

Rhythm & Snooze — Circadian Rhythm Genes

Secrets of the Sequence Video Series on the Life Sciences • Grades 9 — 12

Teaching materials developed by VCU Life Sciences

V i r g i n i a C o m m o n w e a l t h U n i v e r s i t y

Classroom Tested Lesson

Video Description

“Secrets of the Sequence,” Show 146, Episode 1

“Rhythm and Snooze” – approximately 9 minutes viewing time

We know that our sleep-wake patterns are regulated by “circadian rhythms” - our internal clocks. And now doctors are learning more about a set of genes responsible for making this clock tick. They are also discovering the “circadian rhythms” for vital organs working in unison to create a living, breathing, walking clock. This system of “circadian genes” is responsible for regulating the sleep-wake cycle of other genes as we are proving that one’s body is comprised of a series of circadian rhythms. This scientific research could lead to new ways of treating those of us who are night owls or daytime larks.

Ward Television

Producer: Fran Victor

Associate Producer: Teri Prestholdt

Featuring: Joseph Takahashi, PhD, Investigator - Howard Hughes Medical Institute, Northwestern University, Phyllis Zee, MD, Director, Sleep Disorders Center, Northwestern University

Lesson Author; Reviewers: Beverly Grimm; Catherine Dahl, Richard Rezba, and Selvi Sriranganathan

Trial Testing Teacher: Pamela Sparks

National and State Science Standards of Learning

National Science Education Standards Connection

Content Standard C: Life Science

As a result of their activities in grades 9-12, all students should develop understanding of

- Molecular basis of heredity
- Behavior of organisms

Content Standard E: Science and Technology.

As a result of their activities in grades 9-12, all students should develop

- Abilities of technological design
- Understandings about science and technology

Content Standard F: Science in Personal and Social Perspectives.

As a result of their activities in grades 9-12, all students should develop understanding of

- Personal and community health

Selected State Science Standards Connections

Use <http://www.eduhound.com> (click on "Standards by State") or a search engine to access additional state science standards.

Virginia

BIO.5 The student will investigate and understand life functions of archaeobacteria, monerans (eubacteria), protists, fungi, plants, and animals including humans. Key concepts include

- e) human health issues, human anatomy, body systems, and life functions; and

BIO.6 The student will investigate and understand common mechanisms of inheritance and protein synthesis. Key concepts include

- c) cell specialization
- d) prediction of inheritance of traits based on the Mendelian laws of heredity;
- h) use, limitations, and misuse of genetic information, and
- i) exploration of the impact of DNA technologies.

Florida

Processes of Life

Standard 1:

The student describes patterns of structure and function in living things. (SC.F.1.4)

1. knows that the body processes involve specific biochemical reactions governed by biochemical principles.
7. knows that organisms respond to internal and external stimuli.

Standard 2:

The student understands the process and importance of genetic diversity. (SC.F.2.4)

2. knows that every cell contains a "blueprint" coded in DNA molecules that specify how proteins are assembled to regulate cells.

Overview

Circadian rhythms are the "inner clocks" that keep pace with the twenty-four hour cycle of night and day. These clocks maintain an organism's daily routine by controlling daily activities such as sleep and alertness patterns, mating, and other behavioral functions. Though circadian rhythms have a genetic basis, a change in routine (i.e. staying up late, waking up early) may result in altering the circadian clock.

A key aspect involved in the altering the circadian rhythm is light. Light has been known to reset the biological clock. Circadian rhythms have been observed in *Drosophila melanogaster*, the fruit fly. Two clock genes, timeless and period, produce two proteins, TIM and PER, that function during the day. These proteins control activity and mating. Two other proteins, Clock and BMAL1 function at night. Light-induced changes alter levels of TIM protein.

An animal needs a steady routine each day for its biological clock to work effectively. There are many similarities between the human and fruit fly biological clock, such as activity rhythms that have a twenty-four hour cycle, and similar proteins that are reset by light. Therefore, a study of circadian rhythm-induced disorders in fruit flies may indicate treatment for disorders in mammals. Two such disorders are jet lag and seasonal depression. Jet lag is caused by fast travel over multiple time zones. Seasonal depression is caused by the decrease in light during the winter. One question is whether supplements like vitamin C, B₁₂, D, calcium, iron, and niacin have a stabilizing effect on circadian rhythms showing a stable routine of behavior.

