Biol 792 Project

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**Data Modification for Analyzing Circadian Activity**

**Introduction**

The circadian clock drives overt rhythms in physiology and behavior, controlling sleep/wake cycles, hormone secretion, and immune function across taxa. This clock is controlled by a feedback loop of oscillating core genes and is entrained to environmental cues, such as light (*1*). Our core understanding of the circadian rhythm is based on genetic components regulating almost half of all protein-producing genes (*2*).

Artificial light at night disrupts the circadian rhythm and decreases organismal health (*3*). Previous work has shown that although behavioral rhythms are changed the underlying core circadian proteins are not (*3*). The neurological pathways leading to these effects are unknown. We are attempting to unravel these pathways by staining for immediate early gene expression. Immediate early genes, such as cFos and avian ZENK, alter gene expression in activated neurons when organisms are exposed to novel stimuli.

**Methods**

Thirteen birds were entrained to a light dark period of 12 hours each for four weeks. Birds were randomized into three groups, control night, control day, and artificial light at night. Control day activity was included because previous research demonstrated that birds are active at night even with dim light (*3*), therefore we accounted for awake and active neuronal activity. Birds were perfused two hours after night light exposure or at a comparative time for controls. Brains were sliced and imaged for cFos and ZENK staining. Additionally, perches registering a hop collected activity data daily, showing number of hops per minute by hour from each bird.

The daily files use columns for minutes and rows for hours. The first 24 rows represent the first bird, the next 24 the second bird, and so on, with an empty line in between. The output then is a list of files for each experimental day, a useless setup (Figure 1). Also, the file names contain spaces.

Table

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**Figure 1. Example layout of raw hop data.** Sample data of raw hop output. Each file has all birds per day. Rows represent number of hops per minute.

I want to take these data and changes them into a useful format. I will use **Git and GitHub** to collaborate with Valentina Alaasam and have easily accessible code for future students in the Ouyang lab.

The new files will be formatted using **python** code. We will change the files to being a sequential number, date, time, and following number of active movements for each bird individually (Figure 2). We can then use these files to run through a popular circadian analysis program to receive output of rhythm period, power, percent rhythmic, and much more.

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**Figure 2. Example layout of new file.** An image of a file received from fly activity data monitors. Each file is an individual fly and the rows represent each minute of activity.

**Results**

The final adjusted format uses four python scripts, a jupyter notebook, and a few Linux commands to clean up the file. Hopefully, future work will include refining this process more, but for the moment it works, although it’s complicated. The following scrips turn the original output file (see Figure 1) into the file shown in Figure 3.

To accomplish this, the first script, bybird.py, separates all the files into individual birds for that date. The output files are the renamed to have a number at the beginning. There are 24 monitors, therefore there will be 24 files for each date, (e.g. 1\_July 01, 2021.txt). The next script, dates.py, adds all the first bird into file with the dates and times. This matches the first few columns found in Figure 2. Next all the other birds are added up by date into a separate file using rest\_birds\_dates.py. Then using the Altering\_Hop\_Data jupyter notebook, the first birds with dates file is converted to a csv. And the rest of the birds are added in as columns. The output is saved as a csv file. The user then needs to open the file and remove the header row and first column. Then the script csv\_to\_txt.py converts the file back to a text file. Unfortunately, this step creates from errors in the file. The user must then manually clean up the file by running the follow codes on the terminal:

tr -d '\ \"' <Monitor02.txt> Monitor03.txt #to remove quotes and extra space, also renames file

sed -i 's/Jul/\ Jul\ /g' Monitor03.txt #Add spaces around all the dates in the file again

sed -i 's/\t$//g' Monitor03.txt #Removes tab at the end of each line

sed -i 's/01\ Jul/1\ Jul/g' Monitor03.txt #Need to fix script to only use one digit days, this replaces

sed -i 's/02\ Jul/2\ Jul/g' Monitor03.txt

sed -i 's/03\ Jul/3\ Jul/g' Monitor03.txt

sed -i 's/04\ Jul/4\ Jul/g' Monitor03.txt

sed -i 's/05\ Jul/5\ Jul/g' Monitor03.txt

sed -i 's/06\ Jul/6\ Jul/g' Monitor03.txt

sed -i 's/07\ Jul/7\ Jul/g' Monitor03.txt

sed -i 's/08\ Jul/8\ Jul/g' Monitor03.txt

sed -i 's/09\ Jul/9\ Jul/g' Monitor03.txt

Table

Description automatically generated**Figure 3. Resulting output file.** The resulting output file reorganizes the data to fit the standard activity monitor output files.

The new data format allows to easy data analysis. These results show that our experimental ALAN birds were 100% rhythmic before ALAN exposure. Their periods ranged from 23.9 to 24.1 with a mean power of 435.7, a display of normal activity levels (Figure 4).

Diagram

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**Figure 4. Experimental birds show normal activity levels before ALAN exposure.** A double plotted actogram showing activity levels over a 10-day period in 12 hours of light and 12 hours of dark.

**Discussion**

Science has long recognized the importance of the circadian system for diverse biological processes, unraveling its molecular building blocks; from moth to mouse, blood pressure to body temperature (*4*). Disrupting the clock leads to many health deficits, including diabetes, sleep disorders, and even some forms of cancer (*5-7*). In a modern world with a continual shift to 24/7 life and constant night lighting, it has never been more important to understand these detrimental effects.

Lab based experiments are an instrumental part of understanding our circadian rhythm and its underpinnings. Our lab has perches that monitor bird hops to determine circadian periods. The codes created have helped with data analysis of their activity for our current project. And will also help with future ones. Future work will focus on streamlining the codes by simplifying scripts and removing steps. Additionally, I will build R codes for data analysis of the output files.

The ability to quickly determine rhymicity and period length in our experiments will give our papers statistical validity common to the field of circadian biology. Future experiments in our lab will be looking at activity levels under constant bight light, which is much more difficult to determine circadian rhythm. These codes will standardize our data to circadian field. Furthermore, I will make my GitHub repository publicly available, so others who collect activity data from monitored bird perches can use this these steps for analysis also.

**Work Cited**

1. J. C. Dunlap, Molecular bases for circadian clocks. *Cell* **96**, 271-290 (1999).

2. R. Zhang, N. F. Lahens, H. I. Ballance, M. E. Hughes, J. B. Hogenesch, A circadian gene expression atlas in mammals: Implications for biology and medicine. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 16219-16224 (2014).

3. V. J. Alaasam *et al.*, Effects of dim artificial light at night on locomotor activity, cardiovascular physiology, and circadian clock genes in a diurnal songbird. *Environmental Pollution* **282**, (2021).

4. R. W. Logan, D. K. Sarkar, Circadian nature of immune function. *Molecular and Cellular Endocrinology* **349**, 82-90 (2012).

5. M. Schilperoort, P. C. N. Rensen, S. Kooijman, Time for Novel Strategies to Mitigate Cardiometabolic Risk in Shift Workers. *Trends in Endocrinology and Metabolism* **31**, 952-964 (2020).

6. A. Silva, M. J. dos Santos, D. L. G. Gitai, J. Coelho, T. G. de Andrade, Depression and anxiety symptoms correlate with diurnal preference, sleep habits, and Per3 VNTR polymorphism (rs57875989) in a non-clinical sample. *Journal of Affective Disorders* **277**, 260-270 (2020).

7. K. C. G. Van Dycke *et al.*, Chronically Alternating Light Cycles Increase Breast Cancer Risk in Mice. *Current Biology* **25**, 1932-1937 (2015).