Chapter 3

Responses to Stimuli: The Basis of Behavior

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Introduction

The objectives of this exercise are to provide students with an opportunity to observe simple taxic responses, and to demonstrate that certain stimuli are more critical than others in eliciting behavioral responses. The exercise is written to test responses of two common invertebrate animals: one aquatic and one terrestrial.

Adult brine shrimp were selected as the aquatic test animal because they are inexpensive and readily available from tropical fish stores or hobby shops. In addition, they are sufficiently large to be easily viewed in test tubes. Daphnia can be substituted using test chambers of smaller size. Any aquatic invertebrate that can be secured in quantity could be used, but the brine shrimp provide better results for students.

The milkweed bug was selected as the terrestrial animal to study because it can be handled by inexperienced observers. These insects do not fly or run about rapidly. At Nassau Community College, cultures of the milkweed bug are maintained in order to provide ample material for approximately 30 laboratory sections (see Appendix A). Other insects that can be handled could be substituted. A species of sow bug, an isopod that is common in most areas, may be used in a modification of this exercise, but these animals move slowly, and do not show marked responses to several stimuli.

This exercise, designed for a 3-hour laboratory session, can be conducted using standard laboratory equipment. It is a low technology/maximum student participation experience.

Materials

The following materials for the study of brine shrimp and milkweed bug are adequate for one class of 24 students:

Live Materials

Adult brine shrimp (800 adults per session): Two cartons from a tropical fish dealer are adequate for one lab. Three or four cartons would be needed for two labs of 24 students at about $1.50 US per carton. Milkweed bugs: one vigorous colony (100–200 adults)

Chemicals

Salt (1 box of Instant Ocean should be more than ample)
Egg (Mix the albumin with about 5 ml saline to prepare a dilution of egg white) (1)
Crushed ice (1 or 2 gallons)
5% acetic acid
5% alkaline solution (NaOH or KOH)
5% sugar solution (sucrose)
Food coloring (to be used as a marker, any color)
Cigarette (for nicotine preparation, prepared by students)
Perfume or lipstick
Bic pens (24)

Glassware

Large test tubes (10" length) (24)
Small test tubes or vials (24)
Small culture dishes or finger bowls (4" diameter) (24)
Large culture dishes (8" diameter) (24)
Watch glasses (24)
Eye droppers (48)
Battery jars or 1-gallon plastic beakers (to collect animals after testing) (3–4)

Other

Thermometers (12)
Ring stand and ring (3"–4" diameter) (1)
Corks (for large test tubes) (12)
Timer (large wall clock is satisfactory)
Aluminum foil (1 small roll is adequate)
Light sources (plug in sockets and 60 or 75 watt bulbs are satisfactory) (12)
Petroleum jelly
Cotton swabs
Black felt squares (about 15 × 15 cm) (12)
Dark cloth
Plastic spoons (12)
Rulers (30 cm) (12)
Rubber bands
Index cards (1 package, 3" × 5" size)
Carboy or other container for mixing (a 1- or 2-gallon carboy with a spigot is most convenient)

Microscopes

Several binocular dissecting microscopes (1 per table or available in demonstration areas)

Student Outline

Background Information

The major purposes of these studies are:

1. To observe the responses of an animal to various environmental stimuli.
2. To determine conditions which comprise an optimum environment for the animals studied.

All animals continually monitor their environment and make adjustments as conditions around them fluctuate. Environments are constantly changing, as from night to day, summer to winter, wet to dry, etc. Animals are equipped with a variety of sense organs, each specialized to respond to particular forms of energy (light, sound, pressure, chemicals, heat) which are the stimuli (singular, stimulus) that prompt the animal to make an adjustment, or adapt. Such adjustments usually result in a more compatible relationship between the animals and the environment; a situation that requires less energy expenditure and keeps the animal as nearby as possible in optimum conditions. The ultimate benefit of adaptive responses is survival of the individual or survival of the species.

