Assignment 1: Can SUML glutamate neurons drive REM Sleep Theta Activity? A Coding-Based Exploration. (15%)

You are a trainee in the lab studying circuits that control Rapid Eye Movement (REM) sleep. In particular, you are interested in whether lateral supramammillary area (SUML) neurons generate theta oscillations. Theta oscillations are rhythmic brain waves typically in the 4-8 Hz frequency range, and they are a hallmark of REM sleep (Peever & Fuller, 2017). You are interested in studying them because they play a key role in memory consolidation and emotional regulation, making them a vital component of healthy brain function (Hutchison and Rathore, 2015).

To manipulate SUML glutamate neurons and test whether they are important for theta generation, you injected these mice with an excitatory opsin in the SUML and implanted an optic fiber in the same region to activate these cells **(see Lecture 2 - Optogenetics)**. Additionally, you implanted an electrophysiology apparatus to record the mouse's brain waves **(see Lecture 1 - Electrophysiology)**. You notice that every time you activate these neurons during NREM sleep, the EEG shifts from NREM sleep (delta-rich state) into REM sleep with theta dominant frequency shown in the FFT. **(Fig 1)**

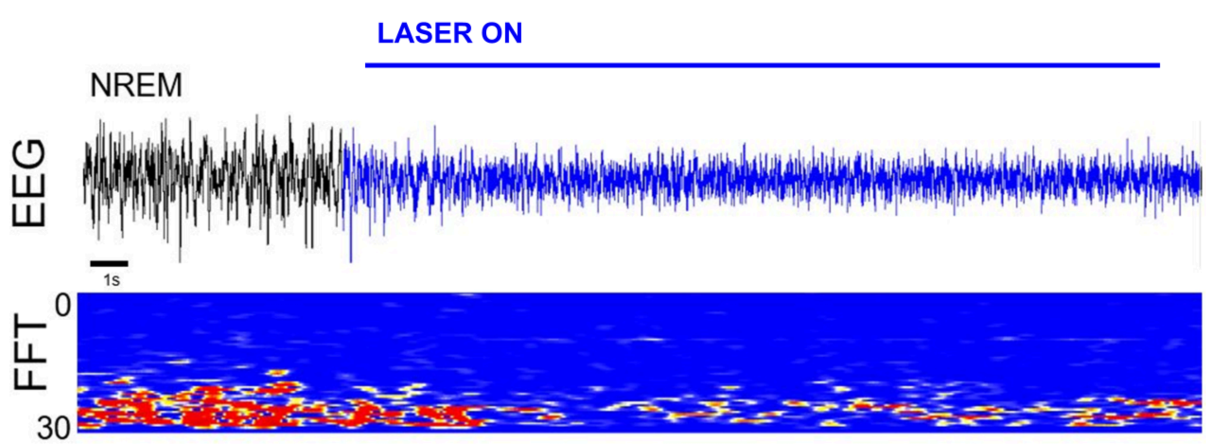


Fig 1: Example trace of EEG Recording during REM Sleep for one laser trial

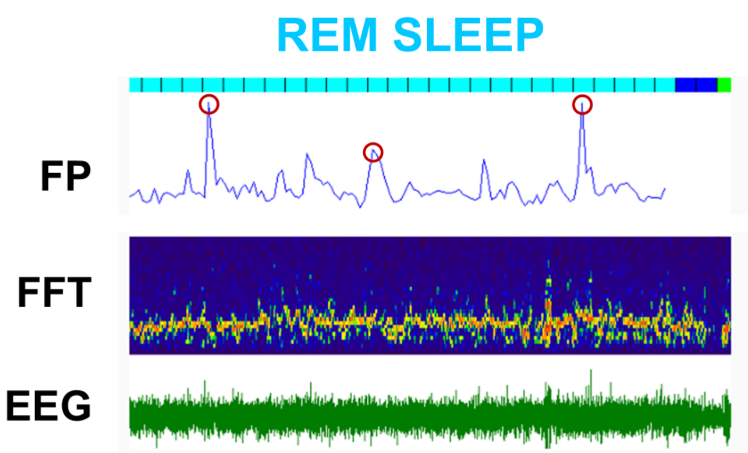
When you extracted your data files for your experimental mice, you realized that you will need to write a code in Python to convert your EEG signals into meaningful quantitative data, as calculating theta activity for 30 trials by hand would take you ages to complete!

1) Write a Python code that extracts theta range for baseline and laser-on trials for each mouse.

