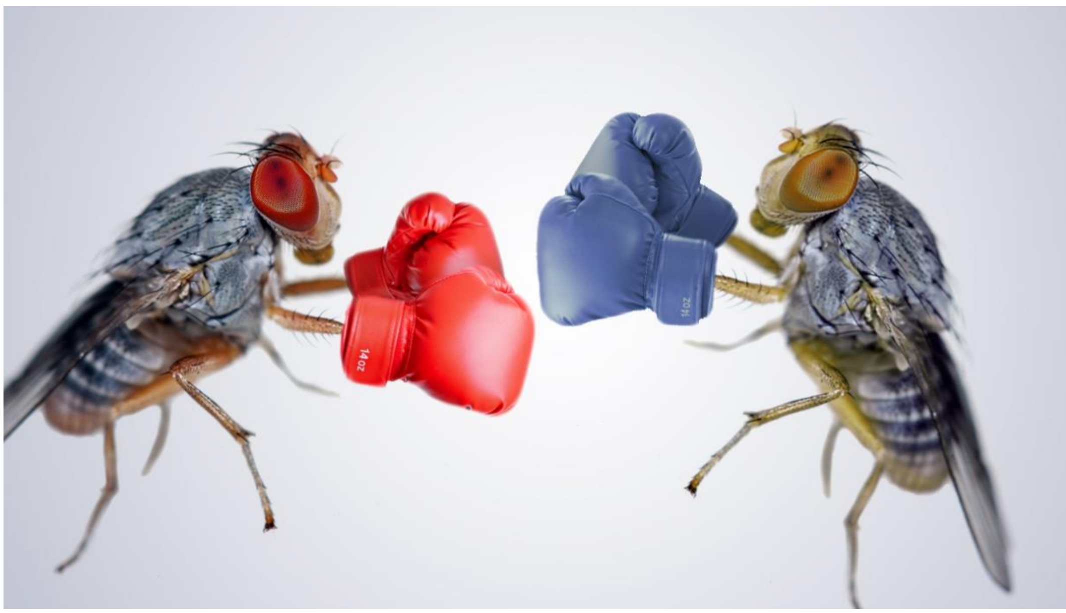
Aggression of fruit flies steered by their microbiome

Granda: the fruit flies dive-bomb me when I disturb them!



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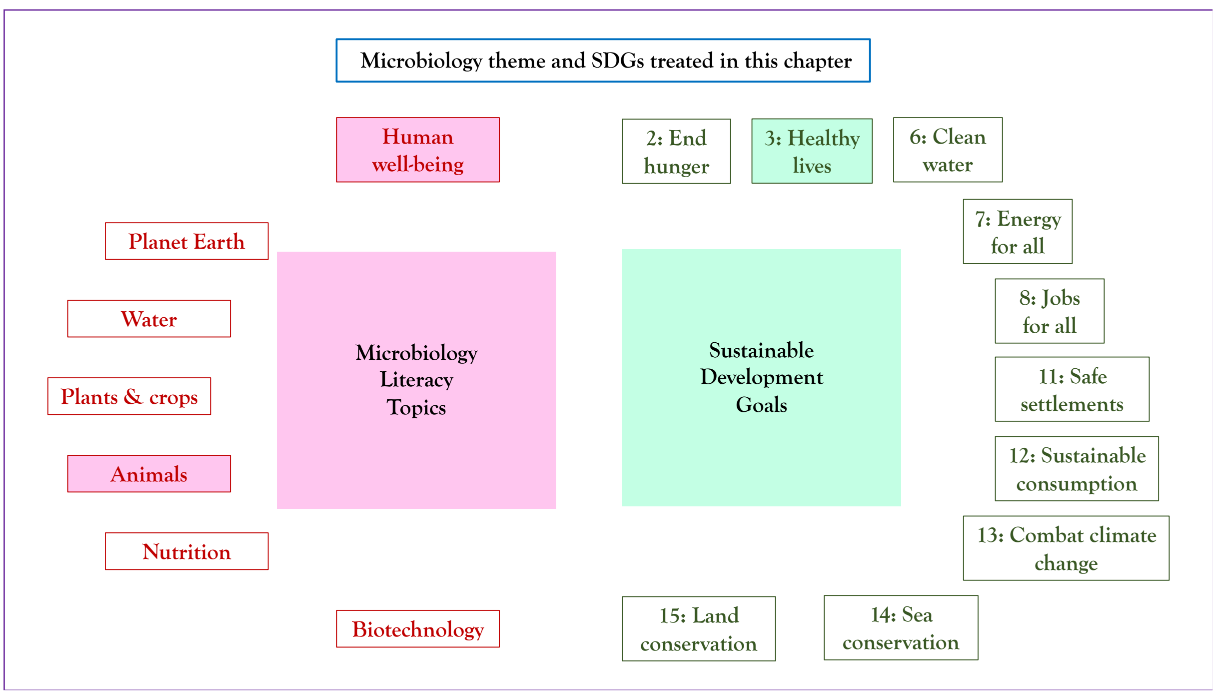
Storyline