The phenomenon of responding to the environment is the basis of animal behavior. The simplest behavior patterns keep an animal away from hazardous situations where it might be too hot, cold, dry, lacking in food, or prevent reproduction. The most complex behavior patters may lead, through elaborate courtship activities, to mate selection and rearing of young. Behavior of any animal species is distinct from all other animal species.

Animal responses to stimuli are called taxes or taxic responses. (In plants they are called tropisms, or tropic responses.) Responses are further described as positive when the animal moves toward the stimulus, and negative when the animal moves away from the stimulus.
The following taxic responses will be studied:

Response	*Stimulus*
Thermotaxis	Temperature
Phototaxis	Light
Chemotaxis	Chemicals
Rheotaxis	Currents
Geotaxis	Gravity
Thigmotaxis	Touch or pressure

Responses of Brine Shrimp

The brine shrimp, Artemia salina, is an invertebrate animal (phylum Arthropoda, class Crustacea) that lives in salt water. It is used as fish food for tropical fish and is readily obtained commercially. These animals are in perpetual motion. They swim upside down (unusual in the animal kingdom) propelling themselves with synchronized strokes of 11 pairs of appendages. Examine a specimen with a dissecting microscope. Note the wide distribution of hairs and bristles (setae) on the body. The setae possess sensory receptors. On the head there is a conspicuous pair of black compound eyes and two pairs of antennae, the latter being more difficult to identify. All of these sense organs are used in monitoring environmental conditions.

In this exercise, brine shrimp will be tested for their response to a variety of stimuli. Only one stimulus will be tested at one time. The observations for each test can be kept on the work sheet provided. After all the tests are completed the results should show the preferences of this species and indicate the optimum environment for brine shrimp.

Procedures

The standard procedure to be used for each test is described below. The test for each stimulus requires one special condition added to the standard procedure. Follow the standard procedure for a trial run.

1. Obtain a large clean test tube, 10 inches long, and a small finger bowl.
2. Fill the test tube 2/3 full of sea water (prepared according to directions given). Prepare a quantity of sea water if the supply has been depleted.
3. Place six brine shrimp in the tube. Do not agitate the tube unnecessarily.
4. Tilt the tube, resting the lip on the edge of the small finger bowl.
5. Allow at least 5 minutes for the animals to explore the new environment.
6. For 2 minutes observe the behavior. Do the animals congregate or swim about independently? Are they distributed throughout the chamber or do they spend more time in a particular area? Are they consistent back swimmers?
7. When these observations have been completed, pour the contents of the tube into the large container provided. Rinse the tube with deionized water.
8. This trial will familiarize you with the appearance and movements of the brine shrimp. It will also serve as a control for comparing behavior that develops in the following tests.
9. A new group of six animals will be used for each of the following tests.

Test 1: Phototaxis (response to light)

1. Wrap black felt around the lower half of the tube. Keep felt in place with a rubber band.
2. Use the wall socket and bulb as a light source. Shade the bulb with aluminum foil to direct more light toward the tube. This will also shade the eyes of the investigator from uncomfortably bright light.
3. Arrange the test tube holder (the finger bowl) so that the tube, when tilted, will not have light from the bulb penetrating the dark chamber at the end of the tube.
4. Fill the tube 2/3 full of sea water and add six fresh brine shrimp.
5. Tilt the tube, resting the lip on the edge of the small finger bowl.
6. After the appropriate conditioning time, observe the behavior of the animals for 2 minutes. Count the number of animals that show a positive and/or negative response.
7. Record your observations in Table 3.1.

8. Empty tube, rinse with deionized water and prepare for the next test.

Table 3.1. Observations on behavior of brine shrimp, *Artemia salina*.

