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Neuroscience 2 – Miniproject (Weeks 10-14)

The miniproject is a Matlab-based* analysis of a subset of V_m data from Dr. Taro Kiritani (Kiritani et al., manuscript in preparation). There will be a total of 10 marks for the miniproject, counting towards 25% of your final grade.

* Python will be possible but only with minimal support

Week 11

18 November, 13:15-14:00 – Miniproject introduction (Carl Petersen, Zoom)

18 November, 14:15-15:00 – Miniproject precise goals (Sylvain Crochet, Zoom)

Before Friday 20th November please download:

- ***BIO482_MiniProject*** zip file at :

<https://drive.google.com/file/d/19WbSeUFd2gCHit6A53Zg0n0WJu8ID2Qr/view?usp=sharing>

and

- ***MiniProject_2020_Q*** word file

20th November, 13:15-15:00 (Sylvain Crochet and Tas, Slack and Zoom)

On Friday 20th November we will first explore the dataset using the DataViewer on your own computers.

Wednesdays 13:15-15:00 (Sylvain Crochet and Tas, Slack and Zoom)

Fridays, 13:15-15:00 (Sylvain Crochet and Tas, Slack)

18th December (before 17:00) send your Miniproject report by email to (Sylvain Crochet, sylvain.crochet@epfl.ch)

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

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 the 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” trials from each cell of the MiniProjectData structure, detect all the APs and compute the mean firing rate in Hz for each cell. Then compute the mean firing rate for each cell class (EXC, PV, SST, VIP). Report your results in a table (1 mark)

	Cell ID	Cell type	Mean Firing rate
0	TK545_2	EXC	0.875000
1	TK532_1	EXC	1.274725
2	TK490_1	EXC	0.540000
3	TK539_1	EXC	0.900000
4	TK473_1	EXC	0.066667
5	TK364_1	PV	20.739496
6	TK407_1	PV	7.333333
7	TK479_2	PV	36.045455
8	TK479_1	PV	32.066667
9	TK478_1	PV	18.483333
10	TK506_2	SST	12.550000
11	TK355_1	SST	3.108333
12	TK358_2	SST	2.550000
13	TK471_1	SST	13.383333
14	TK472_1	SST	6.866667
15	TK462_2	VIP	16.800000
16	TK461_1	VIP	28.406250
17	TK455_2	VIP	4.566667
18	TK454_1	VIP	4.732143
19	TK523_2	VIP	26.771429

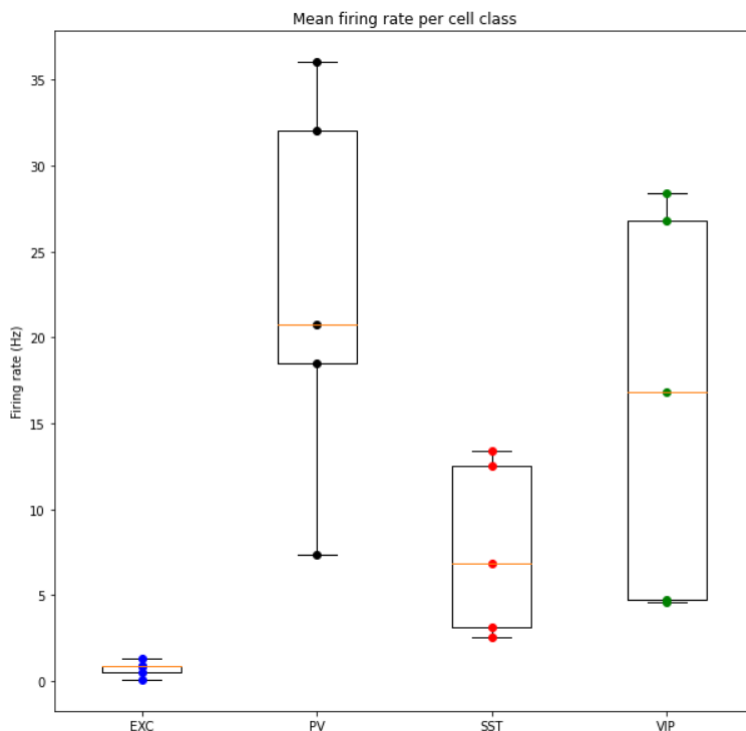
Table showing the mean firing rate per cell, the cells are sorted by cell type

Cell type	Mean Firing rate	SD of the Firing rate
EXC	0,731278388	0,453528821
PV	22,93365674	11,43810433
SST	7,691666667	5,102028464
VIP	16,25529762	11,48845664

Table showing the mean firing rate per cell class, and the firing rate Standard Deviation per cell class

Which cell type has the highest mean firing rate? The lowest mean firing rate? Is the mean firing rate of a neuron a good predictor of its cell class – justify with one graph and one sentence? (0.25 mark)

Answer: We can see that PV cells have the highest mean firing rate, and EXC cells have the lowest one.



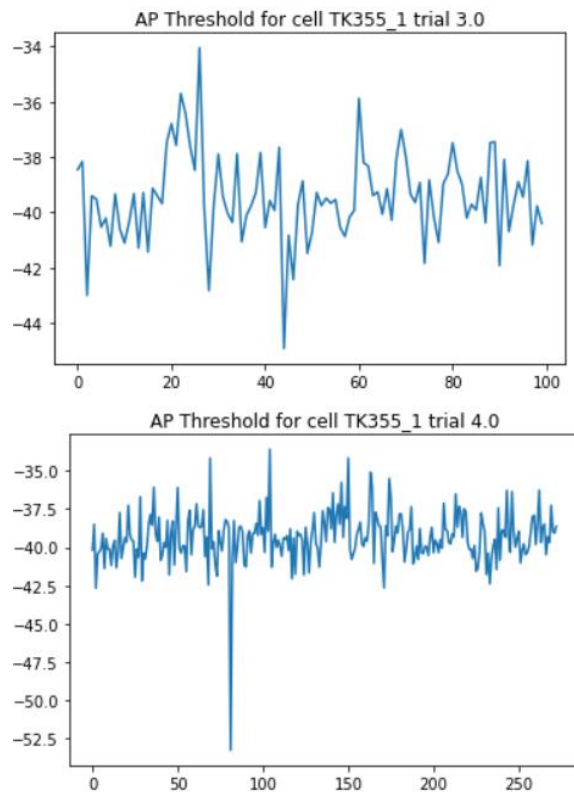
We can see from the above graph, where we have plotted the cell type (x-axis) vs the cell's firing rate (y-axis), that the EXC neurons are clustered around 0 up to 1 as a mean firing rate (MFR) value, and none of the other cells from the other types ever have these low values. We can also see that the PV cells can have a high MFR value (>30) which could be used as an indicator for the PV cell class as none of the other cell types reach these high values. For the SST neurons, we can see that their MFR values are between 2 and 14, however if we see a cell firing at this rate, we can not be certain that it is an SST cell, as it's possible (with lower probability) for it to be a VIP or EXC cell.

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 when the Vm instantaneous change (1^{st} derivative of the Vm) crosses an absolute threshold of 25 V.s^{-1} (in most of the cortical neurons, the maximum instantaneous Vm change for synaptic event does exceed 10 V.s^{-1}).

Using the same 'free whisking' trials, compute the AP threshold for each AP. **Then compute and report in a table the mean AP threshold for each cell and each cell class. Is the AP threshold a fixed value for across neurons? For each individual neuron? Illustrate with on example (1 mark)**

	Cell ID	Cell type	Mean AP Threshold
0	TK545_2	EXC	-40.450037
1	TK532_1	EXC	-39.391526
2	TK490_1	EXC	-39.913340
3	TK539_1	EXC	-31.392161
4	TK473_1	EXC	-43.284448
5	TK364_1	PV	-39.390856
6	TK407_1	PV	-39.450154
7	TK479_2	PV	-41.258838
8	TK479_1	PV	-38.558044
9	TK478_1	PV	-39.210636
10	TK506_2	SST	-40.649234
11	TK355_1	SST	-39.380713
12	TK358_2	SST	-42.185066
13	TK471_1	SST	-36.115199
14	TK472_1	SST	-40.570030
15	TK462_2	VIP	-39.136162
16	TK461_1	VIP	-39.291837
17	TK455_2	VIP	-41.715366
18	TK454_1	VIP	-41.665798
19	TK523_2	VIP	-37.227929

Cell type	Mean AP Threshold per cell class
EXC	-38.938287
PV	-39.456704
SST	-39.794825
VIP	-39.827443



The range for the AP Threshold is small between -43 to -29, so the values are quite similar for all cells. The AP Threshold is however not a fixed value across different neurons as we can see from the table, no two entries have the same value. The AP Threshold is also not a fixed value for each individual neuron, which we can notice from the fluctuations after plotting for example the (non-averaged) AP Threshold values for 2 different trials for the cell id 'TK355_1', we see that the values are different as shown in the graphs above.

Once the AP threshold time determined for each AP, we will compute the AP duration. To limit the variability due to changes in AP amplitude, we will compute the AP duration at half-amplitude. 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.

