Addendum

Links between the gut microbiota, metabolism, and host behavior

Catherine E. Schretter

Division of Biology & Biological Engineering, California Institute of Technology, Pasadena, CA, USA

ABSTRACT

The gut microbiota is known to regulate multiple aspects of host physiology, including metabolism and behavior. Locomotion, which is closely intertwined with metabolism, is an important component of complex behaviors, such as foraging, mating, and evading predators. Our recent work revealed that certain bacterial species and their products modulate motor behavior in the fruit fly Drosophila melanogaster via metabolic and neuronal pathways. In the context of our previously published findings and recent work by others, I will discuss potential avenues for future research at the intersection of the microbiota, metabolism, and host behavior.

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Introduction

Diet and metabolic state, which are features of an organism’s environment, can influence its behavior. For example, a fruit fly’s decision to choose between two foods is significantly altered based on its diet.1 Recent work in mice and flies has also linked the gut microbiota to host behavior.2-5 Further, specific gut microbial species have been shown to modulate host metabolism.6 Due to the known links between metabolism and motor behavior, we explored the connection between the microbiota, metabolism, and locomotion, and found that certain gut bacteria and their products modulate host speed via changes in sugar metabolism and octopamine signaling.7 As the investigation of host-microbial interactions in the context of behavior is emerging, I will discuss strategies for further exploring the microbial- and host-specific pathways involved, as well as potential future directions.

Bacterial effects on host behavior

Previous work in an infection study found that bacterial effects can extend beyond the immune system to include changes in host behavior, including food ingestion.8 In addition to studies involving pathogens, recent research in organisms spanning from choanoflagellates to mice has revealed commensal bacterial influences on a variety of host behaviors, including social behavior, food intake and preference, pain sensation, and locomotion.2,9-14

To examine microbial effects on host behavior, researchers often compare animals lacking a microbiota, referred to as germ-free or axenic, to those with a complex microbiota, or conventional. Another way of reducing microbial numbers and diversity is through the administration of antibiotics either in the food or water. As antibiotics can be given at any point during the lifespan of an organism, they facilitate the ability to uncover additional influences on host behavior, including developmental contributions. In our work, we found that flies treated with antibiotics early in adulthood exhibited similar increases in locomotion as axenic flies, in comparison with conventional flies.7 Since comparable changes were found regardless of the timing of microbial coloni
dation, we proposed that our phenotype was the result of active signaling rather than a developmental effect.

In both mice and flies, there is a diverse set of bacteria in the gastrointestinal tract that could contribute to particular phenotypic changes in the host. Recent work has used probiotic treatments and
mono-assocation experiments to reveal connections between a defined behavior and specific bacterial species. For example, the administration of *Lactobacillus rhamnosus* (JB-1) was found to decrease anxiety-like behavior in mice. Furthermore, certain microbial byproducts, such as short chain fatty acids, have been implicated in altering stress-related behavior and satiety. Different strategies have been employed to identify the species and molecules involved in mediating microbial effects on host physiology. We took a reductionist approach in our study, applying cultures of single bacterial species or their products to axenic flies. Through this strategy, *Lactobacillus brevis* and its product, xylose isomerase, were found to decrease speed and activity in axenic female flies. This methodology was chosen to minimize contributions introduced by fluctuations in the microbial community of conventional flies, which can occur during changes in temperature or diet. While this strategy limits these potential confounding factors, it is difficult to apply this approach to broader examinations of the bacterial species and products involved. Therefore, using community-wide comparisons could uncover correlations between additional microbial species and host behavior. Potential hits identified from a wider screen may then be tested through mono-associations or the transplantation of artificial communities into axenic animals. Metabolomic analysis of microbial communities or activity-guided fractionation of their metabolites could further identify products that modulate host behavior. For example, activity-based strategies have uncovered molecules that regulate avoidance and swarming behavior in *Caenorhabditis elegans* and choanoflagellates, respectively. In addition to demonstrating contributions by specific organisms and their products, another important area of interest in both our work and the field is exploring host mechanisms that facilitate these behavioral effects.