All organisms have a complement of microbes, called the microbiome. The microbiome is not passive, with microbes simply feeding on the food sources they find on us, but dynamic and interactive, influencing many aspects of our biology. Exciting recent findings have shown that our microbiome can also have major impacts on our brain and its activities: the so-called gut-brain axis. Impacts include mental health, moods, vulnerability to stress, and some forms of behaviour. Because of the difficulties inherent in human studies, we often use model organisms for initial studies. The fruit fly is one of these model organism proxies. Here we discuss recent gut-brain-microbiota research in flies, focusing on how the microbiome affects aggression.

The Microbiology and Societal Context

The microbiology: microbiome; microbial metabolites acting as hormones-pheromones; gut-brain axis; microbial impact on animal behaviour. *Sustainability issue*: health



Fruit flies and behavior: The microbiology

1. ***Microbiomes, dysbiosis and animal models of disease.*** Microbes occupy essentially all surfaces of all larger organisms, including us: these microbial partners are called the microbiota. Microbiota are an essential part of all organisms, whether in the gut, mouth, or elsewhere in or on the body. Researchers have already found evidence of interactions between the host microbiota and the brain. In some cases, specific microbes are associated with certain mental

health disorders. In others, a general disturbance of the normal microbiota, in particular a reduction in its diversity – microbiota dysbiosis – underlies the disorders.

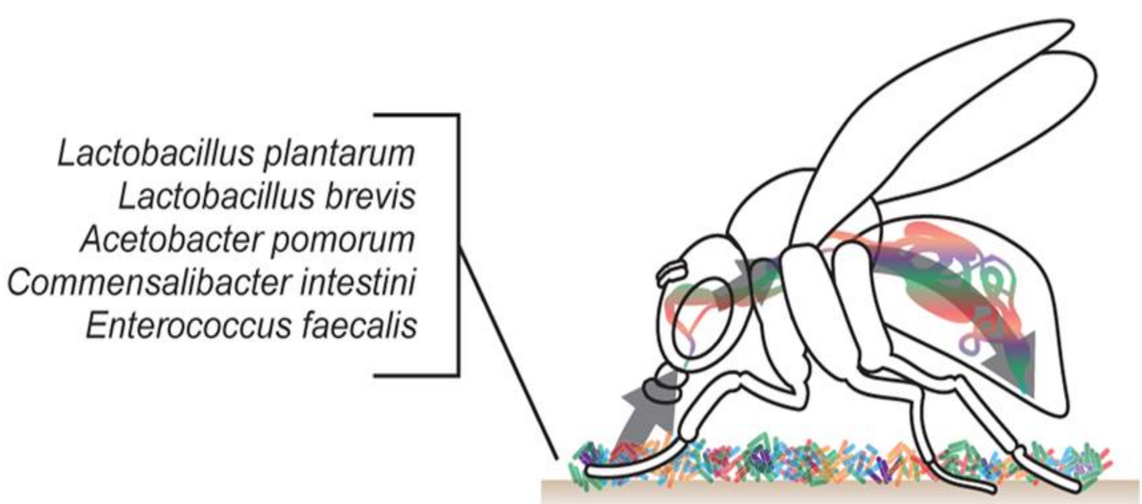
The dynamic gut-brain-microbiota axis is an active topic of study as researchers search for mechanisms underlying this crosstalk. Much research on improvement of human mental health is carried out in model organisms. Mice are traditional models for gut-brain axis research, but recent work suggests that the fruit fly, *Drosophila melanogaster,* is also an ideal study species.

