CARDIOACCELERATORY ACTIVITY BY THE HYPERTREHALOSEMIC HORMONE

Introduction:

Hypertrehalosemic hormone (HTH) is a neuroendocrine product of the intrinsic neurosecretory cells of the **corpora cardiaca** that activates the fat body to synthesize trehalose from stored glycogen (we will study this in next week's laboratory). The hormone is a decapeptide (10 amino acids) with the following structure:

pGlu-Val-Asn-Phe-Ser-Pro-Gly-Trp-Gly-Thr-NH_2

HTH also works in a second manner – it stimulates the heart to increase its rate of beating (cardioacceleration), as well as the amplitude of the beat (strength). This may assist in moving hemolymph, which carries trehalose to the muscles during periods of intense muscular activity (e.g. escape from a predator). Remember that HTH is secreted from the **corpora cardiaca** (Figure 1). The corpora allata, located behind to the copora cardiaca, secretes juvenile hormone (JH).

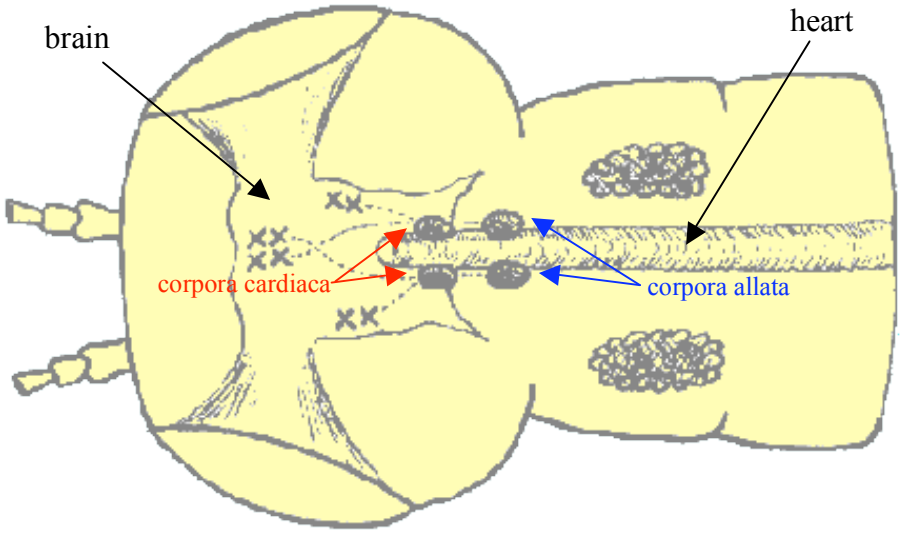

Figure 1. Important organs, and their locations, in the head of a cockroach.

Objectives:

In this lab we will explore the effect of HTH on the heart *in vivo* by applying corpora cardiaca extracts to the exposed dorsal vessel of a dissected cockroach. This laboratory has 3 key objectives:

1) To demonstrate the effect of hypertrahalosemic hormone (HTH) on an insect's heart.
2) Illustrate a hormone bioassay.
3) Illustrate how physiologists can work with insect tissue *in vivo*.

Materials and methods:

Cockroaches	Razor blade	Saline solution
Centrifuge	Pipettes	Pins
Dissecting kit	Dissecting tray	Eppendorf tubes
Sonicator	Pipette tips	Microscopes

STEP 1: Extraction of HTH from corpora cardiaca

1) Decapitate a cockroach (*Blaberus discoidalis*) and use the head for dissecting the brain to isolate the corpora cardiaca. **Place the abdomen in an empty beaker for later use**.
2) Place the head with the frons (face) upwards on the paraffin wax surface of the dissecting tray.
3) At the base of the antenna align the razor blade perpendicular to the frons (see figure 1).
4) With a quick push of the razor blade cut through the head and into the paraffin. **Keep your fingers out of the way!**
5) Discard the top portion of the head and grasp the lower part of the head by the mouth parts. Using your thumb nail, squeeze the head upwards as if squeezing tooth paste (use just enough pressure). This will push the cranial viscera out of the cut.
6) Using **fine tip forceps** remove the viscera that you've just squeezed out.

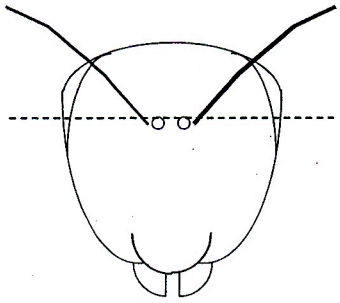

Figure 2. The head of a cockroach. The dotted line is the plane upon which razorblade should cut.