Test #	Stimulus	Animal response			Remarks
		Positive	Intermediate	Negative	

Test 2: Thermotaxis (response to temperature)

1. Wrap a 2.5-cm (3") band of black felt around the upper half of the tube.
2. Fill a finger bowl about 3/4 full with ice.
3. Fill the tube with water and add six fresh brine shrimp.
4. Take a temperature reading of the contents of the tube.
5. Push the bottom of the tube into the bowl of ice. Tilt the tube and bring the upper end close to the lighted bulb. Use an aluminum foil shield to direct light toward the felt-covered section to increase temperature at this end of the tube. Also, shield the cold end from excessively bright light from the bulb.
6. After the appropriate conditioning time, observe behavior of the animals. Record observations.
7. Before emptying the tube, take a temperature reading of tube contents at the top of the tube, taking care not to stir. Push the thermometer to the bottom of the tube to measure temperature at the cold end. Record readings.
8. Empty tube, rinse with deionized water, and prepare for the next test.

Test 3: Chemotaxis (response to pH)

This test requires two procedures: one tube using an acid solution to determine response to H+ ions and a second tube using a basic solution to determine response to OH- ions. If additional tubes are available these tests can be run simultaneously with six animals in each tube.

1. Fill the tube with water and select six fresh brine shrimp.
2. Tilt the tube over the edge of the small finger bowl. Be sure the tube is bathed with diffuse light from the ceiling and not the bright light source at the table. Place a paper towel beneath to provide a white background.
3. Allow 5 minutes for conditioning the animals.
4. During this 5 minute period, prepare the acid and/or basic solution as follows: Pour about 5 ml of 5% acetic acid solution into a watch glass. Add a few drops of food coloring (any color available) to deeply color the acid. The basic solution is prepared the same way.
5. Use a pipet (eye dropper) to add about three drops of the acid solution to the contents of the tube. *Be careful not to stir the water in the tube*.
6. Observe the reaction of the animals as the interface between the acid and the water moves down the tube.
7. Record your observations.
8. Empty the tube, rinse in deionized water and repeat this same procedure using the alkaline solution instead of the acid solution. If both tests have been performed, proceed to the next test.

Test 4: Chemotaxis (response to chemical substances)

This test will determine the responses to two food substances: a sugar solution and a protein substance prepared from egg albumin. This procedure will use the interface between the two solutions as in the pH test just completed. It will save time to run the two tests simultaneously.

1. Fill the test tube with water and select six fresh brine shrimp.
2. Tilt the tube over the edge of the small finger bowl. Use a paper towel for a white background.
3. Condition animals for 5 minutes.
4. During the conditioning interval prepare the sugar solution. Pour 1 ml of the 5% sugar solution into a watch glass. Add a few drops of food coloring. The egg solution is prepared the same way.
5. Use a pipet to add three drops of the sugar solution to the tube. *Do not stir the contents of the tube.*
6. Watch the interface between the two solutions (water and sugar or egg white) and observe the reaction of the animals.
7. Record your observations.
8. If you have not observed the reaction to both food substances, repeat this test using the second food substance.

9. When this test is completed, empty tube contents, rinse tubes in deionized water and leave them at your table.
10. A different procedure is followed for the next test.

Test 5: Rheotaxis (response to water currents)

This is really responses to pressures exerted on the animal by flowing water.

1. Fill the finger bowl with water.
2. Place six fresh brine shrimp in the finger bowl.
3. Allow 5 minutes for conditioning.
4. With the pipet create a steady, but gentle, current of water in the bowl. This can be done by submerging the tip of the pipet and gently squeezing the bulb. Watch the shrimp carefully so that the current is not so strong that the shrimp are forced along by the water. A steady current can be created (clockwise or counterclockwise) around the dish by placing the pipet at the side. Directing the pipet across the middle of the dish will create two swirls which will give the animals more choice.
5. Observe the behavior of the animals and record observations.
6. Empty contents of dish. Clean all equipment. Leave glassware clean and dry at the center of your table.

Results

Record your observations on the board to prepare a class summary of the five tests. Characterize optimum environmental conditions for the brine shrimp based on the results of this exercise.

Questions

1. Distinguish between positive and negative taxic responses.
2. List environmental stimuli other than those tested that brine shrimp might encounter.

