LAB OVERVIEW

The Big Picture: The nervous system receives information from the outside environment, integrates that information, and causes a response. Chemosensation, the ability to detect different chemicals in the external environment, is one very important sense that provides many organisms with information about their environment. Today we will investigate the sensation of taste in the Arthropod, Manduca sexta, by performing a behavioral test to determine if these animals prefer sugar or caffeine.

| Before lab: | • Background Information:  
    Biology: The Dynamic Science. CH 38, 39 |
| During lab: | • Analysis of Taste in the Arthropod Manduca sexta |
| Assignments (Due at the end of lab today) | • Manduca Lab Worksheet |
Thought and discussion questions:

- What types of information does the sensory nervous system provide?
- What is taste? Why is it important?
- How can the behavior of an adult animal provide information about its anatomy and development?

Learning objectives:

1. Be able to recognize and identify basic anatomical features of *Manduca sexta*.
2. Be able to quantify taste preference in *Manduca sexta*.
3. Be able to understand the importance of replicates in experiments.
4. Be able to conduct and interpret t-tests.
TASTE IN THE ARTHROPOD MANDUCA SEXTA

BACKGROUND

The animal, *Manduca sexta*, is a member of the Phylum Arthropoda and has a life cycle in which it goes through many different developmental stages (Figure 1). *Manduca sexta* first develop from eggs into larvae. As larvae, they molt five times, going through 5 instar stages, after which they enter a pupal phase. After a number of days as pupae, they eclose and emerge from their casing, having developed into adult moths. In nature, *Manduca sexta* mainly feed on solanaceous plants such as tobacco or tomato. Eating the leaves of these plants gives the larva its distinctive green coloring. Today, we will be working with larvae that have been raised in the lab on a wheat germ-based diet. The lack of pigments in the laboratory food causes the larvae to take on a slightly bluer color instead.

![Figure 1: Life cycle of *Manduca Sexta*. In lab today, we will examine the feeding behavior of a 5th instar larva. Diagram modified from: http://insected.arizona.edu/manduca/PDFs/Posters.pdf](http://insected.arizona.edu/manduca/PDFs/Posters.pdf)
Manduca sexta are important scientific model organisms. A model organism is a particular organism that is studied by scientists to help them investigate a broad biological question, with the expectation that what is learned from studying this model organism will be widely applicable to other organisms. Manduca sexta are particularly good model organisms because they are small, easy to rear in a laboratory setting, and have a relatively short generation time. Manduca sexta have become a particularly important model organism for studying the function of the sensory nervous system, in particular the study of gustation (i.e., the sense of taste). Manduca sexta are well-suited to study gustation because they have a less complex nervous system than vertebrates, but are still large enough so that one can observe and record from the cells that mediate the gustatory response.

As you have been discussing in lecture, the sensory nervous system is responsible for detecting external environmental signals. Environmental signals can come in many forms: light, sound, heat, etc. Chemosensation, the ability to detect different chemicals in the external environment, is one very important sense that provides many organisms with information about their environment. To detect chemicals in our environment, humans use specialized sensory neurons called chemoreceptors in our mouth (taste receptors) and our nose (olfactory receptors). Similar to humans, Manduca sexta use specialized chemoreceptors located in their head to sense chemicals in their environment (Figure 2). These chemoreceptors are exposed to the external environment and relay information to the animal about the type of environment it is in (whether there is food, toxins, etc). Although these caterpillars do have a visual system, they rely heavily on chemosensation to detect important information about their environment.

![Figure 2: Anatomy of Manduca sexta. On the right is a diagram of a Manduca sexta larva (Modified from: http://insected.arizona.edu/manduca/PDFs/Posters). Shown on the left is a representation of the sensory structures of the head of the animal. (Modified from Glendinning, et al., 1999).](image-url)
*Manduca sexta* can use the information that it receives from its chemosensory neurons to modify aspects of its behavior. For example, when the caterpillar tastes glucose, an energy-providing food source, it will commence feeding. However, when the caterpillar tastes a toxin, it will not continue to feed on that substance. This is similar to how humans can detect different types of tastes (sweet, sour, salty, etc.) which in turn affects our eating behavior.

Today in lab, we will examine a behavioral response mediated by the sensory nervous system in *Manduca sexta*. To do this, we will use a previously-established assay (experimental procedure) to determine if *Manduca sexta* prefer to eat the sweet-tasting sugar alcohol, inositol, or the non-nutritious and bitter-tasting substance, caffeine. To test their preference, we will present them with each of these options and observe which substance they choose to eat in a specific period of time.

