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BIOL 476

Actogram Report (100 pts):

Exploring circadian rhythms: complete the below pre-defined questions and experiments using the following two tools:

- Mouse Actogram Model, Project Neuron, University of Illinois (free online tool)
  https://projectneuron.illinois.edu/games/mouse-actogram-model

- Sleep Diary or any tracker for measuring human sleep/wake activity, from which you can download data for further analysis.

Findings from these actogram experiments must be written up by each student in this report form. Be specific and <u>ELABORATE</u> on your findings! (hint: use relevant circadian literature to support your findings)

1) Mouse Actogram:

Using the Project Neuron online activity tool, explore the mouse actogram by designing your own experiment. There are three different scenarios you can explore, or make up your own. Choose **one** and develop a hypothesis, prediction and experiment, perform the experiment, and analyze the results. Incorporate your generated actograms as images (e.g. jpeg, tiff) in your report with proper legends and brief descriptions.

Three scenarios (A, B or C):

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

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

C) 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 <u>subjective daylight</u> period (when the mouse expects light and is sleeping).

 • Introduce the pulse at the beginning of the mouse's <u>subjective 'night'</u> (when the mouse expects dark and is active).

 • Introduce the pulse at the end of the mouse's subjective night.

<u>Experimental Design:</u>

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

Answer the following questions (be specific, and <u>elaborate</u> on your findings):

1) Hypothesis and prediction: What do you think will happen and how will you test it?

If a mouse were to be entrained to a 12-hour light and 12 dark cycle for two weeks, and then afterwards introduced to constant 24 hours of light for another two weeks, I hypothesize that the mouse's activity will start to freerun as the mouse will start its activity period later each time throughout the 2-week period. Additionally, the mouse's sleep/wake cycle will also freerun, in a similar manner, as the mouse will sleep later each time throughout the 2-week period.

My prediction is that the mouse will still have the same sleep and wake cycle (the 12-hour period the mouse sleeps and the 12-hour period the mouse is awake will be self sustained), in addition to, its activity period (measured by mouse wheel duration) will not be changed. However, these circadian rhythms will be shifted to the right more every 24 hours (free run with phase delay) as there is nothing entraining the rhythms such as light.

While the mouse's circadian rhythms are freerunning, they are not necessarily "broken" as we can return the mouse back to its regular cycle by entraining it with the introduction of its original 12-hour:12-hour light-dark cycle.

2) What are your control and treatment groups?

- My control group would include 30 mice that would only be exposed to the 12-hour light and 12-hour dark cycle.
  - Control → 30 mice only exposed to 12:12 light-dark cycle
  - Duration → 1 month (30 days)

- My treatment groups would include 30 mice that would be expose to the 12-hour light and 12-hour dark cycle for the first half of the experiment (15 days), which is followed by constant 24-hour light for the rest of the experiment (15 days).
  - Treatment → 30 mice exposed to 12:12 light-dark cycle then constant light
  - Duration → 15 days (12-hour:12-hour light-dark cycle) and 15 days (constant light)

- Future Experiment: Introduction of additional control group (12:12 light-dark cycle → constant light → 12:12 light-dark cycle)
  - This would test whether the rhythm can be reverted to its original cycle as by entraining it with a zeitgeber (light)
    - Verifies if it's a circadian rhythm (as circadian rhythms can be entrained)
  - Control group would consist of 30 mice that would be first exposed to 12-hour light and 12-hour dark cycle for 10 days, then followed by constant light for 10 days, and finally back to 12-hour light and 12-

hour dark cycle for another 10 days.

3) 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? How many animals do you choose to examine. How much lux, etc)

For my experiment, I would setup two separate areas for the control and treatment groups of mice. These two areas will have identical setups as pertaining to food access (availability), activity sectors (mouse wheel), temperature, humidity, and gravity. The only difference between these two areas will only be the photoperiod in which light is on for the treatment group. In addition, each area will have 30 individual cages in which each mouse will be isolated to prevent social interaction that could add another variable. Furthermore, each mouse will be taken from a batch of homebred mice that I will be cultivating for at least 5 generations (this process is added to reduce any genetic anamoly, in addition to, different fitnesses that mice could attain from being in a different batch).

