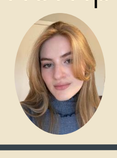
THE BRAIN-GUT AXIS OF HONEYBEES

TESTING HOW MICROBIOTA AFFECT INDIVIDUAL AND SOCIAL BEHAVIOUR

Anna M. Chernyshova[1, 2], Shenella A. George[1], Julia F. Lacika[1], Graham J. Thompson[1]


[1] Department of Biology, Western University, London ON Canada..
[2] Department of Microbiology and Immunology, Western University, London ON Canada.



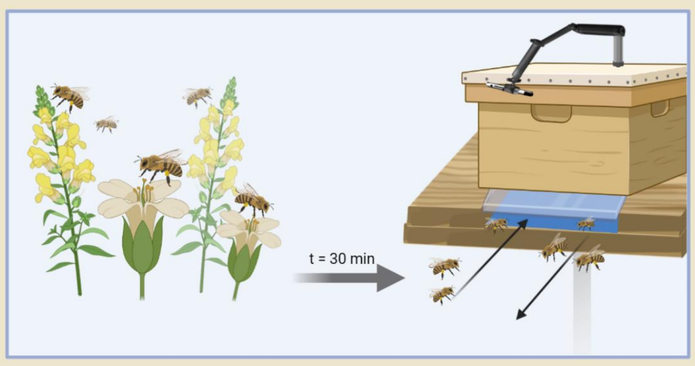

Gut microbiota can influence host behaviour through a **functional axis that connects the health and activity of the gut to the brain**. For social animals, the gut-brain axis takes on an added complexity if its influence scales-up to the **collective behaviour** of entire colonies **(Figure 1)**.

In this study, we describe three behavioural assays focused on the Western honeybee that attempt to detect the influence of a brain-gut axis within individual worker bees and within the colonies in which they live and function as altruistic helpers **(Figures 2-4)**.

We expect **antibiotic depletion** or **probiotic enrichment** of gut fauna to affect worker behaviour in predictable and potentially tractable ways **(Figure 1)**, for example, by improving or diminishing the activity of workers relative to untreated control bees.

BEHAVIOURAL ASSAYS

Foraging assay



- Workers forage on behalf of the colony.
- We can record timing and even success of worker foraging trips using pollen traps

Figure 2. As workers leave through the colony entrance to forage, a GoPro camera continuously records the number of bees at the colony entrance.

Defense assay

- Workers sacrifice themselves to defend their colony. We can measure defensiveness by video-recording the number of workers that attack or sting our standardized threat display

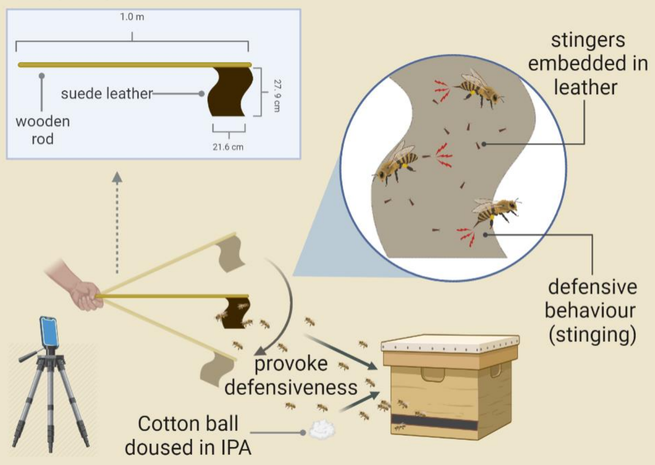
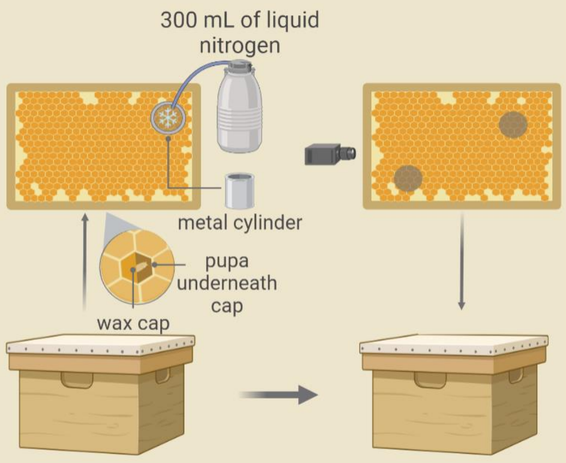


Figure 3. We can provoke defensive behaviour using an alarm chemical (Isopentyl alcohol) and a standardized disturbance (i.e., waving a suede patch in front of the hive).