REFERENCES:


http://insected.arizona.edu/manduca/PDFs/Posters

http://insected.arizona.edu/Manduca/

http://www.acad.carleton.edu/curricular/BIOL/resources/rlink/

http://creatures.ifas.ufl.edu/field/hornworm.htm
PROCEDURE PART A: USING A MICROPIPETTER

1. **Work in groups of two.** Sign-out a yellow-capped micropipetter from your instructor: (200 µl volume).

2. To perform the taste preference assay, you will need to measure very small volumes of liquid using a **micropipette** (Figure 3). Micropipettes are instruments that allow us to accurately measure very small volumes of liquid (microliters, abbreviated µl). 1 L = 1000 ml = 10⁶ µl. Before you can test taste preference in *Manduca sexta*, it is critical that you practice accurately use a micropipetter by following the steps below. **These are delicate and expensive instruments. Please handle them with care.**

![Diagram of a micropipette](image)

Figure 3: Diagram of a micropipette. To use, a tip must be placed on the end of the tip. Then, the volume is set by twisting the push button handle. The diagrams on the right show the approximate levels drawn into the tip for three representative volumes: 5µl, 20µl and 50µl.
3. Pick up the micropipetter (yellow-capped). This micropipetter is calibrated to measure volumes between 40 to 200 µl; thus, you should only use the yellow-capped micropipetter if you want to measure a volume between 40-200 µl. You can determine the volume a micropipetter is calibrated to measure by looking for the white label on the blue handle (you should see 40-200 µl written in white letters).

4. To set the volume, find the volume scale located on the side of the handle. Notice that there are 3 numbers (150 for example). Set the volume scale by twisting the yellow-capped knob on the top of the pipetter. Move the knob clockwise to decrease the volume, and move it counter-clockwise to increase the volume. Note that you can adjust the volume in 1 µl increments. **Turn the handle only as far as it moves freely; forcing it will damage the micropipetter.** Set the volume to 100 µl (volume scale should read 100).

5. To use the micropipetter, hold it by the handle and point the yellow-capped end up, which means the white end should be on the bottom. Next, fit a disposable plastic tip snugly on the white end of the pipetter. **Never use the micropipetter without at tip.** The operation of the micropipetter is controlled by the yellow-capped plunger, which should be operated slowly at all times. Get a feel for the plunger before you start dispensing liquids. Slowly depress the yellow-capped knob of the plunger until it stops the first time; this is the **FIRST STOP.** Then using a little more force, depress it further until it stops at the **SECOND STOP.** It is important that you are aware of these two stopping places.

6. Obtain a beaker of colored water. To measure 100 µl of water, start by depressing the plunger to the **first stop.** **DO NOT PUT THE MICROPIPETTER IN THE LIQUID UNTIL YOU HAVE REACHED THE FIRST STOP.** Then, still holding the plunger at the first stop, place only the tip of the tip (do not get the pipetter itself wet at any time) under the surface of the water. **Slowly** release the plunger to draw the measured volume of colored water up into the pipette tip. Remove the tip from under the surface of the water. If there are drops of liquid stuck on the outside of the tip, gently touch the tip to the side of its container to remove them. You should now have the appropriate volume in your pipette tip.

7. Obtain a clean, empty microcentrifuge tube and label it “100”. To deliver the 100 µl of water to this tube, place the tip of the tip against the bottom of the microcentrifuge tube. **Slowly** depress the plunger to the first stop to let out most of the water, pause, and then depress the plunger to the second stop. **This will blow out a small amount of air to make sure that every last bit of the water in the tip has been delivered.** Then, **while still holding down the plunger, remove the tip from the liquid.** If you let the plunger come back up before you have removed the tip from under the surface of the liquid, you will suck the liquid back up into the tip. Check to make sure the tip is empty to ensure that you have successfully delivered the amount intended. If there are drops on the outside of the tip, gently touch the tip to the side of the container to remove them.

8. After you have dispensed the liquid, remove the tip by depressing the tip-removal plunger, located below the yellow-capped plunger. This blue tip-removal plunger controls movement of the bottom white portion of the micropipetter, removing the tip from the end.
9. Now set the volume of the yellow-capped micropipette to 200 µl by turning the yellow-capped knob (volume scale should read 200). Be careful not to turn the knob past 200 as this is the largest volume this micropipette can measure. Fit a new tip on the white bottom portion of the micropipette.

10. Once the volume is set to 200 µl and a tip is on the pipetter, repeat the above steps to place 200 µl of water in a clean, microcentrifuge tube labeled “200”.