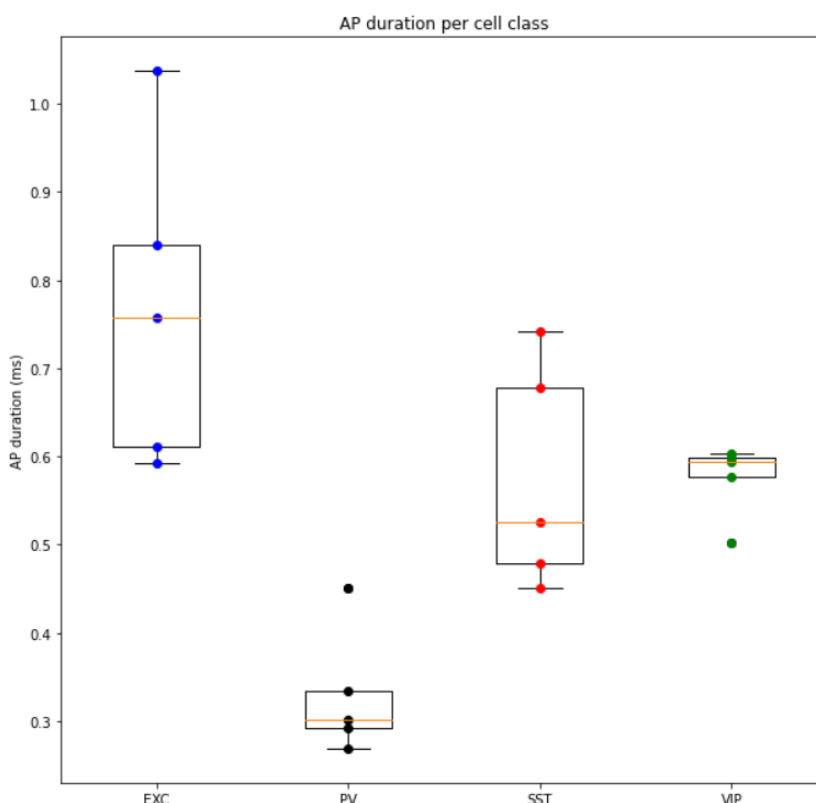
Compute the mean AP duration for each cell and each cell class and report your results in a table. (1 mark).

	Cell ID	Cell type	Mean AP duration
0	TK545_2	EXC	0.610714
1	TK532_1	EXC	0.757759
2	TK490_1	EXC	0.592593
3	TK539_1	EXC	0.839352
4	TK473_1	EXC	1.037500
5	TK364_1	PV	0.268628
6	TK407_1	PV	0.291411
7	TK479_2	PV	0.301248
8	TK479_1	PV	0.334436
9	TK478_1	PV	0.450913
10	TK506_2	SST	0.677656

11	TK355_1	SST	0.451340
12	TK358_2	SST	0.525082
13	TK471_1	SST	0.741314
14	TK472_1	SST	0.478034
15	TK462_2	VIP	0.502133
16	TK461_1	VIP	0.594252
17	TK455_2	VIP	0.603741
18	TK454_1	VIP	0.576415
19	TK523_2	VIP	0.597892

Cell type	Mean AP duration per cell class
EXC	0.767583
PV	0.329327
SST	0.574685
VIP	0.574887

To what extent the AP duration is a good indicator of the cell class – justify with one graph and one or two sentences? (0.25 mark).



After plotting the AP duration vs the cell type we obtain the graph above, where, we can see that for PV neurons, the AP duration is the lowest 0-0.3, so given a low duration, it would imply, with a high probability, that it is a PV cell. Some EXC cells reach high values over 0.9 (unlike other cell types), so a high AP duration could be an indicator of this cell type. The AP duration of VIP is clustered around 0.5 to 0.6 (given an AP duration in this range, we can deduce it's most probably a VIP cell, but with a possibility of it being another cell type as well – like SST). The AP duration of SST cells from the provided data ranges from 0.4-0.8,

As we can see from the first graph (plotted both the AP duration and firing rate on the y axis using different symbols – see the legend), as well as from the second (x axis-AP duration, y axis Mean FR) that using both the AP duration and the firing rate would be a better cell classifier (however not a deterministic one), as we are able to separate (cluster per cell) some of the points. With a lower firing rate (around 0) and a higher AP duration there is a high probability it is an EXC neuron (we can see a “cluster” in graph 2), a low duration and high firing rate indicates most probably a PV cell. If the AP duration is between 0.5-0.6 and the firing rate is within the normal range, there is a higher probability it is a VIP cell, but it could as well be an SST cell.

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.

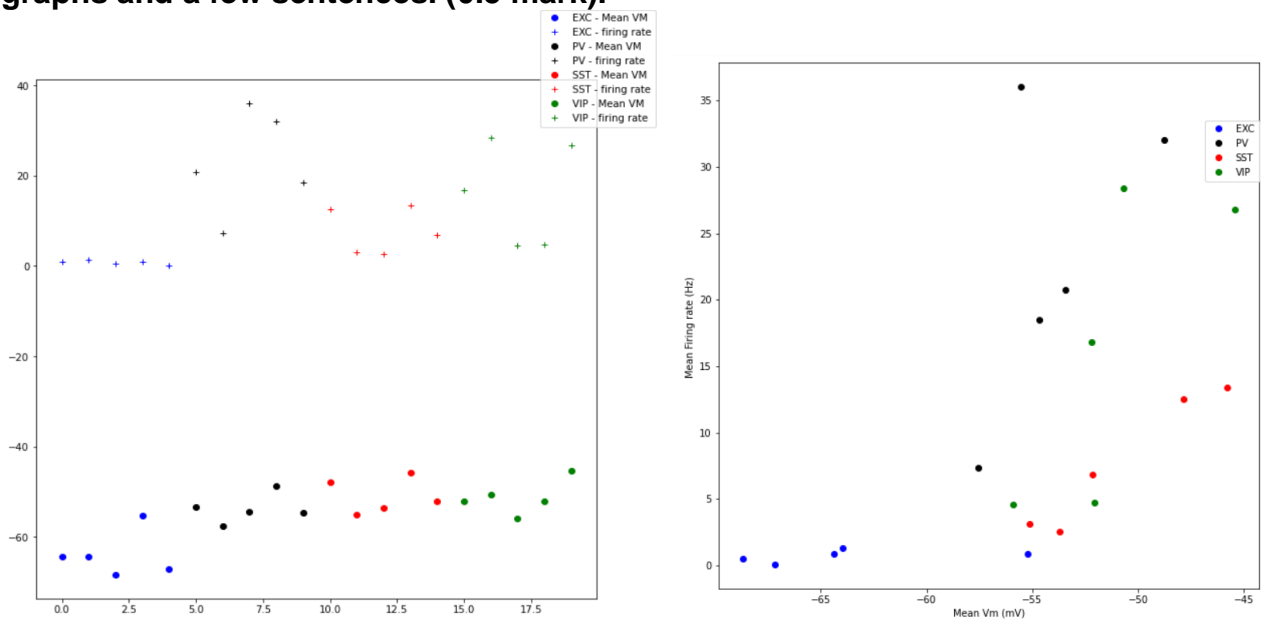
First, we will limit the impact of the suprathreshold activity on our measurements by ‘removing’ the APs. Using the codes written to extract the APs, we will identify each AP and the AP threshold and cut each AP using a linear regression between the AP threshold and the Vm 2 ms after AP peak time. Then, 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.

Report in a table the mean Vm and SD of the Vm for each cell and for each cell class. (1 mark).

	Cell ID	Cell type	Mean Vm	Mean SD of Vm
0	TK545_2	EXC	-64.375546	5.595157
1	TK532_1	EXC	-63.958400	5.698467
2	TK490_1	EXC	-68.669149	6.897227
3	TK539_1	EXC	-55.222641	5.750780
4	TK473_1	EXC	-67.160110	5.991924
5	TK364_1	PV	-53.445895	6.892784
6	TK407_1	PV	-57.555623	6.358850
7	TK479_2	PV	-55.523841	9.964408
8	TK479_1	PV	-48.754100	6.333102
9	TK478_1	PV	-54.648284	9.210296
10	TK506_2	SST	-47.854555	3.871420
11	TK355_1	SST	-55.125863	4.763113
12	TK358_2	SST	-53.702511	3.551605
13	TK471_1	SST	-45.764187	4.663399
14	TK472_1	SST	-52.133431	4.648754
15	TK462_2	VIP	-52.214935	6.610714
16	TK461_1	VIP	-50.673885	7.005845
17	TK455_2	VIP	-55.912123	4.510946
18	TK454_1	VIP	-52.046909	4.166978
19	TK523_2	VIP	-45.435655	5.315009