2. ***Fruit flies as a model organism.*** Fruit flies - here we refer almost exclusively to the species *Drosophila melanogaster* - are a popular study organism. They are used in research on genetic activation and manipulation, neuroscience, and microbe-host interactions. They are morphologically simple but have many shared **pathologies** with humans: approximately 75% of known disease-causing **genes** are conserved in a similar form in flies. This means that similar mechanisms are at work in disease onset and progression, despite large physiological differences between humans and flies. Similarities have been found for cancer, Parkinson's disease, diabetes, metabolic disorders, and wound healing, amongst others.

Further, therapeutics can be tested in flies in early stages of drug trials with minimal bureaucratic hurdles, in contrast to studies conducted with rodent models. There is also conservation of mechanisms underlying biological properties and processes not related to disease, including brain function, aging, circadian rhythm, memory, and learning.

In addition to their biological similarity to humans, flies have a short **life-cycle** and are easy to grow. This means they mature and reproduce quickly. The faster a model organism grows, the quicker results are obtained from experiments, and the more rapid the scientific progress. Fast growth also makes it feasible to raise large colonies and carry out experiments that require a large number of test animals.

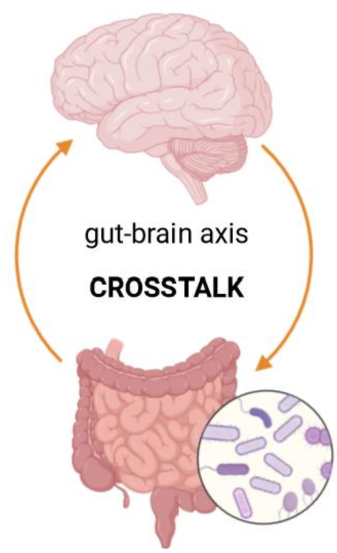
There are well established methods for gene manipulation in this species, as well as a variety of generally accepted behavioral tests, making research accessible and streamlined.



Fruit flies have simple microbiomes, with 5 species dominating the gut microbiome.
Image credit: Leitao-Goncalves *et al*, doi: 10.1371/journal.pbio.2000862.

Flies are also attractive as a model species for microbiome research because the fly microbiome is easily manipulated - flies can be made 'germ-free' through a multi-step, but essentially simple, procedure that includes washing eggs in bleach and sterilizing food and the vials in which they are kept during the experiment. In addition, the microbiota of flies are highly responsive to antibiotics which can thus be used to render them germ-free. This allows researchers to examine how the absence of microbiota affects the host. And – importantly – fruit flies have simple microbial communities dominated by just 5 species of bacteria. Because flies have such simple microbiomes, germ-free flies can be recolonized with each of the microbiota stains separately, or with specific combinations thereof, to examine how individual bacterial strains affect the host. This ability to test for an effect in the presence and absence of specific microbes allows researchers to establish causalities, i.e., strong evidence that an effect X is directly or indirectly caused by microbe Y.

3. ***The gut-brain axis and the microbiome.*** The **gut-brain axis** refers to the **crosstalk**, or interaction, between what is happening in the gastrointestinal (digestive) tract and what goes on in the brain. The central nervous system, which is responsible for controlling most bodily functions, communicates with the enteric nervous system, which controls behavior of the gastrointestinal tract, in a bidirectional manner. Furthermore, because the gut wall is **permeable**, substances can pass through it into the blood stream. Microbial **metabolites**, compounds produced in the gut by the digestive and biosynthetic activities of its rich microbiota, can therefore reach the circulatory system, eventually travelling to the brain, and even crossing the **blood-brain barrier**, a layer of cells that keeps dangerous matter from penetrating the brain. We are now learning that some microbial compounds have hormone-like activities and some are psycho-active – they can affect brain functioning. This added dimension of the microbiota to the gut-brain axis is termed the gut-brain-microbiota axis, though it is often referred to simply as the gut-brain axis.

