Exploring circadian rhythms

Introduction: Getting familiar

1. When you open the application, you will see several features including an animated mouse at the top.
   a. Is the mouse typically active at times when the light is on or off?

   b. What is the term used to describe animals active during that time?

   c. In the wild, why is it an advantage for the mouse to be active during this time?

2. On the right side of the screen you will see an actogram, a tool used by scientists to study patterns in circadian rhythms. The bar above the actogram shows the current light-dark cycle in a 24 hour period; the white and gray regions in the bar represent the light and dark periods, respectively.
   a. At what hour does the dark period begin? At what hour does it end?

   b. How is activity (and lack thereof) represented on the actogram?

   c. What do you think is a simple way in which scientists record the activity of the mouse without having to watch it? (Hint: look at the animation)

3. Below the mouse animation you will see "Choose a light/dark cycle" and a drop-down menu that will allow you to control the light-dark settings for your experiment—but we will use this more later. For now, get familiar with the buttons to the right of the drop-down menu. There is a button for Play, Pause, Copy, and Clear.

4. Press the Pause button and then press Copy. You will see a smaller image of the active actogram appear in the blue section labeled Copied Actograms at the bottom. This is only a temporary location to store your actograms.

5. Save an image of your actogram permanently by right-clicking on the actogram and selecting "Save Image As…" Name the file "test." Only the six most recent copied actograms will stay at the bottom, so it's important to save as you do your work. Finally, press the Clear button to clear the active actogram and start over.
   a. In science, why is it important to keep good records of your data?

The 12:12 Light-Dark Cycle

6. After clearing the actogram, press Play and allow the actogram to run for a few "days," and then pause it. Move your cursor to the bar showing the current light-dark cycle above the actogram. Click on any location on the bar and drag it to the left or right so the dark bar is in a different location. Now press Play again and observe the results.

    a. Using the space below, describe what happens to the mouse's activity according to the actogram. Be as specific as possible.

    b. Copy and save an actogram that shows a shift in the light-dark cycle and the mouse's entrainment to the new cycle. How many days did it take for the mouse to entrain to the new cycle?

    c. What is a real-life example of when this shift occurs in human lives?

When an animal adapts to a particular light-dark cycle, it is described as being <u>entrained</u> to that schedule. We can see that after adjusting to the new light-dark cycle, the mouse starts waking up at the same time every day again.

Constant darkness

7. Now clear and resent the actogram again. After letting the mouse entrain to the 12-12 light-dark cycle, change the setting to "Full darkness." Let the actogram run for more than ten days.

    a. Describe what happens to the mouse's activity. Be specific.

In constant darkness, the mouse is no longer bound by external cues, and its internal clock modulates the timing of daily activity. This internal circadian clock is a bit different in every animal, but generally the length of the <u>subjective day</u> in constant darkness is a bit *under* 24 hours. There is a consistent shift in activity onset that is called "<u>free-running.</u>" Mice in constant darkness begin daily activity a bit earlier every day.

The correlate in humans would be to start waking up "naturally" after being used to waking up to an alarm clock every day. In humans, without external cues that tie us to the environmental schedule, our subjective day-length is a bit *over* 24 hours. For a few days, we may continue to get up at the same time, but over time we may wake up a bit later every morning.

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Exploration

Now you can explore the mouse actogram by designing your own experiment. There are three different scenarios you can explore, or make up your own. Then develop a hypothesis and prediction and experiment, perform the experiment, and analyze the results.

Constant light: Entrain your mouse to a 12:12 light-dark cycle and then introduce your mouse to days with 24 hours of light.

Repeated pulse (skeleton photoperiod): The mouse is kept in constant dark except for a pulse of light at the same time every day.

Single pulse (phase response): First, your mouse is kept in constant dark. Then a pulse of light is introduced for one day and afterward the mouse is kept in constant dark again. When will you introduce the pulse? There are several ways you can do this:
- Introduce the pulse during the mouse's subjective daylight period (when the mouse expects light and is sleeping)
- Introduce the pulse at the beginning of the mouse's subjective 'night' (when the mouse expects dark and is active)
- Introduce the pulse at the end of the mouse's subjective night

Experiment Design

Write or type your answers out on a separate piece of paper.
1. Hypothesis and prediction: What do you think will happen and how will you test it?

2. What are your independent and dependent variables?

3. What are your control and treatment groups?

4. What are your methods? (Describe the steps you will take with detail. At what exact times and exactly how long will treatments be given? How many times will you repeat the experiment? How will you analyze your results?)

Experiment Results

When you are done with your design, then you can run your experiment. Be sure to follow all of your directions that you wrote out in your method section. When your experiment is done, analyze your results.
5. Describe what happened in your results. Was your prediction supported? How did your control group respond differently than your treatment group? Why do you think that happened?

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