The experiment for both groups will start at the exact same time (6 am ~ prevents the addition of another variable). For the first 15 days, both groups will be exposed to the 12-hour light and 12-hour dark cycle (lights off from 6 am to 6 pm and lights on from 6 pm to 6 am). The treatment group after the initial 15 days will have their lights on constantly for the remaining 15 days of the experiment. To prevent human interaction as an additional variable, everything in the experiment will be automated. Food and water dispersion will be automated, and the mice will have access to the food at their own pleasure (food/water availability 24/7). Air humidity, temperature, and gravity will be kept at a constant by AC and humidifier in lab. An area of the lab containing the treatment group will be closed off and filled with black-out curtains to prevent light from escaping. Additionally, to prevent outside interference, the lab will contain foam, as seen in recording studios, to prevent sound from penetrating through the walls. Finally, for both groups, light controls will be automated and made available to monitor remotely. The light source will provide enough lumens to supply a constant of 1000 lux to each individual area.

All analysis and data collection will be handled through an automated computerized system. Like the light and food automation, the mouse wheel will have sensors detecting movement of the wheel. Additionally, cameras will be installed in every cage and a live recording will be saved for future analysis (no privacy for the mice). The results collected will be analyzed to map sleep/wake cycles, in addition, to wheel activity. The data will be plotted in an actogram for each mouse in each group. Afterwards, all the actograms in a particular group (control and treatment) will be averaged to create the control group actogram and treatment group actogram. The phase shifts and robustness will be calculated from the control and treatment actograms, which will be analyzed later in the results. Furthermore, as an extra precaution, a backup of all the computerized systems will be on site to automatically replace the current system if it goes down.

Experiment will be ran at least 3 times to solidify the data collected and to prove that there were no outliers in the results.

Future Experiment: Introduction of additional control group (12:12 light-dark cycle → constant light → 12:12 light-dark cycle)
- This additional control group will be setup similar to the other groups under the same environmental conditions (Food/water availability, humidity,

temperature, lux,duration...etc.)
- Mice will go through 10 days of 12-hour:12-hour light-dark cycle followed by 10 days of dark, then finally 10 days of 12:12 light-dark cycle
  - ○

Experimental Results:

When you are done with your experimental 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. Incorporate your results and actograms in this report.

4) 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? Compare your findings to the relevant literature, and cite these studies in your results section.

Control Group: 12:12 light-dark cycle
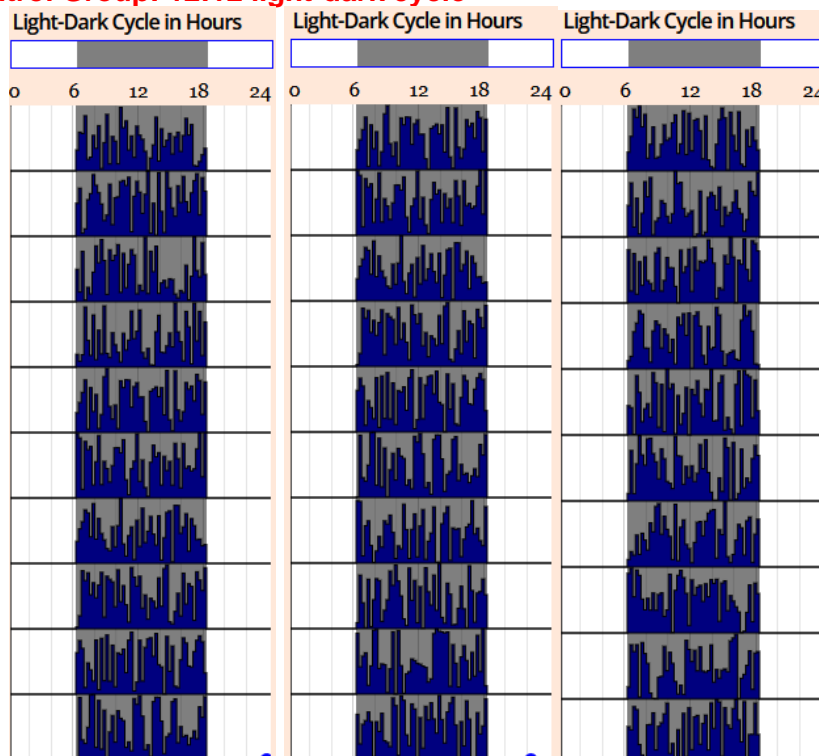
***Experiment ran for 30 days in 12:12 light-dark cycle