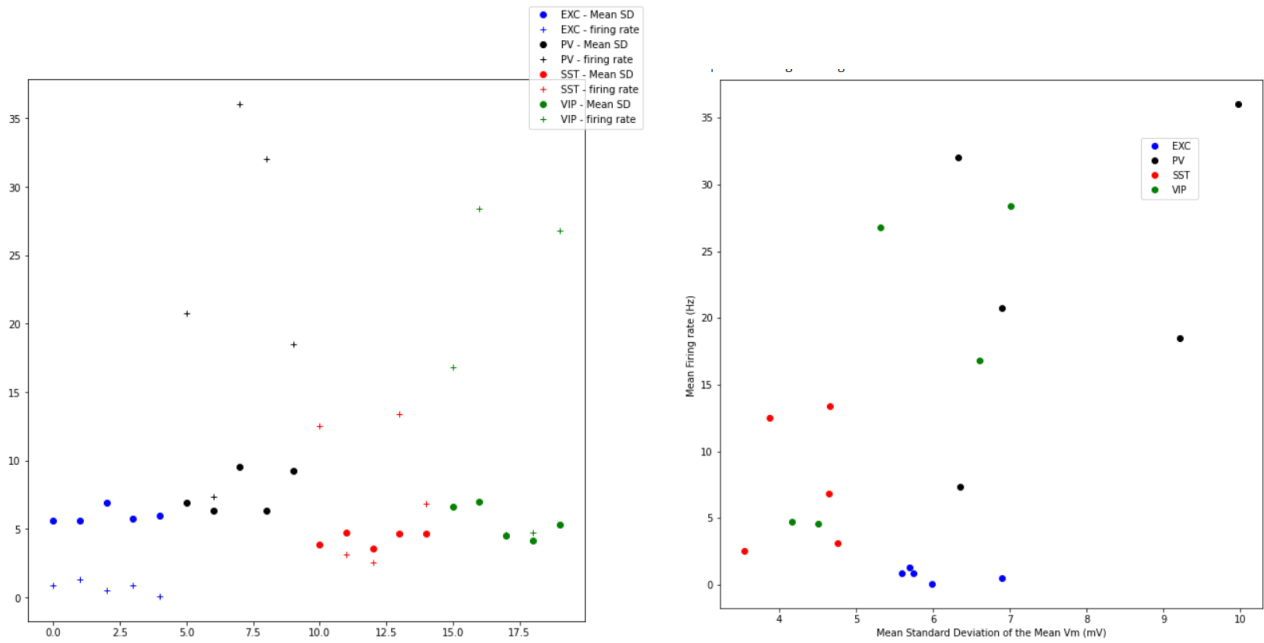
Cell type	Mean Vm per cell class	Mean SD of Vm per cell class
EXC	-63.877169	5.986711
PV	-53.985549	7.751888
SST	-50.916109	4.299658
VIP	-51.256701	5.521898

Explain the possible impact of the mean AP threshold, mean Vm and SD of the Vm on the firing rate of a neuron in a few sentences. Which one of those properties actually account for the difference in firing rate across the different neurons – justify with 3 graphs and a few sentences. (0.5 mark).



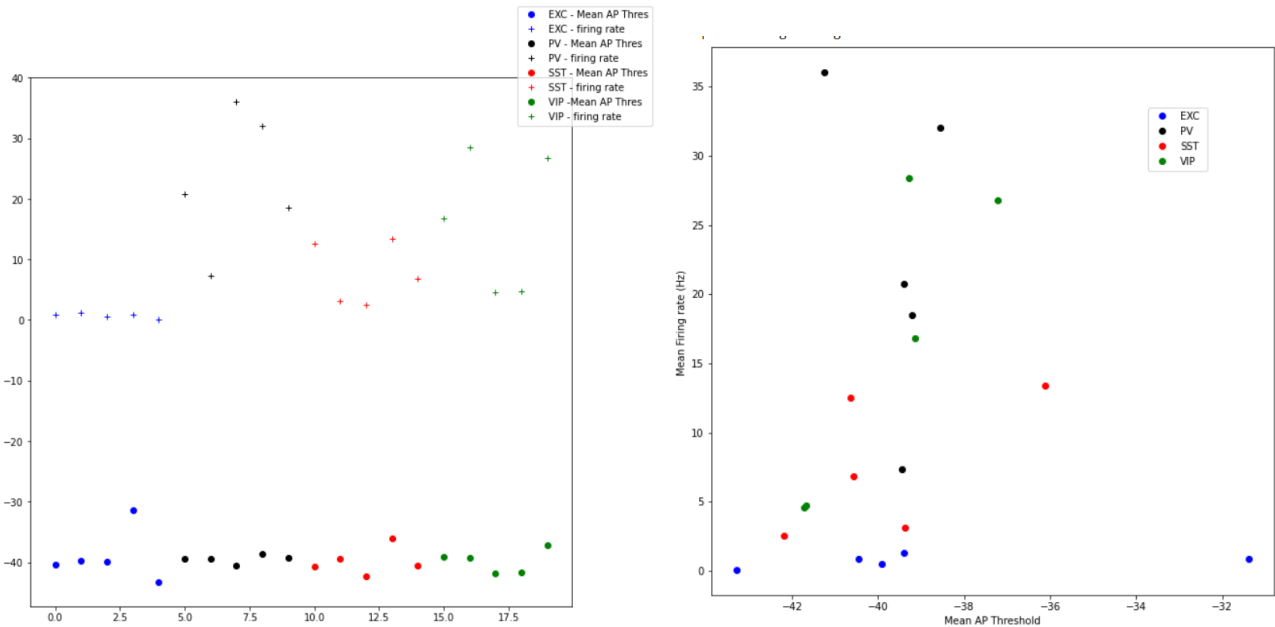
Correlation Coefficient (Mean SD of Vm vs Mean Firing rate) = 0.5813847448282162

We can notice from these graphs quite clearly that the Mean Vm has an impact on the firing rate, namely, we see a certain positive correlation. Whenever the Mean Vm is higher for that cell class, the firing rate is higher as well!



Correlation Coefficient (Mean SD of Vm vs Mean Firing rate) = 0.5542562125187728

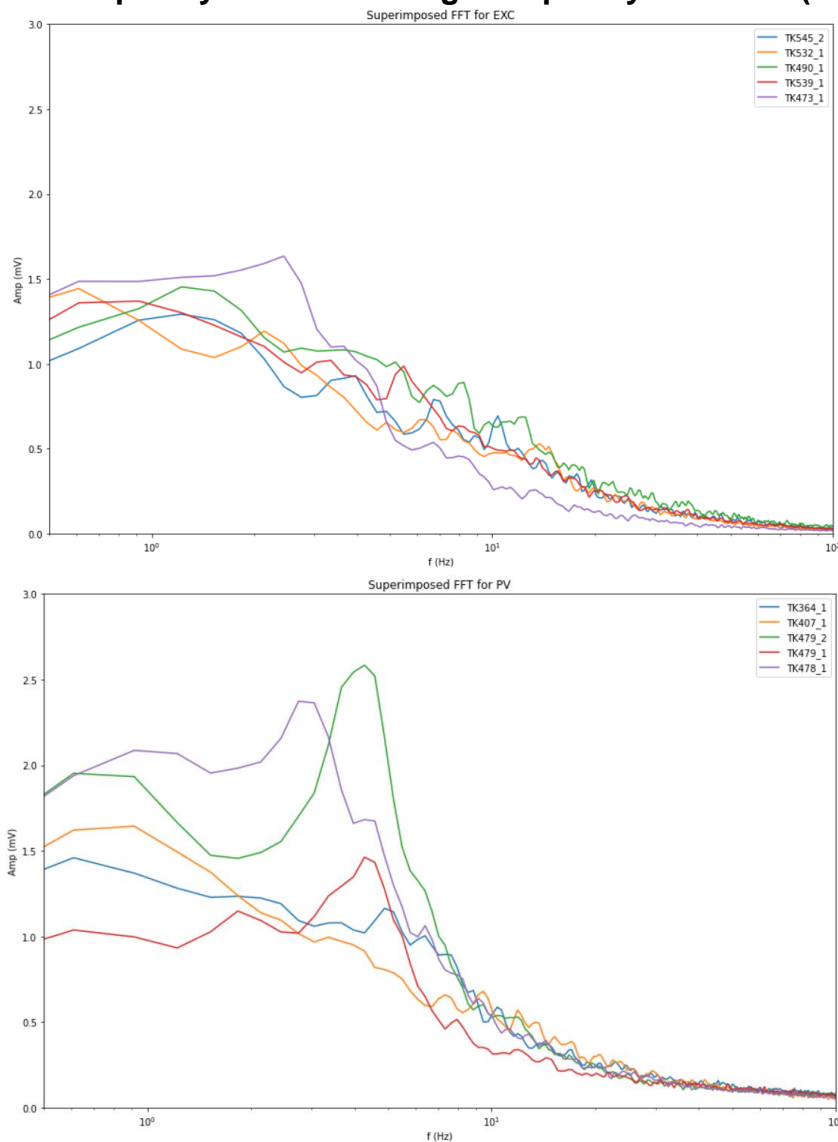
We can not notice such a big correlation when plotting the Standard deviation of the Vm with the firing rate, as we can see on the plots above, there is no noticeable direct link between the change in the SD and the change in the firing rate for the PV and VIP cells, but we do see a negative correlation between the two for EXC cells (SD increases while firing rate decreases and vice versa).