Responses of the Milkweed Bug

Animals that live on land are subjected to more diversity in their environment than the brine shrimp or other aquatic animals. Aquatic habitats such as that of the brine shrimp are relatively stable. Due to the physical characteristics of water, changes in temperature and other conditions come about more slowly. The same factors on land (temperature, chemicals, etc.) can undergo wide and rapid fluctuations. Active animals (as opposed to burrowing and secluded types) are continually alert, receive stimuli, and respond positively or negatively to meet their needs.

The large milkweed bug, *Oncopeltus fasciatus* Dallas (phylum Arthropoda, class Insecta), is a suitably active terrestrial animal for a laboratory study of behavior. This insect lives on milkweed, a wild plant that grows in open fields and along roadsides on Long Island.

Handling the Bugs and Initial Observations

1. Obtain a large finger bowl. Spread a thin layer of petroleum jelly on the inner wall of the bowl about an inch below the rim of the bowl. Place one milkweed bug in the bowl. You can pick up a bug easily by placing the mouth of a test tube over the bug and allowing it to crawl up on the side of the tube. Then slide a piece of paper over the mouth of the tube. When you are ready to introduce the animal, hold the tube over the bowl, remove the paper, and tap the tube gently, to dislodge the bug.

2. Notice the pair of long antennae and the pair of compound eyes on the head of the bug. The wings, with conspicuous red and black markings lie folded on the back but can be lifted for flight. Minute sensory bristles, called setae, are located at different places on the body, but are particularly numerous at the anterior and posterior ends, and on the feet. The antennae and compound eyes are especially useful for monitoring conditions and objects around them, while the bristles on the feet and elsewhere respond to direct contact with solid objects, such as the surface on which the animal is supported. Use a hand lens or the dissecting microscope to observe the distribution of the setae.

3. Identification of adult male and female bugs (see Figure 3.1): Adult bugs are those with fully developed wings. Immature stages are orange in color and have a pair of black wing buds on the back just behind the head. Place a test tube over an adult bug and allow it to crawl into the tube. Close the tube with a cork. Hold the tube in a vertical position and examine the ventral posterior segments of the bug. The region posterior to the legs and thorax is called the abdomen.

   Male: Ventral surface of the fourth and fifth abdominal segments is rounded. Both segments are usually black, although there are exceptions. Posterior end of the abdomen, ventral surface, is round and shiny.

   Female: Ventral surface of the fourth abdominal segment is pointed at the midline. This segment is black. Fifth abdominal segment is black on the left and right, orange on the mid-line. Posterior end of the abdomen, ventral surface, is triangular and slightly “fuzzy.”

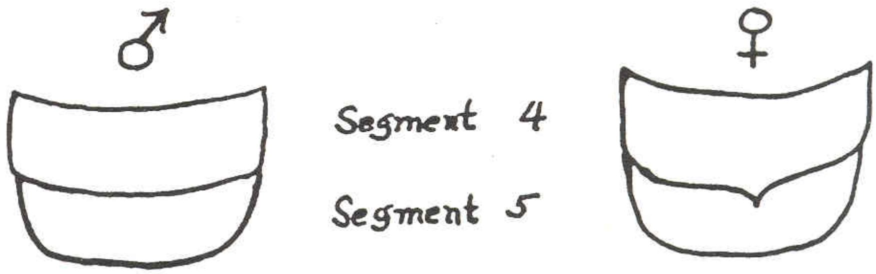

Figure 3.1. Abdominal segments four and five of the male and female milkweed bug.

In this part of the exercise milkweed bugs will be tested for their response to a variety of stimuli. Only one stimulus will be tested at one time. The observations for each test can be recorded on the work sheet provided. After all the tests are completed the results should show some behavioral patterns of this species.

Test 1: Normal versus Stressful Conditions

Part A:

1. Place a bug in a finger bowl and allow it to remain undisturbed for 5 minutes.
2. Measure the rate of antennal movement. Each antennal flick from the vertical to the horizontal is considered a "movement." Count the number of movements for 1 minute.