Hygiene assay



- Workers uncap and remove infected brood to keep the colony free from disease.
- We can score the degree of hygiene for each as a percentage of freeze-killed brood that are uncapped and removed within 48-hours
- Note that we conducted a literature search and devised a plan to conduct this assay in the summer of 2023

Figure 4. The freeze-kill assay is commonly used by beekeepers to select for hygiene as a quantitative trait. We use liquid N_2 to kill a small but standardized number of late-stage pupae, then return the frame to the hive. After 48 hours we photograph each frame.

GUT ASSAYS

Gut dissection + 16S rRNA Sequencing

- In addition to behavioural assays, we will test how the composition of gut microbes varies as a function of pro- and anti-biotic treatments.

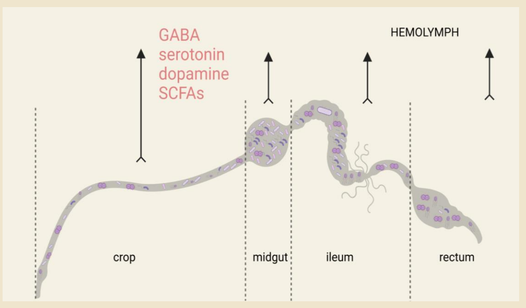
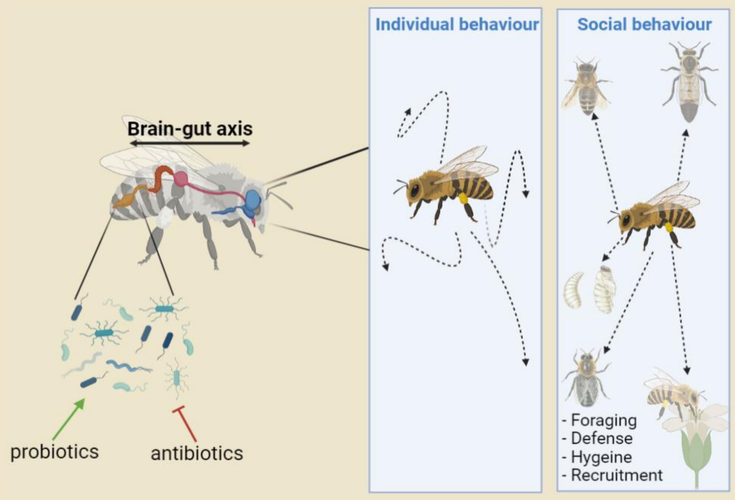


Figure 5. We can dissect the guts of workers to extract microbial DNA. We then sequence **16S rRNA gene amplicons** to estimate the relative abundance of gut microbes (by strain). In addition, **HPLC metabolomics** screens of gut-brain-hemolymph can identify metabolites that mediate information transfer along the 'axis'.

THE BRAIN-GUT AXIS



- We expect a strong effect from the **brain-gut axis** and specifically for host-adapted symbionts to mediate aspects of health, immunity and behaviour, as appears to be the case in humans [1].

Figure 1. If the effect of enrichment (via probiotics), as opposed to depletion (via antibiotics), of gut microbiota is to stimulate the worker bee's brain, then in general we predict more profitable foraging, more aggressive defence, more efficient hygiene and a more active dance by workers.

Brain dissection + histochemical staining

- Pollen patties inoculated with Bombella apis can modify worker's gut microbiome once consumed

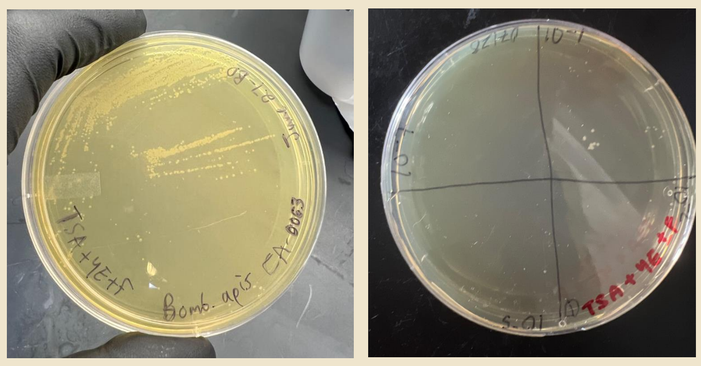


Figure 6. We can monitor the biomass of B. apis in each set of patties by creating CFU plates on a weekly basis

AIMS AND GOALS

Oh the places we'll go!

Our approach is to couple in-field manipulations of gut microbiota with behavioural assays to detect colony-wide evidence of a functional brain-gut axis.

Our aim is to test how our field manipulations of gut microbes can influence the behaviour of highly social insects and, by extension, the performance of societies in which they live.


Our long-term goal is to help advance the **brain-gut axis** framework [2] from its current focus on individuals into the realm of **higher-order interactions**.

REFERENCES
1. Vernier, C.L. et al. (2020). *Science Advances* 6, eabd3431
2. Liberti, J. and Engel, P. (2020). *Current Opinion in Insect Science* 39, 6-13

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