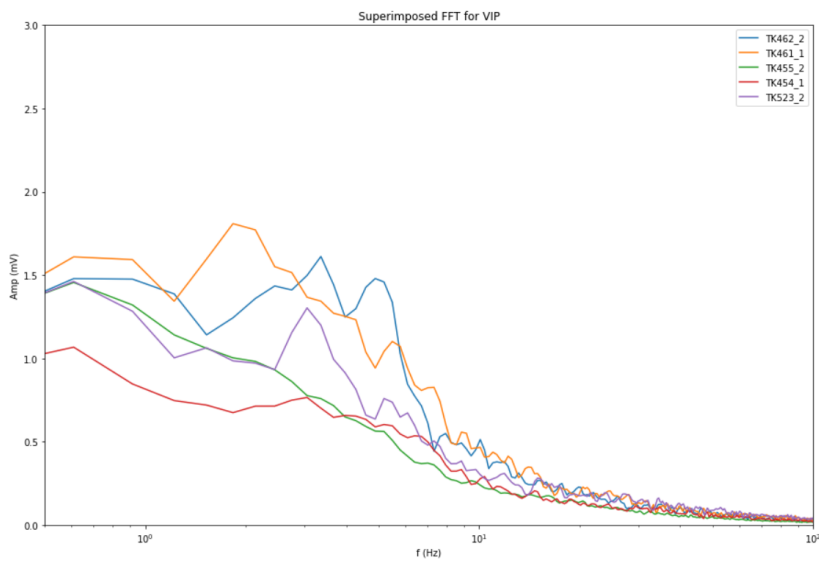
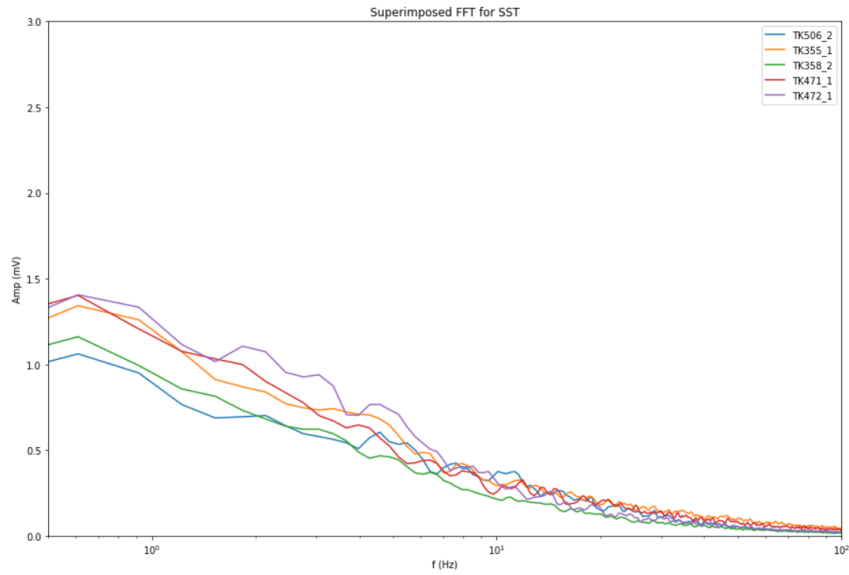


Correlation Coefficient (Mean AP Threshold vs Mean Firing rate) = 0.09538516443872253
 After inspecting this plot, we can see that the AP Threshold does not have any visible impact on the firing rate of the cell either, namely, we cannot link the change of the AP Threshold values to any change in the firing rate of the cells.

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 'free whisking' trials, 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.

Compute the mean FFT for each cell and plot the FFTs for each cell class. Report in a table the mean FFT amplitude for each cell for the two following frequency bands: low-frequency 1-10 Hz and high-frequency 30-90 Hz. (1 mark).



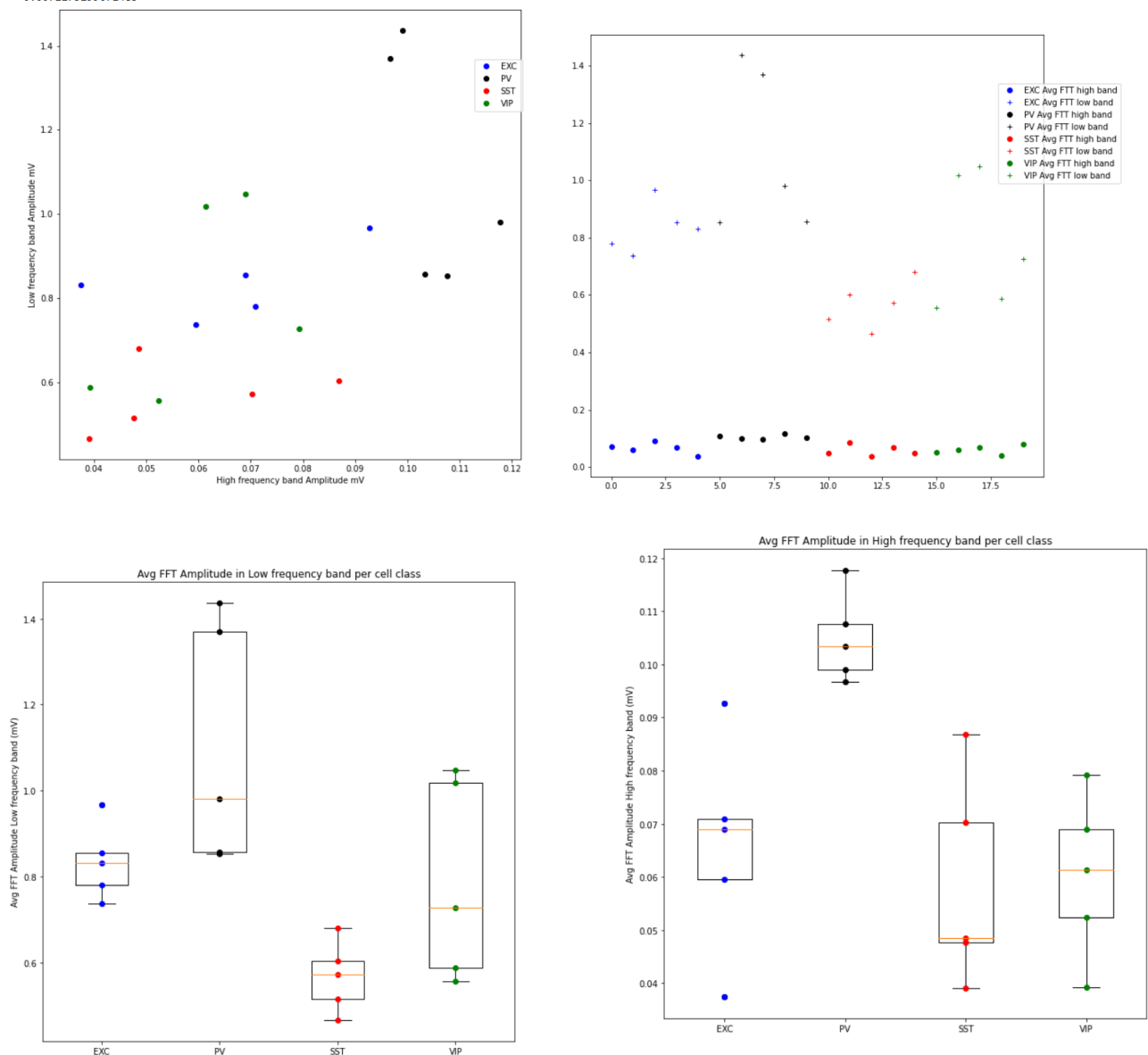


	Cell ID	Cell type	Mean FFT Amp low f. (Hz)	Mean FFT Amp high f. (Hz)
0	TK545_2	EXC	0.780286	0.070925
1	TK532_1	EXC	0.736786	0.059503
2	TK490_1	EXC	0.967021	0.092676
3	TK539_1	EXC	0.854386	0.068949
4	TK473_1	EXC	0.831272	0.037422
5	TK364_1	PV	0.979839	0.117709
6	TK407_1	PV	0.856923	0.103396
7	TK479_2	PV	1.437184	0.099073
8	TK479_1	PV	0.852830	0.107637
9	TK478_1	PV	1.370183	0.096744
10	TK506_2	SST	0.515135	0.047667
11	TK355_1	SST	0.602434	0.086901
12	TK358_2	SST	0.465154	0.039007
13	TK471_1	SST	0.572356	0.070226
14	TK472_1	SST	0.679348	0.048508
15	TK462_2	VIP	1.018590	0.061377
16	TK461_1	VIP	1.048219	0.068941
17	TK455_2	VIP	0.586459	0.039264
18	TK454_1	VIP	0.554905	0.052436
19	TK523_2	VIP	0.727165	0.079215

Which of those two frequency bands contribute the most to the Vm fluctuations – justify with a few sentences? (0.5 mark).

We can see that the low frequency band contributes the most to the Vm fluctuations, as there are higher values (and more peaks), and the values in the high frequency band are quite small and have no significant contribution.

Is spectral analysis informative to distinguish between cell types? justify with one or two graphs and a few sentences? (0.25 mark).



After plotting the mean high frequency vs the mean low frequency band (first 2 plots) of the averaged FFT for each cell, we can see spectral analysis does give some information on

the cell types, however it is not enough to deterministically predict all the cell types. As we can see higher values in both frequency bands imply an PV neuron, while lower values in both frequency bands (especially in the low f. band) would, with a higher probability, imply a SST neuron. For EXC neurons we can also see a certain correlation between the high and low f. band values.

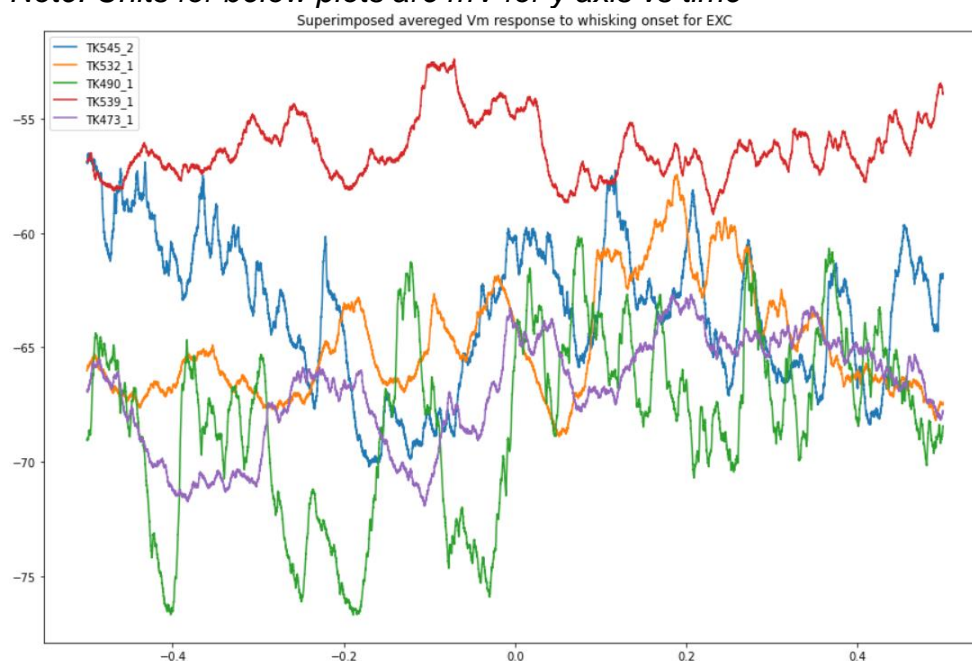
After observing the last two plots we can see that SST cells are characterized by a low FFT Amplitude in the Low freq. band (quite lower than the other cells), PV cells have a much higher avg FFT Amplitude in the High Freq. band. For the other two cell types it's harder to make any assumptions, as they have interleaved values.