Part B:

1. Remove this bug from the container (use the test tube) and place it on the table top. Observe and record the reaction of the bug. Read the following directions before beginning your observations.
2. Direction of movement (walking): Observe walking in relation to the window, or a "landmark" on the table, such as a textbook, or two beakers, one with hot water, the other with cold. Use improvised barriers such as a ruler to keep the bug in an area measuring about 60 cm (24 inches) in diameter. Record in Table 3.2 each new direction the bug takes or diagram its walking movement for 5 minutes.
3. Rate of antennal movement: Record in Table 3.2 the number of up and down movements.

   Antennal coordination: Do both antennae move up and down at the same time or does one move down while the other moves up?
4. Proboscis movement: The proboscis, or stylet, is a tiny rod-like structure, located at the "chin" region of the head. Normally it is held against the mid-ventral surface of the thorax (mid-section of the body). Record in Table 3.2 the position and/or movement of the proboscis as the bug moves across the table during the 5 minute period.

Table 3.2. Observations on behavior of the milkweed bug.

Structure observed	Movements observed on table top		Remarks
Walking (5 minutes)	Number of directions		
Antennae	Time (minutes)	Number of movements up and down	
	1st		
	2nd		
	3rd		
	4th		
	5th		
Proboscis	Time (minutes)	Number of movements	
	1st		
	2nd		
	3rd		
	4th		
	5th		

Test 2: Thigmotaxis (response to touch or pressure)

1. Place the milkweed bug in the center of your finger bowl. Does it tend to remain in the center or does it move to the edge of the bowl? Observe for 3 minutes before drawing a conclusion. Does the bug exhibit positive thigmotaxis? Record your observations in Table 3.3.
2. Now place the bowl with the bug in the knee space under your table. Be sure the bug is in the center of the bowl. After 3 minutes is the bug still in the center? Does the milkweed bug exhibit positive thigmotaxis? Is the response of the bug entirely the result of thigmotaxis?
3. Repeat this observation by placing the bowl (with the bug in the center) on a different side of the table where light enters at a different angle. Observe after 3 minutes.
4. Can the bug exhibit negative thigmotaxis?

Test 3: Phototaxis (response to light)

1. Cover one-half of the dish with a dark cloth. Does the bug prefer the light half or dark half? Use a large test tube for this.
2. Move the bowl to a different part of the room and record your observation of the bug after it has 3 minutes to acclimate.

Test 4: Thermotaxis (response to temperature)

Place the tube, still partially covered with a dark cloth, near an incandescent bulb to raise the temperature in the tube. Note if the bug selects a particular part of the temperature gradient.

Test 5: Chemotaxis (response to chemical substances)

1. Remove the tobacco from a cigarette. Place the tobacco in a test tube and fill the tube with warm water. Allow this mixture to stand for at least 30 minutes. Remove the tobacco fragments by pouring the solution through a strainer or several thicknesses of cheesecloth. While waiting for the preparation of the tobacco solution, continue with other observations.
2. Place a drop of perfume or a swab of lipstick on a cotton swab. Bring the swab close to the antennae of the bug (first, near the left, then the right, then between). Does the bug move in response to this stimulus? Next test a bug of the opposite sex with the same stimulus.
3. Dip a swab into the 5% acetic acid solution. Bring the swab near the antennae (first near the left, then right, etc.).
4. Bring the swab with perfume near the left antenna and the swab with acetic acid near the right antenna. How does the bug react?
5. Repeat the procedure with a Bic pen. Other ball point pens may not work as well.
6. Repeat the procedure with the tobacco solution. Be sure this is the last test for chemotaxis (left antenna, right antenna, in between).

Table 3.3. Observations on behavior of the milkweed bug in response to different taxes.