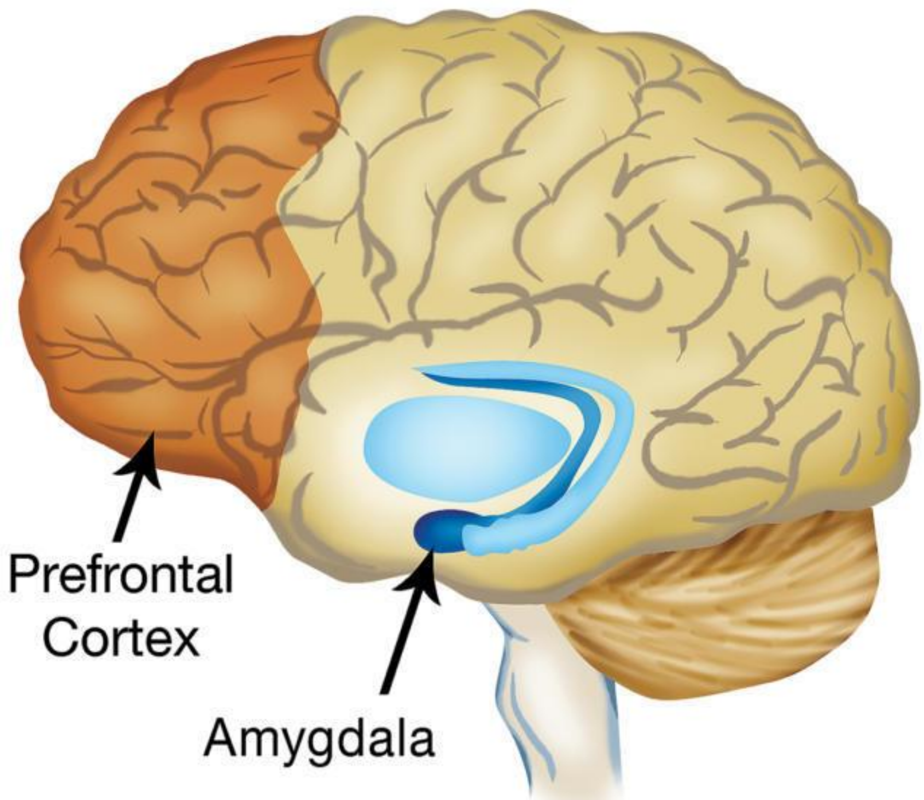


4. ***Effects of gut-brain crosstalk on the host.*** The crosstalk between one's brain and microbial community is an emerging research field in humans and model organisms. There is evidence that the gut microbiota is implicated in various behaviors, including aggression, mating patterns, and other social behaviors, as well as in mental conditions like autism, Alzheimer's, ADHD (attention deficit hyperactivity disorder), and schizophrenia.

To better understand how this crosstalk works, and in search of treatments for and prevention of mental disorders, researchers have begun studying the specific mechanisms by which the microbiota affects behavior - they are actively identifying metabolite by-products of the microbiota and their role in physiological pathways related to behavior.

Because the human body is very complex, and because it is normally unethical to perform studies on human subjects, scientists use model organisms to study the gut-brain crosstalk. Initially rodents were the main model species, but years of research also support the use of flies as a model for a number of human pathologies, pathways, and behaviors. The overlap between fly and human behavior is observed on many levels, not only in a microbiota-mediated manner. In this framework, we will focus on one behavior in particular: **aggression**.

5. ***Brain control of aggression.*** Aggression is defined as violent or hostile behaviors or attitudes towards others. Biologically, aggression is controlled in several regions of the brain. The brain region called the amygdala perceives environmental stimuli, like smells, facial expressions, and threats and, in response, signals the release of chemicals called neurotransmitters, which control behaviors associated with, e.g., stress and aggression. In humans, the prefrontal cortex can mediate aggressive behavior such that when it is activated, aggression is decreased. Research has also found that some aggressive acts, such as murder, are associated with reduced activity in the cerebral cortex.



6. ***Overlaps in aggressive behaviors in humans and flies.*** While the fruit fly's brain is not structured like a human brain, the building blocks, **neurons**, are the same. Neurons are the cells that make up the brain. They come in different shapes and sizes and have a range of functions. The human brain is made up of ~100 billion neurons, whereas the fly brain has ~100,000. While in flies, the division of brain regions is less clear than in humans, there are neurons for olfaction (the sense of smell), visual stimuli, behavior control, and so on. Researchers have discovered that by studying neuron shape, activation, and **gene expression** patterns - sets of genes that are activated or functioning in a given cell at a given time - in flies, they can learn about how the human brain functions too. This is because many genes are conserved between the two species.