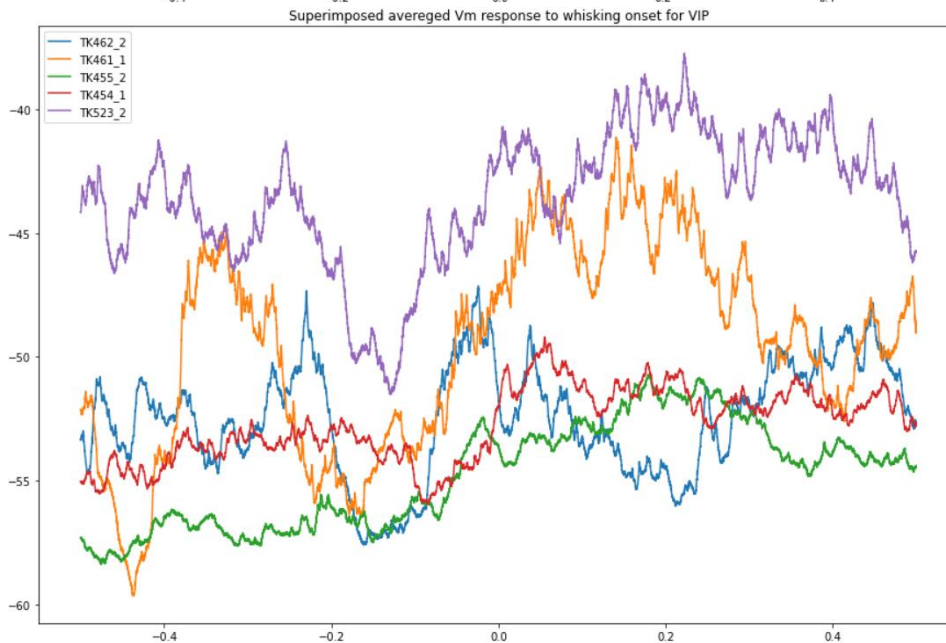
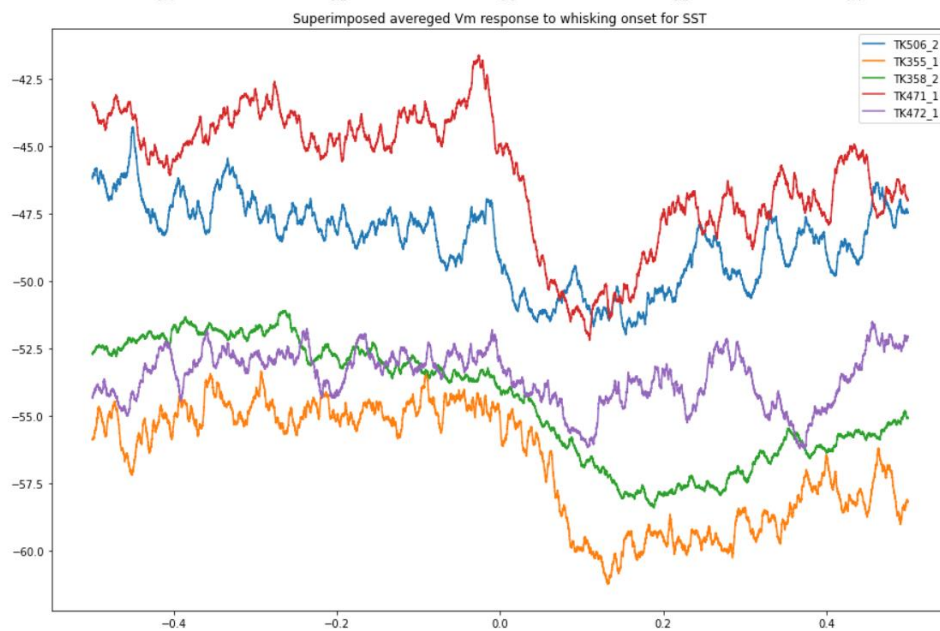
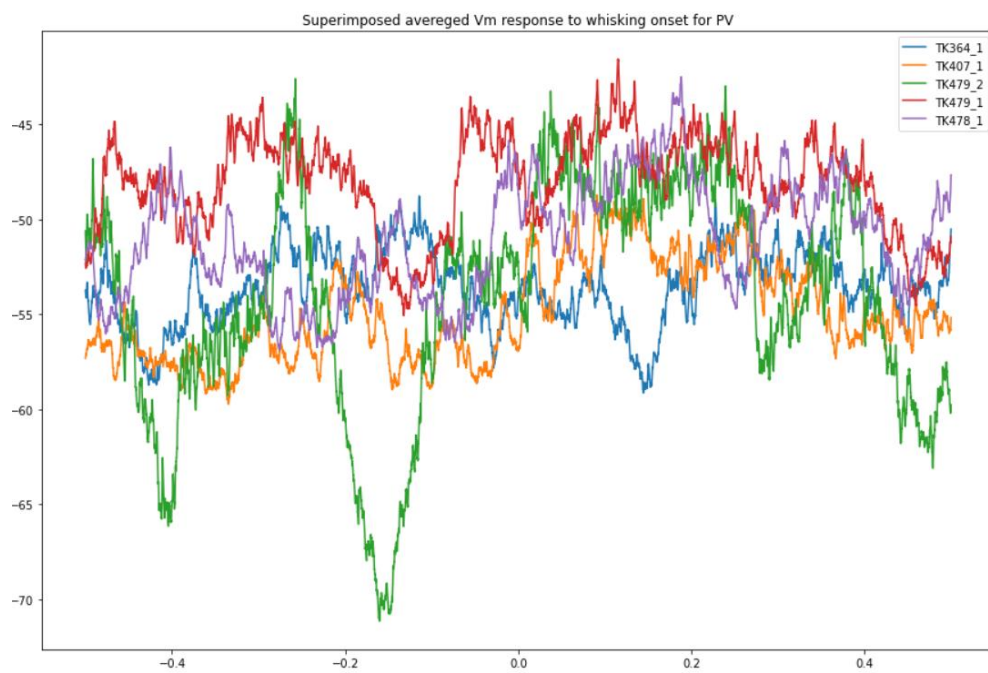
Part 2. Cortical states and motor activity

What is the impact of motor activity on cortical activity? Because cortical neurons receive inputs from many other nearby cortical neurons, Vm dynamics reflect the overall cortical network activity. We can thus assess the impact of motor activity on the cortical activity of the barrel cortex by comparing the Vm dynamics of cortical neurons when the mouse is not moving its whiskers (Quiet) and when the mouse is actively moving its whiskers (Whisking). Here we will use whisking onset times during 'free whisking' trials to evaluate the impact of whisker movement on neuronal activity across cell types. You will first assess the impact of whisking on subthreshold Vm dynamics. First, you will compute the average Vm around (0.5 s before – 0.5 s after) whisking onset times. Then, you will compute and report the change in mean Vm after whisking onset. You will consider only whisking episodes lasting more than 0.2 s with no whisker episode 0.5 s before. The change in mean Vm will be computed as the difference between the mean Vm 500 to 50 ms before whisking onset and the mean Vm 50 to 500 ms after whisking onset.

Report your results in 4 Graphs showing the averaged Vm response to whisking onset for each cell of the 4 cell classes. (0.5 mark).

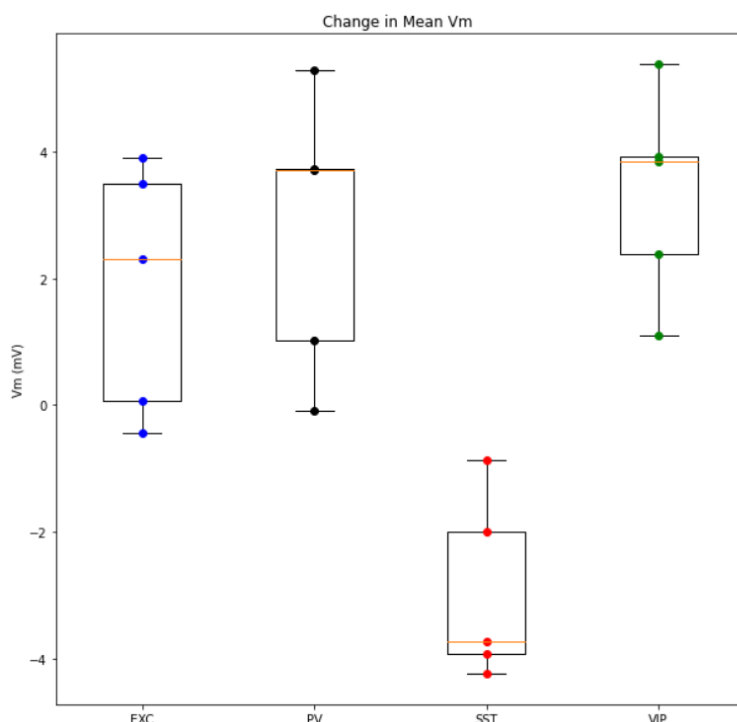
Note: Units for below plots are mV for y axis vs time





Compute and present in a Graph or Table the change in mean Vm at whisking onset. Are the whisking related changes in neuronal dynamics similar across cell types – justify your response in one sentence? (0.5 mark).