In your code, make sure that your functions perform the following:

- Takes raw EEG data as input for both baseline and experimental REM sleep trials
- Applies an appropriate mathematical formula that converts this raw EEG signal into frequency-domain representation **(sampling rate = 1024)**
- Filters the power values that fall within theta range (4-8 Hz)
- Computes the total power of theta and returns it
- Averages theta power for baseline and experimental conditions (each mouse has 8 baseline trials and 8 experimental (laser-on) trials. You will need to calculate the average theta power separately each condition by averaging across the 8 trials)
- Your code returns the average theta power for each condition that can be used later for statistical analysis and graphing.

Given that you saw your previous experiments revealed positive results, you decided to run an additional test to see whether these SUML glutamate neurons are correlated with theta activity. To do that, you took advantage of a technique called fiber photometry (FP) **(see Lecture 3 - Calcium Imaging)**. This technique allows you to record population activity of glutamate neurons and correlate their activity with theta bursts. Therefore, you extracted both your fiber photometry signal and theta power that is temporally aligned with this signal **(Fig on the Left)**.



You notice that there is a burst in the fiber photometry signal, and you are curious whether these bursts are temporally aligned with theta activity. To explore this further, you decided to write a python code to count the number of peaks in both the fiber photometry and theta power signals during REM sleep, and compare whether these bursts occur with similar frequency.

2) Write a Python code that extracts meaningful peaks from fiber photometry and theta power signals.

In your code, make sure that your functions perform the following:

- Takes a list or array of fiber photometry and theta values and returns the total number of activity bursts per REM sleep episode (hint: a burst can be defined as a value greater than a certain threshold. Bonus points for allowing the user to pass in the threshold as a function's argument)

- Each mouse has approximately 8-12 REM sleep episodes. You will need to detect peaks in both the fiber photometry and theta power signals, and then calculate the average number of peaks in each signal across all REM episodes for each mouse.
- Determine whether the number of peaks for fiber photometry signals correlate with the number of theta bursts.

You finally finished preparing all your analysis, and now you want to graph them to see whether there are any significant differences.

3.1) Visualize the Differences

- Create bar graphic to visualize average theta power in baseline and experimental (laser-on) conditions
- Create a linear regression graph to visualize whether total number of peaks in fiber photometry signal aligns with theta bursts per each REM episode
- Ensure your axes for each graph are labelled accordingly.

3.2) Compute the Differences

- Determine a correct statistical test (paired vs. unpaired t-test?) to investigate whether the theta power during REM sleep is significantly different between baseline and laser-on conditions across your animals. Make sure to report test used, t-statistics, p-value and mean in your results.
- Determine if fiber photometry peaks and theta peaks are correlated: run a linear regression to test whether the number of fiber photometry peaks predicts theta peaks counts per each REM episodes
- Make sure to report the test used, correlation coefficient (r), p-value and interpretation of the relations
- Bonus points: plot your graphs with statistical annotations (such as indicating significance, for example, * or n.s, or standard deviation)

3.3) Interpret your results: Are the differences you observed statistically significant? What do these results suggest about the role of SUML glutamate neurons in generating theta oscillations during REM sleep? Does it refute or support your additional hypothesis?

<u>Assignment Rubric</u>

Code Commentary and Readability (2% of your final grade): clear variable names, explanatory comments and structure of the code.
Completion Grade (6% of your final grade): To help you with your final projects, weekly submissions will count toward your completion grade for this assignment. This means you'll receive marks for participating and submitting your work on time, regardless of whether your code is fully working. The goal of these mini-assignments is to help you build your final code bit by bit, following along with the tutorials. <u>Each submission is worth 2% of your final assignment grade.</u> The submission schedule is attached below.

Code Correctness (6% of your final grade): accurate logic, functions and plots; code works as intended and return correct values; proper stats and code execution
Interpretation (1% of your final grade): you should explain clearly what your results mean. This includes interpretation of the final results of your assignments. Make sure to be thoughtful and specific in your explanation (for example, explain why your results support or do not support your hypothesis). One short paragraph is sufficient.

Mini-Assignments Deadline Schedule:
Assignment 1.1: September 15th, 2025 at 11.59 pm
Assignment 1.2: September 22nd, 2025 at 11.59 pm
Assignment 1.3: September 29th, 2025 at 11.59 pm
Final Assignment Deadline: October 15th, 2025 at 11.59 pm

Data is collected and provided by two graduate students in the Peever Lab, Anita Taksokhan and Vasilisa Nikiporets.

References:

Peever, J., Fuller, P.M. (2017). The biology of REM sleep. Curr Biol 20, 1237–1248.

Hutchison, I.C., Rathore, S. (2015). The role of REM sleep theta activity in emotional memory. Front Psychol 6. doi: 10.3389/fpsyg.2015.01439.