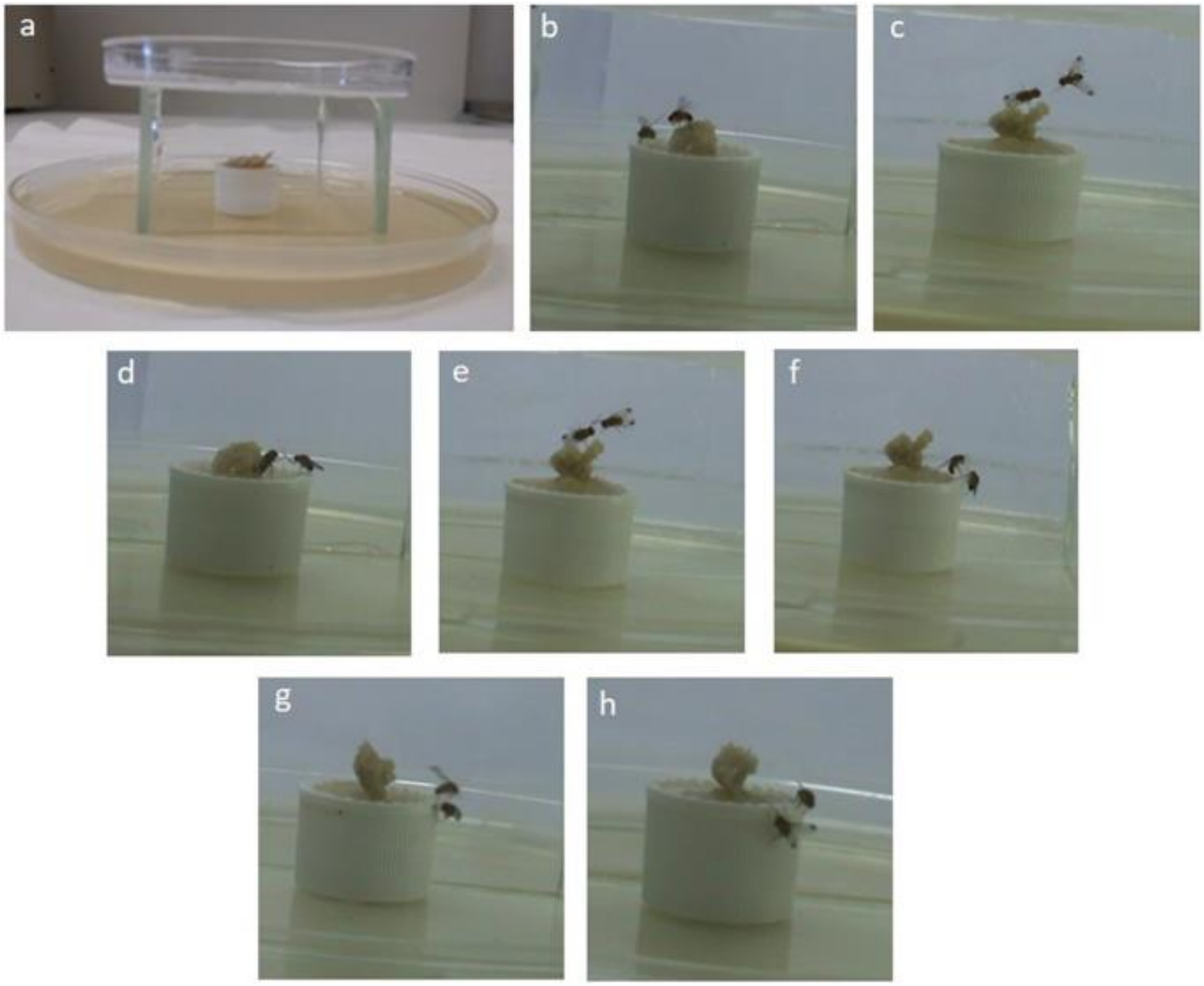
Similarly, there are overlaps in human and fly neurotransmitters and receptors, suggesting aggression signalling is conserved across species.