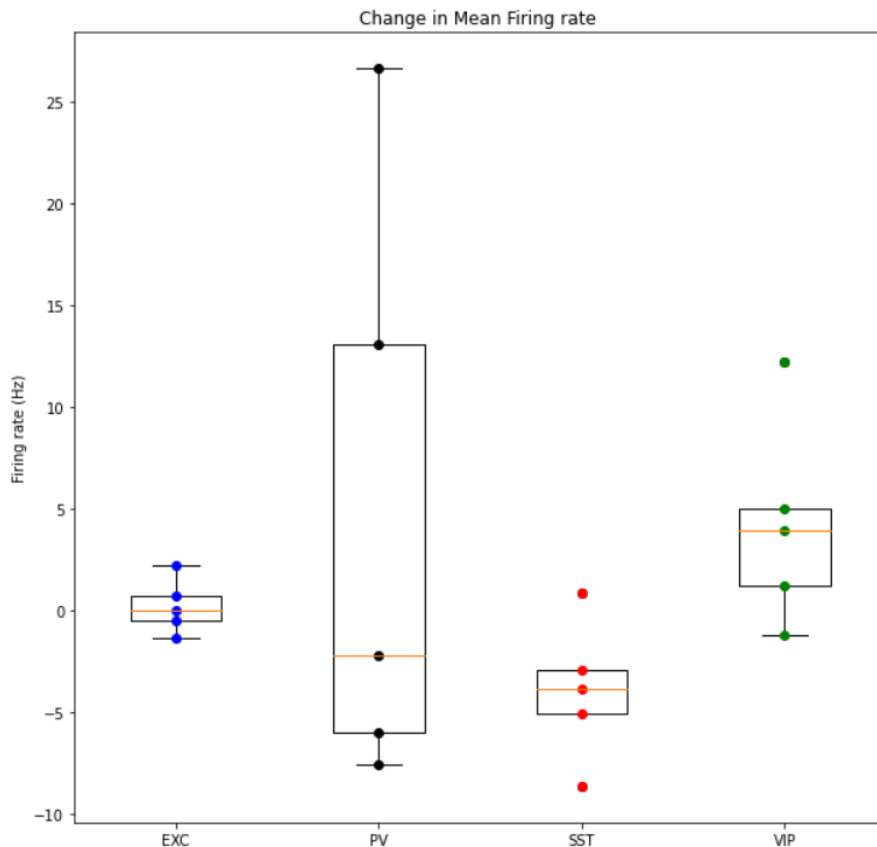
	Cell ID	Cell type	Pre Mean Vm	Post Mean Vm	Diff (Post-Pre)
0	TK545_2	EXC	-63.600218	-63.540461	0.059757
1	TK532_1	EXC	-65.944686	-63.629789	2.314897
2	TK490_1	EXC	-70.016807	-66.120644	3.896163
3	TK539_1	EXC	-56.178636	-56.627754	-0.449117
4	TK473_1	EXC	-68.773931	-65.271517	3.502413
5	TK364_1	PV	-53.471932	-53.557535	-0.085602
6	TK407_1	PV	-56.738504	-53.033593	3.704910
7	TK479_2	PV	-57.196092	-51.914710	5.281382
8	TK479_1	PV	-48.656009	-47.642929	1.013080
9	TK478_1	PV	-53.068251	-49.330379	3.737873
10	TK506_2	SST	-47.485669	-49.491105	-2.005436
11	TK355_1	SST	-54.945408	-58.871124	-3.925716
12	TK358_2	SST	-52.421602	-56.662357	-4.240754
13	TK471_1	SST	-44.336947	-48.067704	-3.730757
14	TK472_1	SST	-53.162853	-54.023151	-0.860298
15	TK462_2	VIP	-53.261666	-52.167304	1.094362
16	TK461_1	VIP	-52.465430	-47.079612	5.385818
17	TK455_2	VIP	-56.853539	-52.934068	3.919472
18	TK454_1	VIP	-53.965125	-51.590211	2.374914
19	TK523_2	VIP	-45.541824	-41.692775	3.849049



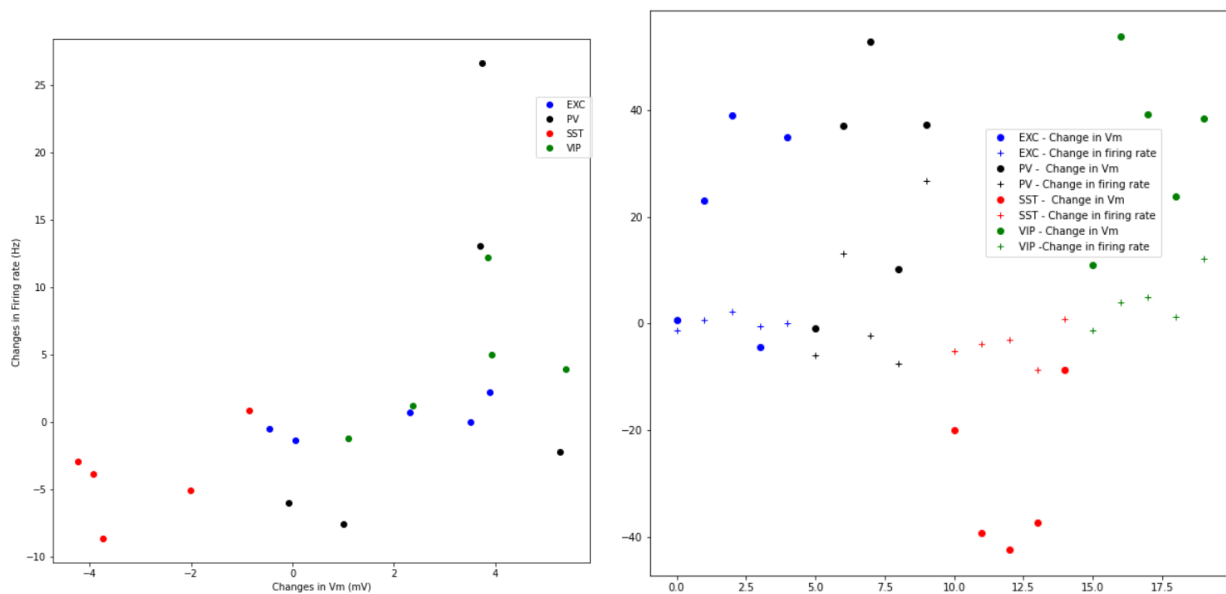
As we can see from the table, and the corresponding graph plotting the whisking related change in Vm per cell class, these changes seem to have similar values for the EXC, PV and VIP cells (so they cannot be distinguished among different cell types), and are quite lower(negative) only for the SST neurons, which could be a way to identify SST neurons.

Next you will compute the change in mean firing rate after whisking onset. For each neuron, you will compute the mean firing rate 500-50 ms before and 50-500 ms after each whisking onset time, and the change in firing rate (FR after - FR before).

You will report in one graph the mean change in firing rates for each cell and each cell class. (0.25 mark).



Do the observed changes in firing rate correlate with the changes in Vm? justify with a graph and a few sentences? (0.25 marks)



Correlation coefficient overall (change in FR vs change in Vm) = 0.5604285203901161

Correlation coefficient EXC = 0.7889472879794839

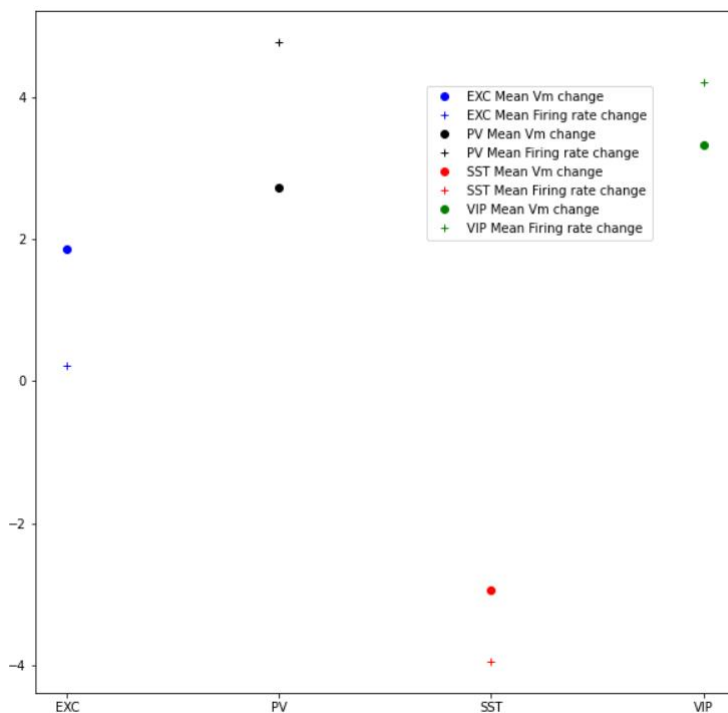
Correlation coefficient PV = 0.4935906797111991

Correlation coefficient SST = 0.5615043241705302

Correlation coefficient VIP = 0.569369395938512

As we can see from the generated graphs (as well as the corr. coefs), there is a certain correlation between the changes in Vm and firing rate for some points, as we can see for EXC neurons, the correlation seems best (mostly when there is a decrease (increase) in the change of firing rate, we see the same effect on the change in mean Vm). However, this correlation seen in general is not very strong, as we can see for SST cells there is almost no correlation.