**Host pathways mediating bacterial effects**

Multiple connections exist between the gastrointestinal tract and the central nervous system in animals, including immune, metabolic, circulatory, and neuronal pathways. Commensal microbes are known to regulate various aspects of each, potentially leading to changes in host behavior. For example, microbes alter levels of pro-inflammatory cytokines produced by macrophages and T cells in the gastrointestinal tract as well as microglia in the brain. Microglia have been linked to behavioral changes associated with neurodegenerative disorders and may thereby be an indirect mechanism through which bacteria influence host behavior. The microbiota also regulates intestinal gluconeogenesis in mice that in turn modulates neural control of host metabolism. Similar metabolic effects by the microbiota are found in flies as certain bacterial species activate systemic insulin/insulin-like growth factor signaling resulting in changes in growth and energy metabolism.

For our work, we examined the involvement of immune, metabolic, and neural pathways in mediating the locomotor effects of *L. brevis* and xylose isomerase. While we did not observe any changes in antimicrobial peptides or dual oxidase (*Duox*) in *L. brevis*-treated flies, we did find lower levels of the sugar trehalose in flies administered with xylose isomerase. Furthermore, transcript analysis, genetic knockouts, and neuronal activation experiments identified the involvement of octopamine and tyramine signaling in mediating the effects of *L. brevis* and xylose isomerase on host speed. As octopamine regulates sugar metabolism, altered trehalose levels may be a result of changes in octopamine. However, additional research into this potential microbial regulation of trehalose via octopamine and the involvement of other parallel pathways is needed.

**Significance and next steps**

In our study, we found that specific bacteria modulate host locomotor behavior through changes in metabolism, and in neurotransmitters previously implicated in the regulation of host metabolism. We additionally discovered that a bacterial product involved in the interconversion of sugars, xylose isomerase, has similar effects on host behavior and related pathways. Given these connections, this work highlights the importance of examining microbial regulation of host metabolism in relation to its impact on host behavior.

Proper maintenance and regulation of metabolism is important in organisms that can experience wide variations in external conditions, such as...
Drosophila melanogaster. Metabolism is closely intertwined with multiple behaviors, including foraging and feeding. For example, flies exhibit increased locomotion during periods of starvation, similar to that found in axenic flies. Furthermore, the neurotransmitters, octopamine and tyramine, have been identified as potential mediators of changes in locomotion following both starvation and the removal of the microbiota. As microbes provide an additional source of nutrients and enzymes that break down components of the host’s diet, the similarities in these phenotypes and potential pathways may suggest that removal of the microbiota results in a starvation-like state. However, more in-depth analysis of the neuronal circuits involved is needed in order to facilitate this comparison. As the microbiota is also known to be important in cases of undernutrition, additional studies are needed to examine this interaction between diet, the microbiota, and behavior.

Another interesting finding from our work was that male flies did not exhibit changes in host speed upon removal of the microbiota or monocolonization with L. brevis. A previous report also found that increased levels of trehalose in axenic flies is sex specific. Sexually dimorphic locomotor behavior has been previously linked to trehalose and insulin signaling. It would therefore be important to next examine the possible ties between sex-specific microbial effects on locomotion, and the differences in metabolic requirements and neuronal circuitry between males and females.

Microbial regulation of host behavior lies at the intersection of various fields and has potential implications for the environment as well as in health and disease states. New tools in neuroscience, immunology, and microbiology will facilitate the mechanistic evaluation of active questions of research in this area. For example, genetically-encoded tools for visualizing neuronal activity in vivo and labeling limited subsets of neurons will allow more detailed examinations of the neuronal circuits involved. As the questions generated in this field span multiple body systems, I also hope that it encourages both collaborations across disciplines and the adaptation of tools previously developed for other uses. This broader approach could produce both insights into identifying consistent themes across distantly related subjects of study, as well as uncovering new avenues for research in previously discrete topics.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

ORCID
Catherine E. Schretter http://orcid.org/0000-0002-3957-6838

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