Testing: A sample related multiple choice item from State Standardized Exams

Soybeans planted early in the spring flower at the same time as soybeans planted early in the summer due to which factor?

- A. age of the plant
- B. length of darkness*
- C. amount of moisture
- D. amount of fertilizer

(Note: It takes a certain number of hours of light within a 24 hour cycle to trigger the mechanism for the plant to bloom.)

Source: Biology Sample Items, North Carolina

Video Preparation

Preview the video and make note of the locations at which you will later pause the video for discussion.

Before Viewing

Ask the following questions:

1. Are you a night person (an "owl") or a morning person (a "lark")?
2. Why do you think you are a Lark or an Owl?
Lark responses may include:
 - Work better in the morning because not tired
 - More energy, inspiration*Owl responses may include:*
 - More energy at night
 - Calm at night so it is easier to see things
 - Not as grumpy at night
 - Already up so do not have to worry about not waking up
3. What time do you get up in the morning? What wakes you up?
Internal alarm, alarm clock, mother wakes me, puppy
4. Is that time the same on weekend, holidays or summer?
Answers vary:
5. Do you sleep soundly through the night? If not, what might wake you up?
Sounds, temperature changes, need to urinate, light
6. How many hours a day do you sleep?
Answers will vary

During Viewing

1. **START** the video.
2. **PAUSE** the video (4:04 minutes into the video) immediately after Dr. Takahashi says: "One of the surprises is that if you isolate those tissues, they can oscillate and show circadian rhythms all by themselves, separated from the body."

Ask: Where is the master circadian clock located and how is it activated?

In a cluster of specialized cells at the base of the brain called the Suprachiasmatic Nucleus (SCN). The clock is activated by light hitting the eye, traveling along the optic nerve and energizing 1000's of neurons.

Ask: Where else in the body are there other special cells that act as clocks?

In various tissues throughout the body including organs such as the liver and the heart.

3. **RESUME** the video and play to the end.

After Viewing

1. Ask: Do all members of your family have the same sleep pattern as you? Find out if your parents, grandparents, aunts, or uncles have always been an owl or a lark, or did they change as they got older?
If there has been a change, what do you think caused a change in their sleep patterns?

Answers will vary

2. Ask: What problems do you see arising from your sleep patterns when you go away to college?

Answers will vary

3. Ask: What problems do people have if their jobs require hours that are different from their preferred sleep patterns?

Not as productive, performance down, sleep problems develop

4. Research has indicated that jet lag and seasonal depression are directly influenced by an alteration in a person's biological clock. Jet lag is caused by fast travel over multiple time zones. Seasonal depression is caused by the reduction of light during winter.

Ask: What recommendations might you make to sufferers of jet lag or seasonal depression?

Increase their exposure to sunlight to reset the clock

5. Conduct the Student Activity: Effects of Light Patterns on Fruit Fly Behaviors

Teacher Notes for the Student Activity: The Effects of Light Patterns on Fruit Fly Behaviors

Materials

For each student group

- Fruit flies
- Two vials with sponge tops for culturing fruit flies. (You can obtain the vials from a biological supply house, such as Carolina Biological).
- Two containers to house vials but allow light to penetrate (such as clear plastic shoeboxes)
- Drosophila Blue Medium (Obtain from a biological supply house)
- Three female fruit flies plus three males for each vial
- 5 cm x 5 cm dark cloth or use a black trash bag
- 20 cm x 20 cm dark cloth

For the class

- Two additional vials - one for holding males and another for females
- Two fluorescent lights - one with a timer to turn 12 hours on and then 12 hours off
- Two thermometers