If we look at the below plot taking into consideration the mean values per cell class (x axis is cell type, y1 is the mean of the changes in Vm for that cell class, y2 is the mean of changes in Firing rate for that cell class) we can see a clear correlation, namely hyperpolarization with decrease in firing rate, and depolarization with increase in firing rate (whenever the class has a higher(lower) mean of change in Vm, it has a higher(lower) mean of change in Firing rate as well).



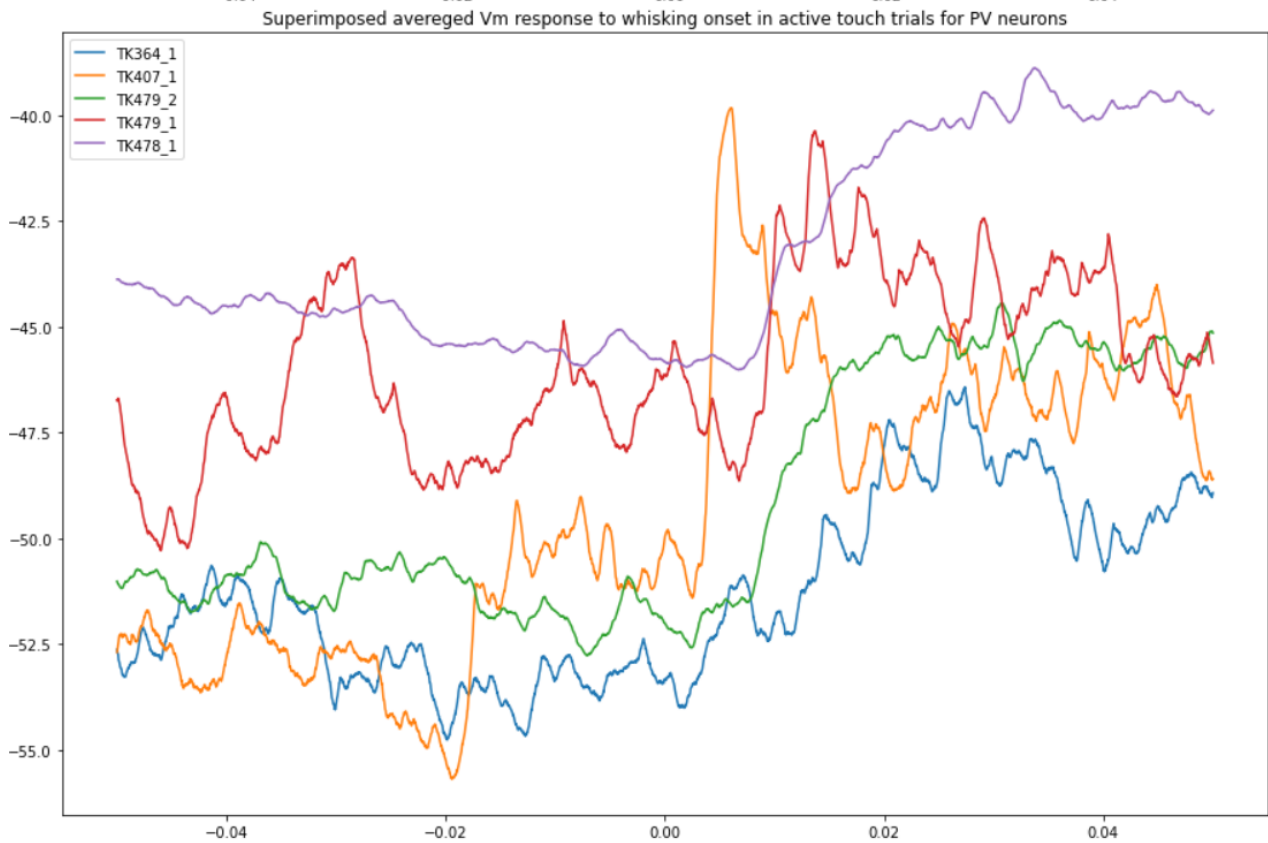
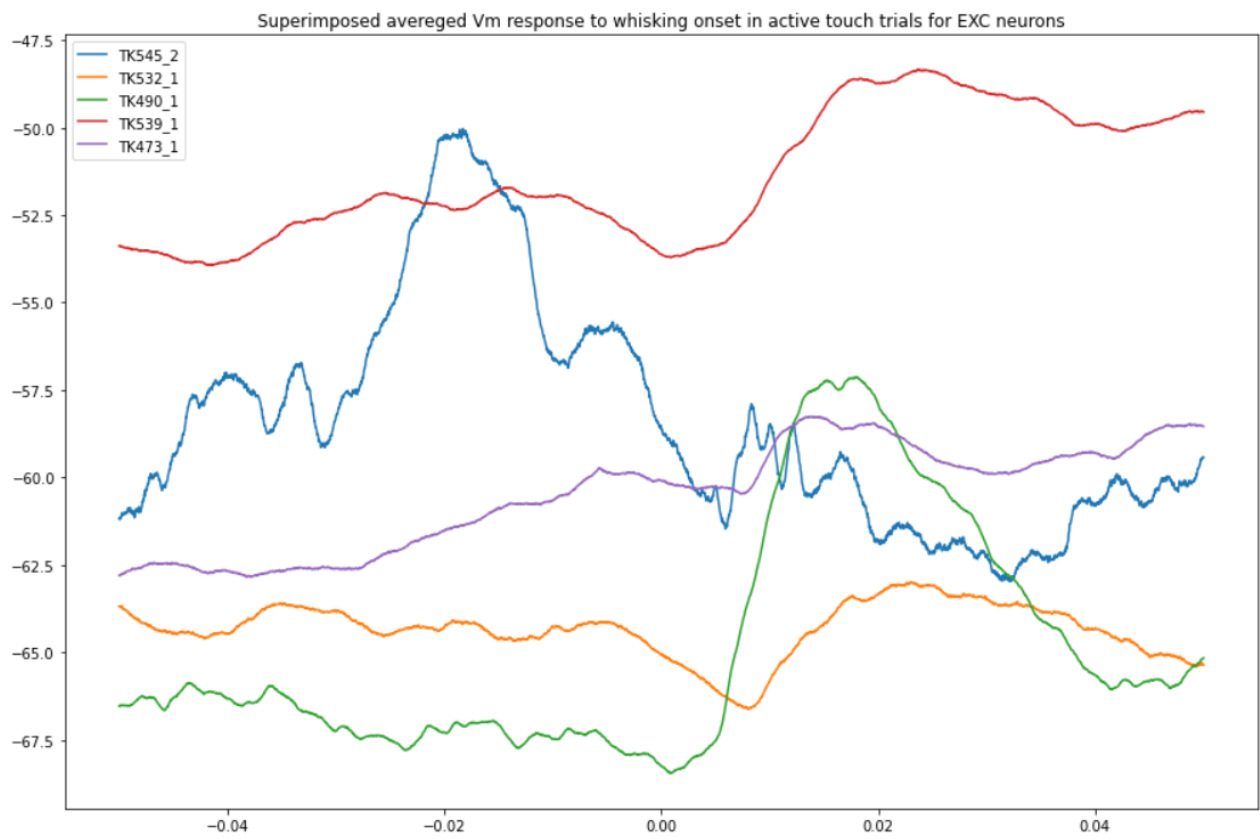
Part 3. Sensory evoked neuronal activity