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7) Place the viscera on a large drop of saline solution (a.k.a., physiological saline) and examine it under a microscope.
8) Carefully remove the fat bodies, muscle and trachea surrounding the opalescent-colored brain. Try to maintain the orientation of the brain in an anterior-posterior position.
9) The corpora cardiaca and corpora allata are located on the posterior side of the brain, and are attached to the brain by two nerves. The corpora cardiaca are the elongate structures closest to the brain and may have a pale blue appearance. Can you see them? If not, ask your TA to help you locate them.
10) **Once you've shown the corpora cardiaca glands to the T.A, place the entire brain, including the corpora cardiaca,** in a 1.5 ml Eppendorf centrifuge tube containing 200 µl of saline solution. Each Eppendorf tube should contain 3 brains.
11) Extract the HTH using the sonicator. Do this until the brain has become homogenized, and the saline solution turns cloudy.
12) Centrifuge the brain sample for 10 minutes, at about 10,000 g, to precipitate solids.
13) Next choose "someone careful" from your group to transfer as much of the supernatant to a clean 1.5 ml Eppendorf tube. **DO NOT DISTURB THE PELLET!**
14) Each group should carefully remove 40 µl of the supernatant with a micropipette and transfer it into a new eppendorf tube.

STEP 2: Dissecting the cockroach to expose the heart

1) Take the decapitated cockroach abdomen and excise the appendages (antennae, wings, and legs).
2) With scissors, slit open the intersegemental membrane between the next-to-last (penultimate) and last abdominal segments.
3) Run the scissors up each side through the lateral intersegemental membrane that connects the sclerites and pleurites – cut from last abdominal segment to the anterior-most edge of the thorax.
4) Grasp the penultimate sclerite of the abdomen with forceps and lift gently. Using the scissors or the tips of another forceps, gently lift away the dorsal surface while breaking any connections between the dorsal and ventral surface (**Note**: these connections are intersegmental muscles that serve to compress the insect for ventilatory movements of air into and out of tracheal sacs and trunks during respiration.). Cut through the leg muscles in the thorax to loosen and remove the ventral thorax. Remove any viscera connected to the dorsal surface.
5) To observe the aorta, invert the dorsal surface and apply 1 ml of 1% NaCl solution (= **physiological saline,** osmotic pressure equal to body fluids) to bathe the heart. The dorsal aorta will appear as a clear area in the midline of the dorsal surface of the abdomen. It is possible to observe the aortal pulses with a microscope for an extended period.

STEP 3: Cardio acceleratory activity bioassay

1) Bathe the heart with 100 µl of saline solution and count the rate of beats per minute, three times, for 1 minute. You'll need to conduct each count under a microscope. Enter your results for each count in the table below, and calculate the average.
2) Aliquot 10 µl of corpora cardiaca extract and add it in to the saline bathing the heart.
3) Allow to equilibrate for 3 minutes.
4) Count the heartbeats of the treated preparation, three times for 1 minute each. Enter your results for each count in the table below, and calculate the average.

5) Rinse the heart preparation three times with saline solution (sufficient quantity to wash away all of the previously applied HTH)
6) Wait for 5 minutes so that the heart gets readjusted to the fresh bathing medium.
7) Apply 100 µl of saline solution and count the rate of heart beats per minute, three times, for 1 minute. Enter your results for each count in the table below, and calculate the average.

Basal Count (60 sec)	HTH Count (60 sec)	After HTH Count (60 sec)
1)	1)	1)
2)	2)	2)
3)	3)	3)
mean count =	mean count =	mean count =

Data Analysis:

Your TA will conduct the statistical analysis. The data and analysis will be posted via email. You will need to generate a single figure (with means and standard errors) once you receive the data:

1) A graph plotting the average heart beat rate (y-axis) against the three different treatments (x-axis): the basal count rate, the count rate after adding HTH, and the count rate after washing away all the HTH.

You will need to specifically address the following questions based upon your observations:

- **What is the effect of adding HTH to insect fat body?**
- **Was the mean count rate for the basal treatment the same as for the after wash count rate? If so, why? If not, why not?**

This lab is due at the start of the week 5 lab (September 30)!!!