Treatment Group: 12:12 light-dark cycle → Constant Light

***Experiment ran for 15 days in 12:12 light-dark cycle followed by 15 days in constant light

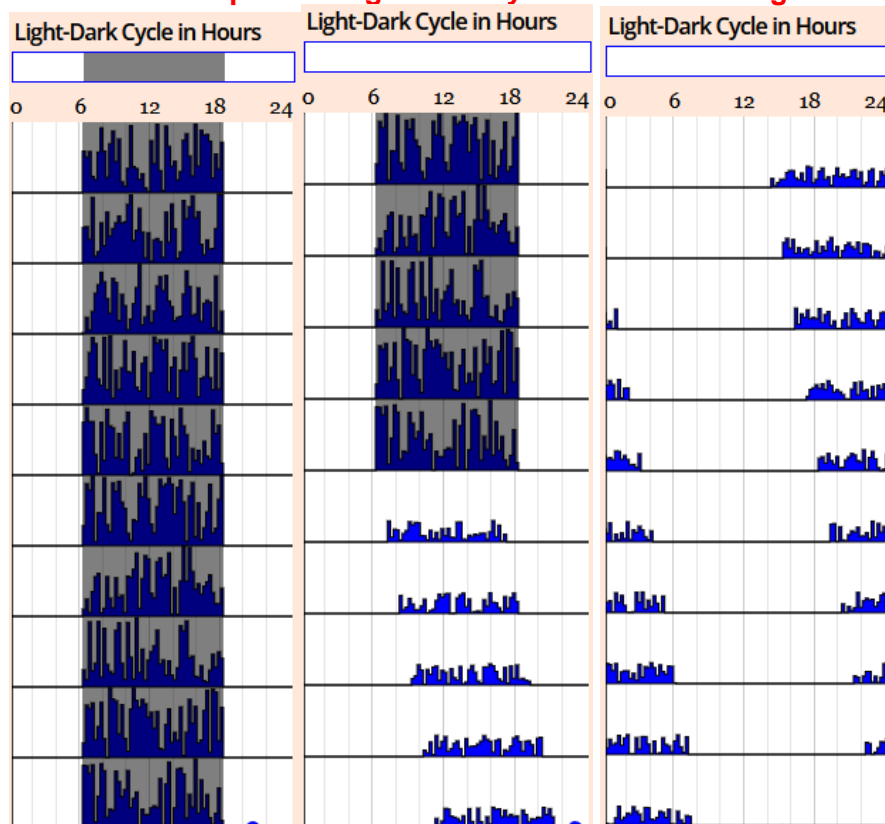Analyzing the data collected from the experiment, my hypothesis is supported by the results as demonstrated by the strong relationship of light entraining the wheel activity and sleep/wake cycles. My prediction were true in the sense that when the mice were introduced to constant light, their cycles began to free run (such with the case of their wheel activity and sleep/wake cycles). The absence of a zeitgeber resulted in their circadian rhythms to be out of phase as with the introduction of the phase delay.

Examining my control group, the mice remained constant in their wheel activity and sleep/wake cycles throughout the experiment. The mice were active at 6 am signified by the blue curves and inactive at 6 pm by lack of blue curves. Interestingly, the data showed the mice to be nocturnal as they were awake when the light was off (grey area) and asleep when they light was on (white area). Comparing this data to the treatment group, we find results varying during the constant light period. For the last 15 days of the experiment, in which the treatment group was exposed to constant light, the data shows a phase delay in their usual active routine. The mice in this group start to wake up later each time throughout the experiment. Additionally, the wheel activity of the mice is shown to be significantly reduced in comparison to the control group of mice. This is partly due to the mice being nocturnal as they are genetically active during the

night time compared to day time. With the light being on constantly 24/7, the mouse is biologically repressed as it is out of its usually active time. However, while the data depicts lower wheel activity rate for the mouse, it still continue to be active for 12 hours signifying its circadian rhthmicity.

The result of the experiment can be easily explained by looking at our literature. Multiple studies have shown that melatonin is released as a result of exposure to light. Melatonin being a hormone primarily in charge of synchronizing circadian rhythms is a big factor in why our treatment group were freerunning in constant light. However, animals (in this case mice) are still controlled by their internal clocks which is supervised by the suprachiasmatic nucleus (SCN). The SCN allows for the regulation of the 24 hour cycle that permits the constant free running circadian activity. For our treatment group, as explained above, the SCN allows for the normal 12 hour active period to happen, however, without light entraining the internal clock to "sleep" and "wake" at a certain times, we are left with a cycle that shifts everyday. On the other hand, the control group that has light and dark cycles, we are able to see the active entrainment of light to the internal clocks of the mice.

In summary, the results supported our hypothesis of light entraining our circadian rhythms. The control group remained constant in their wheel activity and sleep/wake cycles, while the treatment group began to free run with the additional phase delay. Without an entrainment marker such as light, the mouse were only left with their biological clocks to regulate their circadian rhythms.