Procedure

1. Set up the two lighting systems in your classroom. One to cycle on for 12 hrs and off for 12 hrs; the other to remain on continuously. Light cycle is the independent variable.
2. Separate the females from the males to save time, or explain to students how to select females and males:
 - It is easier to have two additional vials with the medium to keep the sexes separate until students need them.
 - To sex fruit flies a dissecting microscope or magnifying glass will be helpful. The male has a darker and narrower abdomen and two dark bands found on the front two appendages called "sex combs". In general, males tend to be smaller than females.
3. Set up clear containers to hold the group's vials. The vials should be in a standing position. The light source should be placed 15 cm directly above the container. Set a timer so that the light will be on for 12 hours and then off for 12 hours over one container. This will be considered normal light. The other container will have continuous light - 24 hours of light. Place a thermometer inside both containers to monitor the temperature. You want to insure that the light does not increase the temperature in the container. Adjust the distance to the light accordingly.
4. Read and explain the procedures described in the Student Handout with your students.
5. Students can make a variety of observations daily as their dependent variable(s) and record those on the Data Sheet supplied in Appendix A. You can select one or all of the following dependent variables, or allow students to design their own experiment under your direct supervision.
 - Interactions: Remove the vial from under the light source; place the vial sideways. Wait 5 minutes and observe the flies for one minute. Record how many interactions occur among the fruit flies. Note any mating and courting behaviors. Repeat this two more times. Each vial should be observed for three one-minute intervals.
 - Orientation: Remove the vial from the container; place the vial sideways on a flat surface under the light. Cover half the vial with a 5 cm x 5 cm dark cloth. If the majority of the flies travel towards the light, record that as a positive (+) orientation. If they travel under the cloth to the darkness, record

that as a negative (-). If there is no clear majority, record that as a zero (0). Ideally, repeat this 25 times for each vial or as time allows.

- **Speed:** Place the vial sideways. Select one fly within a vial, and using a washable marker, trace that fly's track for 5 seconds. Measure the distance traveled in centimeters. Repeat this process a number of times for each chosen fly.
- **Activity:** Rank each vial according to activity level. A rank of 1 indicates little or no activity. A rank of 2 indicates some activity, while a rank of 3 designates an active group.

Additional observations (dependent variables) could be production of larva, pupa, pupa development or adult production. If you wish to monitor genetic crosses or any inherited qualities, place three virgin female flies in each vial. Use only flies that have recently emerged from the pupa stage. This insures that only the sperm from the 3 adult males added to this vial have fertilized the females because females obtain and store all sperm for their lifetime within twelve hours of emerging as an adult.

6. Each day, spend a few minutes discussing the students' observations.

7. Have students complete the "Analyzing your Data" section of their Handout.

Additional Teacher Notes

- Recent results - Constant light tends to result in more activity with random patterns of rest and activity. A higher percentage of flies go towards the light during the orientation test. More larvae are produced than in the normal light. Normal light tends to show normal "courting behavior".
- Adding a second *independent variable* - While challenging to most students, you could add a second independent variable. If you choose an additional variable of adding supplements like iron, calcium, vitamins C, B12, and D, several of the observations under different cycles of light are enhanced. The iron supplement increases the number of flies that will go to the light regardless if it is constant or normal on/off light. Niacin and vitamin D also enhances this behavior but vitamin B12, C, and calcium appear to reverse it. Vitamin C and iron result in a reduced distance in constant light. See Cothron et al, *Students and Research*¹for strategies for teaching about multivariable experiments.
- Supplements can be obtained from a health food store, food store or pharmacy. Grind a small amount to a powder form in a mortar and pestle, mass out 0.4 grams, and add to the 9 mL of water before the water is added to the *Drosophila* Blue Medium in the culturing vial.

¹Cothron, J., Giese, R., and Rezba, R. (2006). *Students and Research – Practical Strategies for Science Classrooms and Competitions*. Dubuque: Kendall/Hunt Publishing Co.

Student Handout:

The Effects of Light Patterns on Fruit Fly Behaviors

In this activity you will test the effects of light patterns on the Circadian Rhythm of fruit flies by observing various behaviors of fruit flies.

