**BIO-482 – Miniproject 2023 (Weeks 10-14)**

The miniproject is a Matlab- or Python-based analysis of membrane potential data from Dr. Taro Kiritani (Kiritani et al., <https://dx.plos.org/10.1371/journal.pone.0287174> ).

**There will be a total of 10 marks for the miniproject, counting towards 1/3 of your final grade.**

* **in Room AAC137**

**Week 10:**

**Friday 17 November, 13:15-14:00 – Miniproject overview (Sylvain Crochet)**

**Friday 17 November, 14:15-15:00 – Miniproject objectives and guidelines (Sylvain Crochet)**

**Before Friday 17 November please download:**

MiniProject\_DATAandCODES for Matlab (**X MB**) <https://drive.google.com/file/d/1oRUwEPkHih3jTOEVUnHaK8qpHCQhh44H/view?usp=sharing>

MiniProject\_DATAandCODES for Python (**X MB**) <https://drive.google.com/file/d/15tHxnbXFHypesKtePAG6ilErgVjOAQH8/view?usp=sharing>

**Weeks 11-14 (Wednesday 22 November to Wednesday 20 December):**

**Wednesday and Friday, 13:15-15:00 – individual assistance on analysis in Room AAC137 (Sylvain Crochet and TAs).**

**Before 22 December (24:00) send your individual Miniproject report by email to (Sylvain Crochet,** [**sylvain.crochet@epfl.ch**](mailto:sylvain.crochet@epfl.ch)**)**

**BIO-482 Miniproject Report**

**Family Name:**

First name:

**SCIPER:**

**We will write Matlab/Python codes to answer the questions below. The number of points earned for each correct answer is indicated in parenthesis.**

**Part 1 – Properties of cortical neurons during quiet wakefulness (3/10 marks)**

*Part1-a. Suprathreshold activity and cell type*

Action potentials (APs) – the *suprathreshold* activity – are high-amplitude and fast events generated by active conductances when the membrane potential (Vm) reaches a threshold for AP initiation. APs can be isolated from the background Vm fluctuations – the *subthreshold* activity – mostly composed of postsynaptic potentials.

In this first part, we will detect and isolate the APs in the Vm recordings based on their amplitude using an absolute Vm threshold. All events with Vm above this threshold will be considered as APs. The time of each AP will be defined by the time at its peak (maximum Vm).

**Using “free whisking” sweeps – periods devoid of active contacts between the whisker and the object – from each cell, detect the APs and compute the mean firing rate in Hz for each cell. Then compute the mean firing rate for each cell type (EXC, PV, SST, VIP).**

The AP waveform is often used to classify cell types. In this second part we will try to measure basic AP properties for each neuron.

The AP threshold is generally considered as the initial time of the AP. AP threshold can be computed in different ways. Here we will consider that the AP threshold correspond to the time/Vm when the Vm instantaneous change (1st derivative of the Vm) crosses a threshold. In most of the cortical neurons, the maximum instantaneous Vm change for synaptic event exceeds 10 V.s-1. Here we will use a specific threshold for each cell (*data.Cell\_APThreshold\_Slope*).

**Using the same ‘free whisking’ sweeps, compute the AP threshold (the Vm at AP initiation time) for each AP. Then compute the mean AP threshold for each cell and each cell class.**

Once the AP threshold time determined for each AP, we will compute the mean AP duration for each cell. For each AP, we compute the AP amplitude as the Vm difference between AP peak and AP threshold. Then we determine (approximate) the time when the Vm crosses the AP Vm at half-amplitude (AP threshold+AP half amplitude) in the rising and decaying phase. The AP duration is the difference in time between the two.

**Compute the mean AP duration for each cell and each cell type.**

*Part1-b. Subthreshold activity and cell type*

Membrane potential recordings allow to access the subthreshold activity of the recorded neuron. The subthreshold activity is determined by the complex interplay between the intrinsic properties of a neurons (input resistance, capacitance, different active or passive conductances) and its synaptic inputs. Here we will try to characterize some basic aspects of the subthreshold activity of the recorded neurons.

First, we will limit the impact of the suprathreshold activity on our measurements by ‘removing’ (cutting) the APs. Using the codes written to extract the APs, we will identify each AP and the AP threshold, and cut each AP using a linear regression between the AP threshold and return to baseline. Then, from the Vm trace after AP cutting, we will compute for each cell the mean and standard deviation of the Vm using non-overlapping 2 s time-windows.