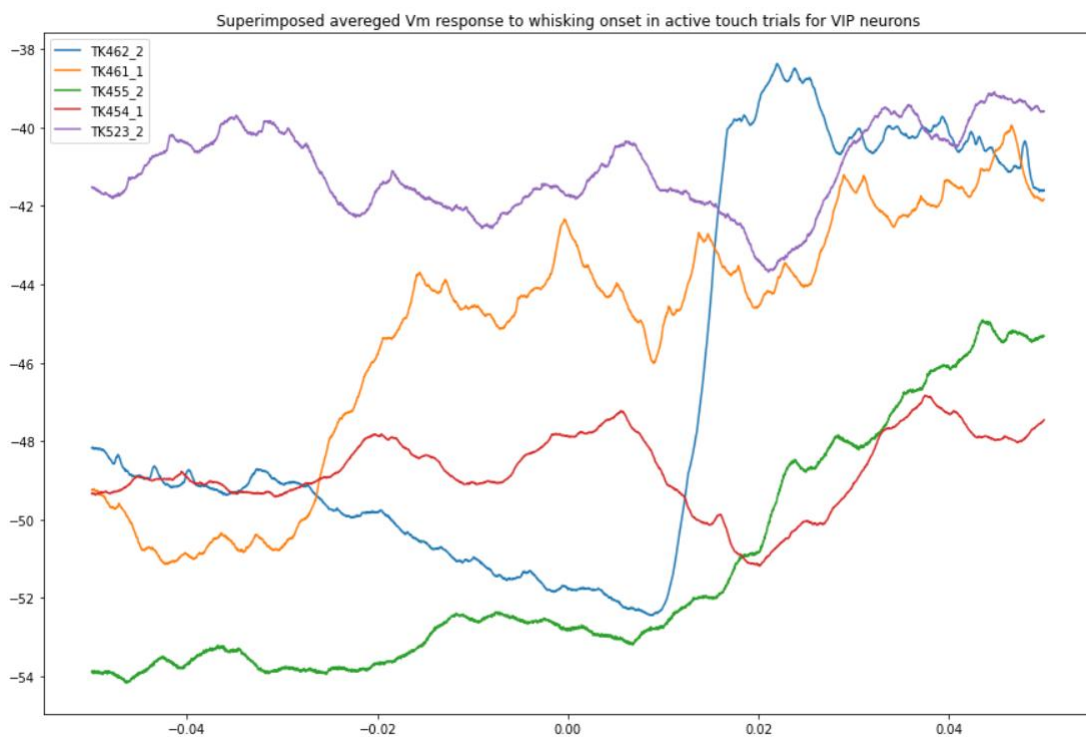
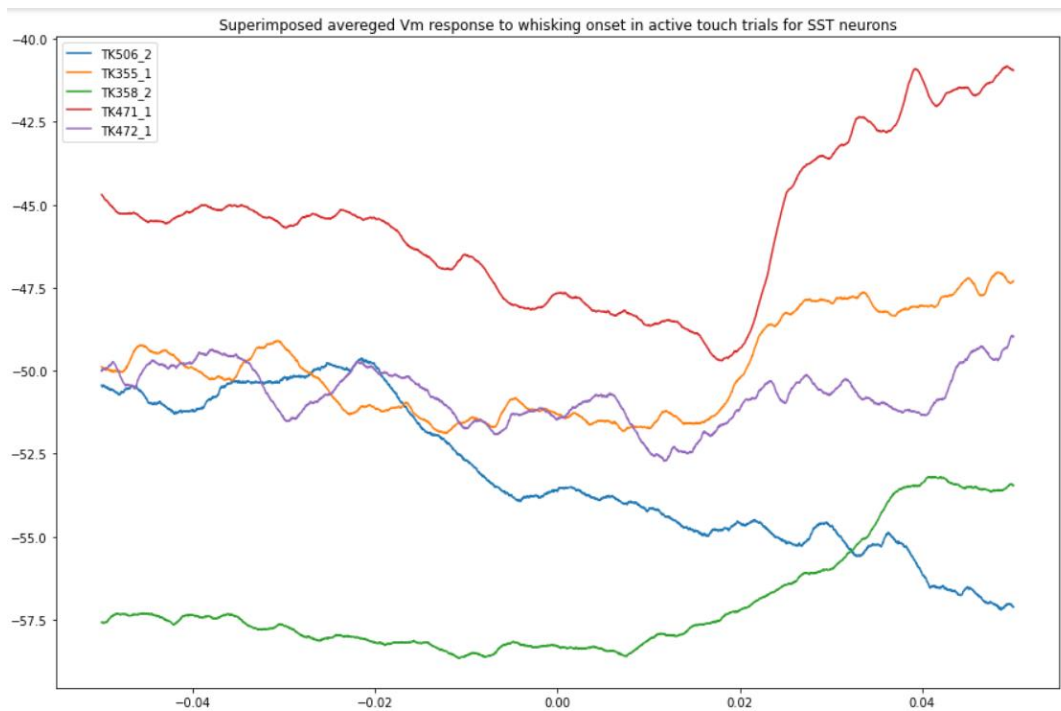
Up to here we have analyzed the neuronal activity in the absence of sensory inputs, comparing periods when the mouse was immobile (Quiet) or moving its whisker in the air (Whisking) in 'free whisking' trials. In other trials, the mouse was presented with an object (a small metal pole) on its right side, 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 barrel cortex neurons. In this last part of the mini project, we will investigate sensory coding for active touch in the different neuron classes.

First we will compute the averaged Vm response triggered by the onset of active touches for each neuron. To do that we will select 'active touch' trials for a given neuron and cut the Vm 50 ms before and 50 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 100 ms before (inter-contact interval > 100 ms).

You will compute the contact-triggered Vm average for each neuron and report your result in 4 graphs (one for each neuron type) displaying the superimposed mean Vm responses for each cell of a given neuron type. (0.5 marks)

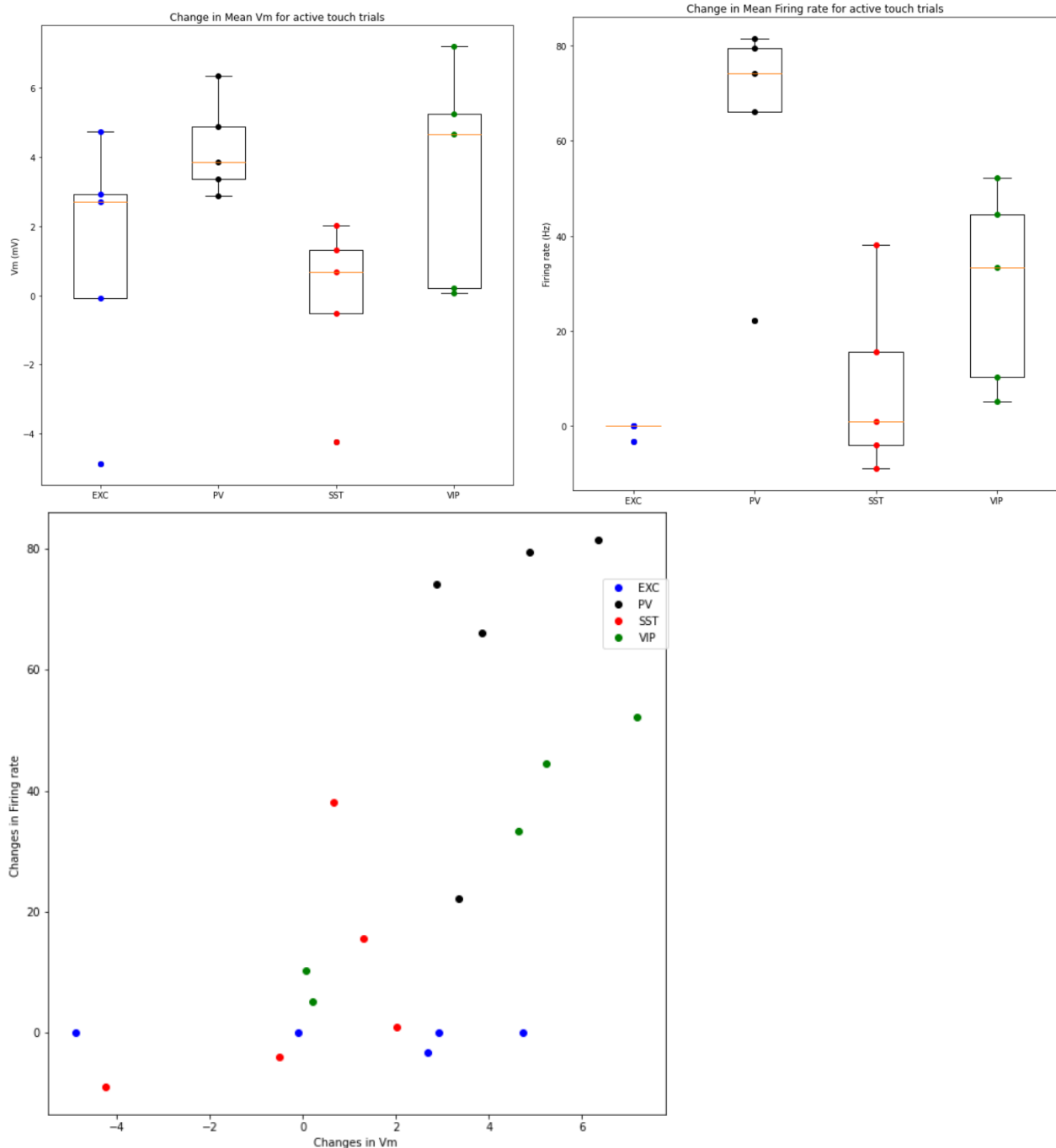
Note: Units for below plots are mV for y axis vs time





Finally, we will quantify the supra- and sub-threshold evoked responses by comparing the changes in mean firing rate and mean Vm (response amplitude) 50-5 ms before and 5-50 ms after contact onset.

For each neuron, you will compute the mean changes in firing rate and mean Vm across all active contacts. You will then compare the sensory evoked responses across cell type. Report your result in 2-3 graphs. (0.75 marks).



Based on your analysis, can you summarize in a few sentences the main characteristics of each cell class. (0.25 marks)

Table containing the summary of observed main characteristics of each cell class

EXC	PV	SST	VIP
Low mean firing rate (0-1Hz)	High mean firing rate (over 30 Hz)	low average FFT Amplitude in the low freq. band (less than 0.55 mV)	AP duration clustered around 0.5-0.6 ms
High AP duration (over 0.8 ms)	Low AP duration (clustered around 0.3 ms)	Reach low values for change in mean Vm (free whisking trials) less than -0.5 mV	Hardest to identify from the analysis so far!
Change in mean firing rate has values around zero (free whisking trials)	Reach high values for the avg FFT Amplitude in the low freq. band (over 1 mV)	Reach low values for change in mean firing rate (active touch trials)	
Strong correlation between the change in Vm and change in Firing rate (free whisking trials)	Reach high values for the avg FFT Amplitude in the high freq. band (over 0.9 Hz)		
Change in mean firing rate is mostly zero (active touch trials)	Reach high values (over 10 Hz) for the change in mean firing rate (free whisking trials)		
	Reach high values (over 50 Hz) for the change in mean firing rate (active touch trials)		