Materials

For each student group

- Fruit flies
- Two vials with sponge tops for culturing fruit flies.
- Water
- Drosophila Blue Medium
- Three female fruit flies plus three males for each vial
- 5 cm x 5 cm dark cloth or use a black trash bag
- 20 cm x 20 cm dark cloth

Procedure

1. Label the vials according to treatment - continuous light or normal on/off light.
2. To each vial add 4.5 g of the Drosophila Blue Medium.
3. Add 9 mL of water.
4. Cover the vial with a sponge top.
5. If your teacher has not already set up separate vials for male and female, repeat steps 2 thru 4.
6. Obtain male and female flies from your teacher. If flies have not been yet been separated by sex, you can sex fruit flies using a dissecting microscope or magnifying lens. The males tend to be smaller, have a darker and narrower abdomen, and have two dark bands found on the front two appendages called "sex combs".
7. Add 3 males and 3 females to each vial. If genetic crosses or any inherited qualities will be monitored, use three virgin female flies for each vial. Use only flies that have recently emerged from the pupa stage. This insures that only the sperm from the 3 adult males added to this vial have fertilized the females because females obtain and store all sperm for their lifetime within twelve hours of emerging as an adult.
8. Place the vials labeled normal on/off light in the container under the light with the timer. Turn on the light. Note the time because this is the time you will make observations everyday for at least 2 days.
9. Check the temperature after 15 minutes and make observations of the fruit flies' behavior. The vials will be exposed to light for 12 hours, followed by darkness for 12 hours. This follows the normal light cycle.
10. Place the vials labeled continuous light in the container under the other light. Turn on the light. Again note the time because this is the time you will make observations everyday. Check the temperature after 15 minutes and make observations of fruit flies' behavior. These vials will be exposed to continuous light for 48 hours.
11. Monitor the temperatures of the containers. They should be equal in temperature and as close to room temperature as possible. If not, adjust the distance to the light accordingly.

Appendix A

Name _____

DATA SHEET

12 hours light/12 hours dark

	Interactions	Orientation	Speed	Activity Rank
Day 1	i) ii) iii)			
Day 2				
Day 3				

Continuous Light

	Interactions	Orientation	Speed	Activity Rank
Day 1	i) ii) iii)			
Day 2				
Day 3				

Additional Resources

Because Web sites frequently change, some of these resources may no longer be available. Use a search engine and related key words to generate new Web sites.

Calcium. (2001, May 16). [Online]. Available: <http://www.gnc.com/wellness/natpharm/supp/calcium.htm>

Chicurel, M. (2001, January 21). *Mutant gene speeds up human clock*. Science 291. 2226-2227.

Circadian rhythms: possible clock messenger identified. (1999, December 24). Science 286. 2434-2436.

Clockwork genes: holiday lectures explore the biological basis of circadian. (2002, November 27). <http://www.hhmi.org/news/112700.html>.

Glick, M. (1996, March 21). *Light sets the molecular controls of circadian rhythm*. <http://www.rockefeller.edu/pubinfo/light.nr.html>

Jet lag. (2001, March 07). <http://www.hhmi.org/grants/lectures/2001/summer/jetlag.html>.

Rosbash, M. (2000). *Researchers identify unique circadian rhythm photoreceptor*. <http://www.hhmi.org/news/robash.html>.

Rosbash, M. (2000, December 27). *Molecular genetics of RNA processing and behavior* <http://www.hhmi.org/research/investigators/rosbash.html>.

Rosbash, M. (2001, February). *RNA processing and behavior* <http://www.hhmi.org/science/genetics/rosbash.html>.

Sehgal, A. (2000, November). *Molecular basis of circadian rhythms*. <http://www.hhmi.org/research/investigators/sehgal.html>.

Sehgal, A. (1999, September 9). *Experiments illuminate workings of biological clocks*. : <http://www.hhmi.org/news/sehgal.html>.

Takahashi, J. (1999, November 25). *Shedding light on circadian rhythms*. <http://www.hhmi.org/news/takahashi.html>.

Takahashi, J. (2001, January). *Molecular and genetic analysis of the mammalian circadian clock system*. <http://www.hhmi.org/research/investigators/takahashi.html>.

Genomic Revolution

http://www.ornl.gov/sci/techresources/Human_Genome/education/education.shtml

The Web site to the government-funded Human Genome Project with links about genomics, the history of the project, and more.

Secrets of the Sequence Videos and Lessons

This video and 49 others with their accompanying lessons are available *at no charge* from www.vcu.edu/lifesci/sosq