11. Now you should have two microcentrifuge tubes, with 100 and 200 µl of liquid respectively. Observe the volume of liquid in each microcentrifuge tube, and make sure that there is less volume in the “100” tube as compared to the “200” tube. Show your instructor to confirm that you have accurately measured each volume before moving on to the chemotaxis assay. It is critical to be able to accurately use this instrument to be able to perform the taste preference assay in lab today.
PROCEDURE B: TASTE PREFERENCE ASSAY

1. After your instructor has checked your pipetting, obtain and lay out Styrofoam board flat on your bench.

2. Obtain 4 large filter paper disks and one acetate template. Lay the acetate template on top of one filter paper disk and mark the location of the 4 holes using a Sharpie. Repeat for all four filter paper disks.

3. Set up four test arenas. Each test arena should look like the diagram below:

   ![Testing arena diagram]

   - Taste disk
   - Moistened filter paper
   - Caffeine-treated
   - Inositol-treated
   - Inositol-treated
   - Caffeine-treated

4. For the next step, wear gloves to prevent any oils from your hands touching the test disks. Assemble the test disks by sandwiching the test disk on a pin between two pieces of acetate disks. Assemble 4 taste-disk sandwiches per testing arena, according to the diagram below.

   ![Taste disk diagram]

   - Pin
   - Smaller acetate disk (above and below taste disk)
5. Firmly press each pin holding the test disk into the Styrofoam board in the middle of the Sharpie marking. The disk should be just a few mm above the large filter paper circle. The disk should be high enough that it is not touching the filter paper circle, but low enough that the caterpillars will be able to reach it easily. Repeat this for all four markings on all four filter paper circles.

6. Add test solutions to the test disks. You will be testing two different solutions: caffeine and inositol. First add the caffeine solution. To add caffeine to the test disk, use a micropipetter to dispense 180 µl of caffeine solution to two test disks that are attached to YELLOW pins located opposite each other in the testing arena. Remember to set the volume and use a fresh tip for your micropipetter. Repeat for all 4 large filter paper circles:

7. Place a fresh tip on your micropipitter. Follow the same procedure above to distribute 180 µl inositol solution to the two other opposite-facing test disks that are attached to BLUE pins. Repeat for all 4 large filter paper circles.

8. Place a caterpillar in the center of the large filter paper disk. Cover with a Petri dish. Repeat for all 4 filter paper circles.

9. Record the time on The Manduca Lab Worksheet. This is the beginning of your taste preference assay.

10. Watch the caterpillars as they walk around the filter paper circles. Which way do they walk? Which disks do they approach first? Which disks do they eat? Observe their behavior and answer the appropriate questions on The Manduca Lab Worksheet.

11. Continue to watch the caterpillars at regular intervals. The assay should continue for 1.5 hours or until 50% of total disk area is eaten.

12. At the end of your assay, estimate how much of each disk has been consumed by the caterpillar. Record your data on The Manduca Lab Worksheet and answer the appropriate questions.
MANDUCA LAB WORKSHEET

1. (2 pts) Think back to the first lab of the semester when you dissected an earthworm and a crayfish. Are Manduca sexta more closely related to earthworms or crayfish? Explain.

2. (2 pts) Before performing the experiment, predict if you think Manduca sexta will prefer inositol or caffeine. Provide a biological rationale for your hypothesis.

3. (4 pts) Watch your four caterpillars as they walk around the filter paper circles. Fill in the table below to record their behavior. At the end of the experiment, provide an estimate of the total area eaten by the caterpillar for the inositol and caffeine disks.

<table>
<thead>
<tr>
<th>Subject</th>
<th>First disk approached (inositol or caffeine)</th>
<th>First disk tasted (inositol or caffeine)</th>
<th>% Inositol disks eaten at end</th>
<th>% Caffeine disks eaten at end</th>
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Time experiment began: __________  Time experiment ended: __________
4. (2 pts) Class Data: Fill in the table below with the data from the entire class.

<table>
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<tr>
<th>Group #</th>
<th>Subject</th>
<th>% Inositol Disks Eaten</th>
<th>% Caffeine Disks Eaten</th>
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Mean:  
Mean:
5. (4 pts) Follow the directions posted above the computers in the lab to use SPSS to perform a paired t-test comparing the mean disk % eaten of the inositol and the caffeine disks. Report the p-value, and interpret your results. According to your statistical analysis, do *Manduca sexta* prefer inositol or caffeine? Explain.

p-value ______________

Interpretation:

6. (2 pts) Based on the amount of variation you found in your group’s data set, why do you think each group examined four caterpillars instead of just one caterpillar?

7. (2 pts) For ~45 minutes before this experiment began, the caterpillars were deprived of any food. Explain why this is an important part of the experimental design.

8. (2 pts) We asked you to use a micropipetter to distribute the inositol or caffeine solution onto each disk. Explain why it is important to measure the same amount of inositol or caffeine on each disk.