**Using the ‘free whisking’ sweeps, compute mean Vm and mean SD of the Vm for each cell and then each cell type.**

Membrane potential dynamics can be described in the frequency domain using spectral analysis. A commonly used method is to compute the Fast-Fourier Transform (FFT) of the signal (the Vm in our case). Because Vm fluctuation is not a stationary signal, one should not compute the FFT for the continuous Vm recording but instead compute the FFT for shorter time windows and averaged the FFTs. Here we will compute the FFT for consecutive 2 s time windows for a ‘free whisking’ trial, then average the FFTs to obtain the mean FFT for a given cell. To quantify and compare the FFT, we can compute the mean FFT amplitude in a given frequency band.

**Using the ‘free whisking’ sweeps, compute the mean FFT for each cell and mean FFT for each cell class. Then compute the mean FFT amplitude in the low-frequency (1-10 Hz) and high-frequency (30-90 Hz) bands.**

**Use your results to answer the following questions. Justify your responses with different graphs.**

1. **Which cell property, or combination of parameters, best distinguish between cell-class?** (1/3 Marks)
2. **Which cell property(ies) contribute most to the difference in firing rates observed across cells?** (1/3 Marks)
3. **Which frequency band contributes most to membrane potential subthreshold activity?** (1/3 Marks)

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Part 2. Membrane potential dynamics and motor activity (2/10 marks)**

What is the impact of motor activity on cortical activity? We can correlate the cortical activity with the mouse whisker motor activity by comparing the Vm dynamics of cortical neurons when the mouse is not moving its whiskers (Quiet) and when the mouse actively moves its whiskers (Whisking). Here we will use whisking onset times during ‘*free whisking*’ trials to evaluate the impact of whisker movements on neuronal activity across cell types.

You will first assess the impact of whisking on subthreshold Vm dynamics (Vm after cutting the APs). **You will compute the averaged Vm response triggered by the onset of whisker movements for each neuron. To do that, you will cut the Vm 500 ms before and 500 ms after each whisking onset time, across ‘free whisking’ trials. Then average each Vm epoch to obtain the whisking onset-triggered Vm average for each neuron. You will use only whisking episods lasting for at least 200 ms and that were not preceded by another whisking episode 500 ms before.**

**Then, you will compute and report the change in mean Vm after whisking onset. The change in mean Vm will be computed as the difference between the mean Vm 0 to 200 ms after whisking onset and the mean Vm 500 to 300 ms before whisking onset**.

Next you will compute the change in mean firing rate after whisking onset. **For each neuron, you will compute the mean firing rate 500-300 ms before and 0-200 ms after each whisking onset time, and the change in firing rate (FR after –FR before).**

**Use your results to summarize the main impact of whisking onset on neuronal activity. Justify your responses with different graphs.** (2/2 Marks)

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Part 3. Sensory evoked neuronal activity (1/10 marks)**

Up to here we have analyzed the neuronal activity in the absence of sensory inputs, comparing periods when the mouse was immobile (Quiet) or moving its whisker in the air (Whisking) in ‘free whisking’ trials. In other trials, the mouse was presented with an object (a small metal pole) on its right side, positioned in the path of the C2 whisker. By moving its whisker forward, the mouse can actively contact – touch – the pole, generating active sensation. These active contacts are encoded in the activity of L2/3 barrel cortex neurons. In this last part of the mini project, we will investigate sensory coding for active touch in the different neuron types.

**First, we will compute the averaged subthreshold Vm response triggered by the onset of active touches for each neuron. To do that we will select ‘*active touch*’ sweeps for a given neuron and cut the Vm 500 ms before and 500 ms after each contact onset time. We then average each Vm epoch to obtain the contact-triggered Vm average for one neuron. You will select active contacts that were not preceded by another contact 200 ms before (inter-contact interval > 200 ms).**

**Finally, you will quantify the sub- and supra-threshold evoked responses by comparing the changes in mean Vm (post-synaptic potential amplitude) and in mean firing rate, 50-0 ms before to 0-100 ms after contact onset.**

**Use your results to summarize the main impact of whisking onset on neuronal activity. Justify your responses with different graphs.** (1/1 Marks)

**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Part 4. Personal question (5/10 Marks)**

**In this last part of the Miniproject, you will devise your own question to study and write codes to answer it.**

**You will briefly explain the rational and relevance of your question based on what you have learned during the course.** (2/4 Marks)

**You will briefly explain the analysis that you have performed and present your results.** (2/4 marks)

**Finally write a brief conclusion to your question based on your findings.** (1/4 marks)