Stimulus	Trial #	Response			Surrounding conditions	Remarks
Thigmotaxis		Center	Intermediate	Side		
Phototaxis		Light	Intermediate	Dark		
Thermotaxis		Warm	Intermediate	Cold		
Chemotaxis		Antennae				
		Right	Left	Between		
Perfume Acetic Acid Bic pen Tobacco						
Thigmotaxis after tobacco		Center	Intermediate	Side		

Geotaxis	1	2	3	4	5	6	7	8	9	10	% negative geotaxis

Test 6: Thigmotaxis (repeat)

Immediately after testing with tobacco solution place the bug in the center of a finger bowl. Does it tend to remain in the center?

Test 7: Geotaxis (response to gravity)

1. Place the open end of a test tube over a bug. After the bug has climbed into the interior of the test tube, cork the tube. A cotton plug may also be used, if corks are not available.

2. After the bug has reached one end of the tube, invert the tube. Repeat this procedure for a total to 10 trials. Is there a consistent response? Does the bug tend to move toward the ceiling? Or toward the floor? What percent of the time did the bug exhibit negative geotaxis (move toward what is now the ceiling of the tube).

3. To calculate the percentage, multiply the number of times it moved toward the ceiling by 10. For example, if it moved toward the ceiling three out of 10 trials: $3 \times 10 = 30\%$ negative geotaxis.

Further Reading

Brine Shrimp

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Milkweed Bug

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APPENDIX A
Rearing the Milkweed Bug Oncopeltus fasciatus

Equipment

Battery jars (2-liter) or other glass containers (1-quart canning jars can be used)
Battery jar covers of nylon netting or gauze
Rubber bands
Small glass jars or vials
Dental wicks or other suitable absorbent material
Small jar caps for food containers
Cotton (non-absorbent is preferable, but not critical)
Paper
Petroleum jelly
Sunflower seeds (shelled) or milkweed seeds (sunflower seeds are available at grocery or health food stores)
Artist's brush (for handling insects)

Contents of the Culture Jar (Figure 3.2)

1. Small jar of water with a cotton wick extending 5–6 cm above the mouth of the jar.
2. A jar cap or other shallow container to hold a single layer of seeds.
3. Several sheets of paper (about 5 × 8 inches) pleated and standing on end for climbing surfaces.
4. A ball of cotton (about 5 cm in diameter) teased to provide a loose mesh for females to deposit eggs.
5. A ring of petroleum jelly 2 cm below the rim of the vessel prevents escape of the insects.
6. Cover with a square of netting held in place with a rubber band.

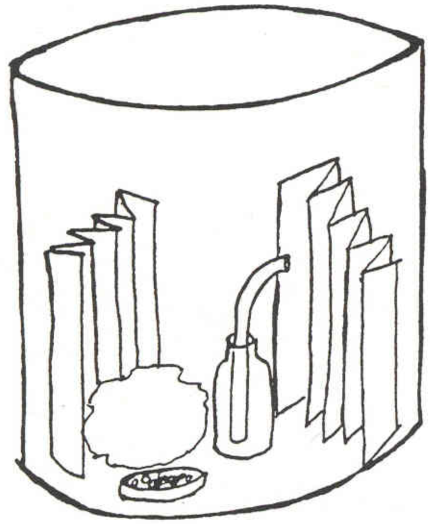

Figure 3.2. Culture jar for rearing the milkweed bug.

Procedure

To start a culture with adults, place several insects in a culture jar. Mating pairs are a good choice because this will insure a favourable ratio of males and females.

Soon after copulation the females begin to lay eggs in the cotton. Batches of yellow eggs are easily recognized and can be removed when desired.

Once a week fresh seeds and water should be provided. If the population in the jar is dense, more frequent changes are necessary. Keep the wick wet at all times. About every fourth week, transfer insects to a fresh jar with clean paper, etc. Be sure there is a ring of petroleum jelly at the top of the jar. Use a small brush for handling the insects. Continuous culturing is carried out by removing batches of fresh eggs from the cotton and placing these in a culture jar to hatch. Development through five instars requires 4–5 weeks, depending on the temperature. One battery jar will accommodate a population of 200 adults.