Future Experiment: Introduction of additional control group (12:12 light-dark cycle → constant light → 12:12 light-dark cycle)
- For future experiments we could introduce another group that could test if the mice would return to their original cycles, if the zeitgeber (light) were to be reintroduced at the end of the experiment
- Soldifies the idea of light entraining our circadian rhythms.

2) Human Actogram:

Create an actogram of your sleep/wake activity in a natural condition. Calculate and compare total scores using the Morningness-Eveningness Questionnaire (MEQ) self-assessment, and sleep diary data (actogram).

1) **Complete the Morningness-Eveningness Questionnaire (MEQ-SA) (see PDF posted on WebCampus). This questionnaire has 19 questions, each with a number of points. First, add up the points you circled and enter your total MEQ score in the table below and on the wiki page (see WebCampus).**

Scores range from 18-86. Scores between 41 and below indicate "evening types". Scores of 59 and above indicate "morning types". Scores between 42-58 indicate "intermediate types".

16-30	31-41	42-58	59-69	70-86
definite evening	moderate evening	intermediate	moderate morning	definite morning

2) Obtain and calculate the mean and standard deviation of the MEQ self-assessment score using scores of students in the class (use the wiki page on WebCampus of the available scores)

# of students	50
Your MEQ-SA score	50
Mean MEQ-SA ± SD (class)	48.52 ± 10.56

3) Based on your MEQ self-assessment score, are you a morning, intermediate or evening chronotype?
   Based on my MEQ self-assessment score, I am an intermediate chronotype.

4) Determine the <u>frequency</u> of MEQ scores of the entire class (no student names), and analyze your results. Hint: create a histogram! Are there any morning and evening types?
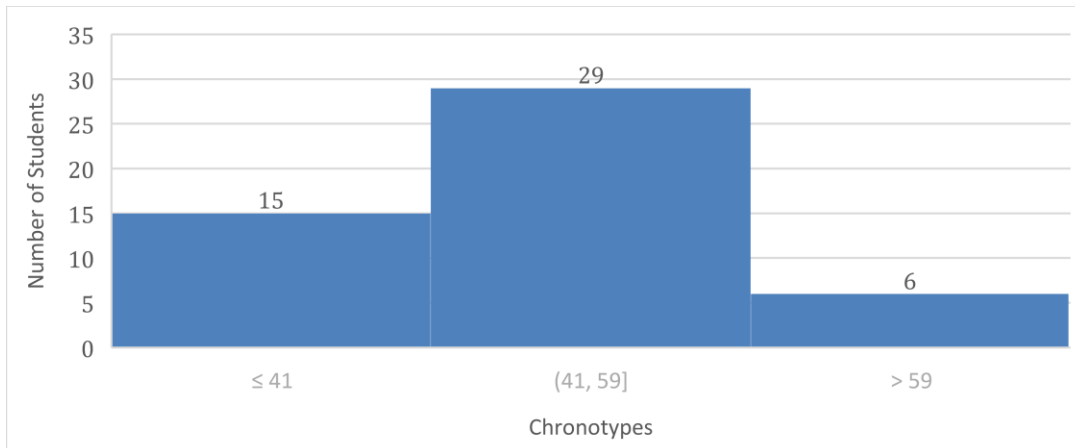
- Morning Chronotype: 6
- Intermediate Chronotype: 29
- Evening Chronotype: 15

The results depicts that the class leaned more towards individuals having an intermediate evening chronotypes.

5) With help of a human sleep/wake activity tracker, record your own sleep and wake cycles in a natural condition. Alternatively, if you are unable to use a sleep/wake tracker, keep a sleep diary (posted on WebCampus) and manually record your sleep/wake time. Record data for at least two consecutive weeks and three weekends. Generate an actogram using the data recorded.

In addition to your actogram, report your sleep variables in weekdays and weekends in the below table:

	Weekdays	Weekends
Sleep onset	8:18 PM ± 2.66 hrs	1:07 AM ± 5.53 hrs
Sleep offset	8:18 AM ± 3 min	2:30 AM ± 4.87 hrs
Sleep duration	7.4 ± 59.4 min	9.13 ± 49.8 min

