Extracting RNA from the Taste and Olfactory Organs of Manduca Sexta

The tobacco hornworm, *Manduca sexta*, is an economically important insect pest that feeds on plants within the family Solanaceae (e.g., potatoes, tomatoes, and tobacco). *Manduca* is also an important model system for understanding the neural basis of feeding behavior in insects. Barnard College has begun an ambitious project (funded by the Howard Hughes Medical Institute) to characterize functionally the taste and olfactory receptor genes of *Manduca*. This project has two goals. The first is to use the taste and olfactory genes to study the logic of this insect’s taste and olfactory systems. The second is to integrate this project into the undergraduate curriculum, enabling the students to participate directly in the discovery process.

My project constituted the first step in the identification of the taste and olfactory genes in *Manduca*. To this end, I spent most of the summer removing the chemosensory organs from fifth-instar *Manduca* larvae. Our goal was to obtain samples of RNA from these organs, and then send the RNA to a genomics center at Cornell University for sequencing. We focused on one olfactory organ (the antennae), three taste organs (the epipharyngeal sensillae and the medial and lateral styloconic sensillae) and one chemosensory organ with both taste and olfactory sensillae (the maxillary palp). After anesthetizing an insect (by immersing it in water), I used iridectomy scissors to excise each chemosensory organ. This was done under a dissecting microscope. The excised tissue was put in a solution (TRIzol) that inhibited the enzymes that break down RNA. Subsequently, the RNA was extracted from the tissues by Professor Mansfield and stored in a sub-zero freezer. Over the course of the summer, I collected chemosensory organs from approximately 300 insects. The tissue is currently being sequenced at the Cornell University Genomics Center.