7. ***In humans, hormones can also affect aggression.*** Hormones are a class of molecules used for signaling inside the body. Whereas control by neurons occurs over fixed routes in the body – those where the neurons are located – hormones can control activities essentially all over the body because they are chemicals distributed throughout the body by the bloodstream and in other body fluids. Neuronal control is like the transport of signals via a train, which can only go to those places where railroad tracks exist, whereas hormonal control is more like the delivery of signals via vans which can go to many places because road systems go almost everywhere. Though hormones may be widely distributed in the body, they nevertheless act highly specifically on target tissues because, to have an effect, they must bind to specific receptor molecules located only on cells of target tissues. Hormones control growth, sexual behavior, and aggression, among other activities.

While animals and insects also produce hormones, many identical to human ones, they also produce molecules called **pheromones**. These molecules act like hormones but in a social manner such that one animal's pheromones cause behavioral changes in a different animal of the same species. Thus, they serve as behavioral signals between individuals. They are hormones released into the environment, distributed by air, and sensed as smells by olfactory organs.

Several studies have revealed that pheromones play an important role in aggressive behaviors in animals and insects. Thus, study of the production of hormones and pheromones, on one hand, and their corresponding receptors, on the other, can help decipher roots of aggression both in the studied species and in humans. It is important to investigate both hormone/pheromone levels and expression of their receptors because, for example, if hormones are overproduced but their specific receptors are produced normally or are underproduced (or vice versa), the downstream effects on aggression will be affected (one of the components of the hormone/pheromone-receptor couple will be rate-limiting for activity).

8. ***Studying aggression in flies.*** Aggressive behavior in flies has been studied from many angles. The first step was to characterize what aggressive behaviors look like. Typically, aggression is expressed through threatening behaviors, like raising wings and attacking behaviors, including tussling, chasing, and lunging. In research, these behaviors are typically measured in male flies in pairs or small groups. Aggressive displays within a given period of time are counted to determine the level of aggression in a specific experimental group. In these behavior experiments, in order to promote aggressive tendencies, flies may be starved for several hours to agitate the animal, or given only a bit of food which creates competition for resources, or a female fly may be added to the experimental vial prior to the behavioral test to create mating competition. In addition to behavior assays, fly brains can be examined for signals of consistent activation in neurons.



Photos taken by video analysis of non-social male *D. suzukii* aggression assay. (**a**) Experimental chamber. (**b**,**c**) Wing threat was observed from different optical angles. (**d**) Low-level fencing in which one fly extends his middle leg, and taps opponent's leg. (**e**) High-level fencing in which both flies extend their forelegs simultaneously, (**f**) Lunging in which one fly rears up on its opposite legs and snaps down the opponent. (**g**) Flying attack in which male suddenly flies and attacks by thrusting its body over the opponent. (**h**) Defensive wing threat in which defender fly flicks its wing to a 45° angle. Adapted from Belenioti, M., Chaniotakis, N. Aggressive Behaviour of *Drosophila suzukii* in Relation to Environmental and Social Factors. *Sci Rep* **10,** 7898 (2020). https://doi.org/10.1038/s41598-020-64941-1

9. ***Flies, aggression, and the microbiome.*** Recently, researchers have been examining the microbiome in their studies of flies and aggression. There is very early evidence that **microbial dysbiosis**, abnormalities in the microbial community composition, as well as complete absence of the microbiome, affects aggression in a range of animals. In some studies, aggression increased when the microbial community was depleted and in others it decreased. Thus, researchers are turning to flies to better understand the role of the microbiota on aggression. This field is still in its infancy and results of the few published studies are thus far also contradictory. One study found increased aggression when flies are depleted of bacteria via antibiotic administration and another found decreased aggression when the microbiome of flies is depleted through a procedure rendering them completely germ free, both with and without the addition of antibiotics. Clearly, more research is needed to begin drawing valid conclusions.

10. ***On-going research into fly aggressiveness.*** The ease of obtaining germ-free flies enables researchers to examine the effects of a healthy microbiota on fly aggression while separately considering effects of antibiotics that may not be directly related to microbiota depletion. The effect of antibiotics, beyond specific therapeutic benefits, is still not fully known, so being able to separate the effect of the drug itself from the effect of an absent microbiome (via the germ-free procedure) is of high relevance.