Sleep Calculations

	Onset	Offset	Duration
M	1:00 AM	9:00 AM	7
T	11:00 PM	7:00 AM	7
W	12:00 AM	9:00 AM	8
Th	11:00 PM	7:00 AM	7
F	12:00 AM	7:00 AM	6
Sa	12:00 AM	12:00 PM	11
Su	3:00 AM	1:00 PM	9
M	1:00 AM	10:00 AM	8
T	11:00 PM	7:00 AM	7
W	1:00 AM	9:00 AM	7
Th	11:00 PM	7:00 AM	7
F	12:00 AM	10:00 AM	9
Sa	1:00 AM	12:00 AM	9
Su	1:00 AM	12:00 AM	9
M	12:00 AM	9:00 AM	8
T	11:00 PM	7:00 AM	7
W	12:00 AM	9:00 AM	8
Th	12:00 AM	7:00 AM	6
F	12:00 AM	10:00 AM	9
Sa	12:00 AM	10:00 AM	9
Su	12:00 AM	10:00 AM	9
M	12:00 AM	9:00 AM	8
T	12:00 AM	7:00 AM	6
W	12:00 AM	9:00 AM	8
Th	12:00 AM	7:00 AM	6
F	12:00 AM	10:00 AM	9
Sa	2:00 AM	12:00 PM	9
Su	2:00 AM	11:00 AM	8

Weekdays					
Onset		Offset		Duration	
Average	Deviation	Average	Deviation	Average	Deviation
8:18 PM	2.6585368	8:18 AM	0.05253097	7.4	0.99472292

Weekends					
Onset		Offset		Duration	
Average	Deviation	Average	Deviation	Average	Deviation
1:07 AM	5.5261121	2:30 AM	4.87360074	9.125	0.83452296

Sleep Diary/Actogram – Kobe Maristela

| | AM | | | | | | | | | | | | PM | | | | | | | | | | | | |
| | Mid | Morning | | | | | | | | | | | N | Afternoon | | | | | Evening | | | | | | |
	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	Sleep quality
M		↓	←	—	—	—	—	—	→	↑														↓	7
T	←	—	—	—	—	—	→	↑																	7
W	↓	←	—	—	—	—	—	—	→	↑														↓	10
Th	←	—	—	—	—	—	→	↑																	7
F	↓	←	—	—	—	—	→	↑																	5
Sa	↓	←	—	—	—	—	—	—	—	—	—	→	↑												10
Su				↓	←	—	—	—	—	—	—	—	→	↑											10
M		↓	←	—	—	—	—	—	—	→	↑													↓	10
T	←	—	—	—	—	—	→	↑																	7
W		↓	←	—	—	—	—	—	—	→	↑													↓	7
Th	←	—	—	—	—	—	→	↑																	7
F	↓	←	—	—	—	—	—	—	→	↑															10
Sa		↓	←	—	—	—	—	—	—	—	→	↑													10
Su		↓	←	—	—	—	—	—	—	—	→	↑													10
M	↓	←	—	—	—	—	—	—	→	↑														↓	10
T	←	—	—	—	—	—	→	↑																	7
W	↓	←	—	—	—	—	—	—	→	↑															10
Th	↓	←	—	—	—	—	→	↑																	4
F	↓	←	—	—	—	—	—	—	—	→	↑														10
Sa	↓	←	—	—	—	—	—	—	→	↑															10
Su	↓	←	—	—	—	—	—	—	→	↑															10
M	↓	←	—	—	—	—	—	—	→	↑															10
T	↓	←	—	—	—	—	→	↑																	4
W	↓	←	—	—	—	—	—	—	→	↑															9
Th	↓	←	—	—	—	—	→	↑																	4
F	↓	←	—	—	—	—	—	—	—	→	↑														10
Sa			↓	←	—	—	—	—	—	—	—	→	↑												10
Su			↓	←	—	—	—	—	—	—	→	↑													10

Instructions: use the symbols below to indicate your sleep times in the grid.
Rate your sleep quality each night from 0 (poor) to 10 (excellent)

↓ go to bed;   ↑ get out of bed;   ←→ actual sleep

6) Using the generated actogram, describe your sleep/wake patterns. Is your MEQ self-assessment supported by the actogram results? If not, please explain.

   a. Looking at the actogram, my sleep and wake patterns looks to be sporadic during the weekdays, and for the weekends, my sleep patterns are shifted towards the right with me waking up later in the day. With my MEQ self-assessment, the data shown does support my MEQ self-assessment score of 50 as I do not necessarily lean towards morning or even, but moreso in the middle. My intermediate chronotype does explain why I am more awake in the middle of the day. Additionally, the data shows that I tend to sleep more during the weekends in comparison to the weekdays by a factor 2 more

additional hours. On the same note, I also tend to wake up later in the day during the weekends in comparison to the weekday.

b. In summary, the data supports my MEQ self assessment of being an intermediate chronotype.