Beyond quantifying the number of aggressive encounters, flies allow for practical examination of the mechanism through which the microbiome works. Gene expression can be examined for specific target genes following microbiota manipulations. Pheromone production can also be measured across treatments focusing both on the pheromones themselves and on the concentration of receptors.

Additionally, researchers may discover that flies with **genetic mutations** causing reduced pheromone or receptor production have altered microbiomes and associated behavioral profiles. Then they can test if supplementation of specific microbial taxa can be used to manipulate aggression, potentially bypassing the pheromone system. From here, researchers can begin to understand specific feedback between the endocrine (hormone-pheromone) system and the microbiota, potentially using microbial taxa to fill hormonal deficits down the line

With its relatively simple biology, the easily manipulated fly model can be used for a range of studies and is becoming more common in the field of microbiome-host interactions. Over the next decade, several important advances on the fly gut-brain axis are expected.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 3. Improve health and reduce preventable disease and premature deaths.** The microbiome not only helps to break down the food we eat, it is also implicated in immune function, behavior, and overall well-being. Understanding how the microbiome affects aggression in flies will allow us to extrapolate to other species with the end goal of understanding the gut-brain crosstalk in humans. In the future, an anti-aggression probiotic can perhaps be formulated to help modulate aggressive behaviors and outbreaks among those who struggle with behavioral control.

Exercises

1. Role-playing: de-fusing aggressive situations
2. Examination of aggressive behaviours in animals: highlight similarities and differences
    a. Deer
    b. Antelope
    c. Tigers
    d. Horses
    e. Cows
    f. Crickets

The Evidence Base, Further Reading and Teaching Aids

Pandey, U. B., & Nichols, C. D. (2011). Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery. *Pharmacological reviews*, *63*(2), 411-436.

Thomas, A. L., Davis, S. M., & Dierick, H. A. (2015). Of fighting flies, mice, and men: are some of the molecular and neuronal mechanisms of aggression universal in the animal kingdom?. *PLoS genetics*, *11*(8), e1005416.

Zwarts, L., Magwire, M. M., Carbone, M. A., Versteven, M., Herteleer, L., Anholt, R. R., ... & Mackay, T. F. (2011). Complex genetic architecture of Drosophila aggressive behavior. *Proceedings of the National Academy of Sciences*, *108*(41), 17070-17075.

Nagpal, J., & Cryan, J. F. (2021). Microbiota-brain interactions: Moving toward mechanisms in model organisms. Neuron. 109.

https://www.kqed.org/science/1494923/these-fighting-fruit-flies-are-superheroes-of-brain-science Includes video of flies fighting

https://www.nytimes.com/2014/02/04/science/to-study-aggression-a-fight-club-for-flies.html Includes video of flies fighting and images of fly brains

https://www.umt.edu/marketing/creativeservices/Research%20View/Spring%202015/Fruit%20Fly%20Fight%20Club.php Includes image of fruit flies fighting

https://www.caltech.edu/about/news/fighting-flies-41791 A general-audience description of an article describing neurons involved in fly aggression

Glossary

aggression:	violent or hostile behaviors or attitudes towards others
blood-brain barrier:	a layer of cells that keeps dangerous matter from penetrating the brain
crosstalk:	bidirectional feedback
gene:	a part of the genome - DNA sequences - that codes a specific function
gene expression:	sets of genes that are activated or functioning in a given cell at a given time
genetic mutation:	a means of manipulating an organism's genetic matter to change the function of a gene (often rendering it inactive)
germ-free:	free of bacteria
gut-brain axis:	the communication between the gut and the brain. This term typically also implies that the role of the microbiota is also considered.
hormone:	a class of molecules used for signaling inside the body
life-cycle:	the series of stages occurring throughout life including birth, reproduction, and death
metabolite:	a compound digested by or produced in the gut by the microbiota
microbial dysbiosis:	abnormalities in the microbial community composition
neuron:	the cells that make up the brain
pathology:	the causes and effects of a disease

pheromone:	similar to hormones, but acting in a social manner such that one animal's pheromones cause behavioral changes in a different animal of the same species
permeable:	porous, allows passage of substances
re-inoculated:	to return one of more bacterial strains, typically after their removal via antibiotics or following germ-free procedures
taxon (pl. taxa):	a taxonomic group of rank; a general term to reform to a genus, species, or strain indiscriminately