

Miniproject: Analysis of membrane potential data in cortical neurons

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1 Questions

1.1 Question 1

Based on what you have learned during the course, explain what could be the impact of the AP threshold, the mean V_m , and the SD of the V_m on the mean firing rate of a neuron.

If the action potential threshold increases, it becomes less likely to occur because a larger excitatory postsynaptic potential (EPSP) is required to bring the membrane potential above the threshold. Therefore, we would expect the mean firing rate to decrease.

At rest, if the membrane potential is higher, a smaller EPSP is needed to reach the threshold, making it more likely for an action potential to occur. This could result in an increase in the mean firing rate.

A small standard deviation in the membrane potential ensures stability over time. In contrast, with a large standard deviation of the membrane potential, two EPSPs of the same amplitude could produce very different outcomes. This makes neuronal transmission less accurate and reliable. As a result, the firing rate will be less predictable if the standard deviation is high and more predictable if it's low. If we now consider the mean firing rate, the instability in the membrane potential could equally bring it closer to the action potential threshold or push it further away. Therefore, overall, the mean firing rate is unlikely to be disrupted by this instability.

Based on the analyses performed in Part 1, identify which property(ies) actually influence the mean firing rate of cortical neurons across cell classes? Justify your answer with some graphs.

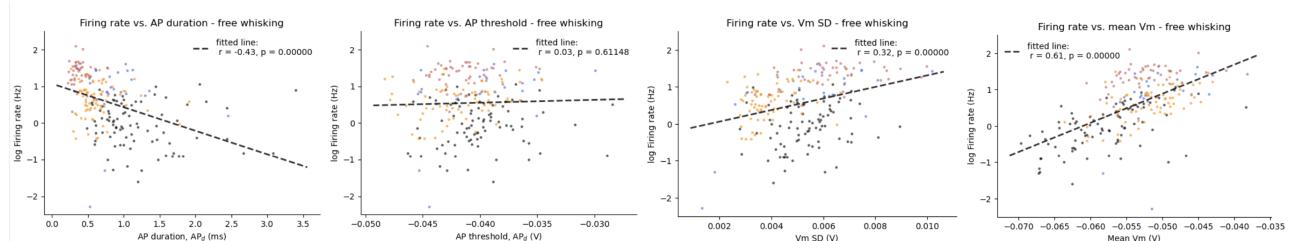


Figure 1: Firing correlation with other features. (Left to right) mean membrane potential, membrane potential standard deviation, action potential threshold, action potential duration.

Let's consider the plots on Figure 1: the Pearson correlation coefficients between the logarithm of the firing rate and the AP duration, AP threshold, membrane potential standard deviation, and mean membrane potential are -0.43, 0.03, 0.32, and 0.61, respectively.

Firstly, the AP threshold appears to be the only one with no significant logarithmic correlation ($r = 0.03$) and no substantial impact on the firing rate of neurons in general ($p\text{-value} = 0.61$).

Regarding the AP duration, it is evident that the AP duration influences the mean firing rate: the longer the AP duration, the lower the firing rate. The extremely small p-value further supports

this and demonstrates a significant relationship between these two features. The linear regression in the same graph shows a moderate negative correlation, described by the equation $\exp(-0.64t + 1.07)$. Additionally, we observe that excitatory neurons tend to have considerably longer AP durations and lower firing rates, whereas PV-GABAergic neurons exhibit the opposite behavior. However, this relationship is not strictly deterministic, as there is a distribution of excitatory neurons with low firing rates but also short AP durations, and similarly, PV-neurons show the opposite effect in some cases.

Similarly, variations in the Standard deviation of the membrane potential influence the firing rate, exhibiting an exponential positive correlation ($\exp(156.09x - 0.25)$). However, these variations do not appear to differentially impact the firing rate across different cell types.

Finally, the mean V_m also has a significant impact on the firing rate, with a p-value < 0.05, indicating a strong correlation with the logarithmic firing rate. Thus, as with the previous analyses, the relationship between the firing rate and the mean V_m can be described by the equation $\exp(156.9x + 0.5)$.

1.2 Question 2

Based on the analyses performed in Part 1, what are the specificities of each class of cortical neurons allowing to best distinguish excitatory vs inhibitory neurons? and between the different subclasses of inhibitory neurons? Justify your answers with some graphs.

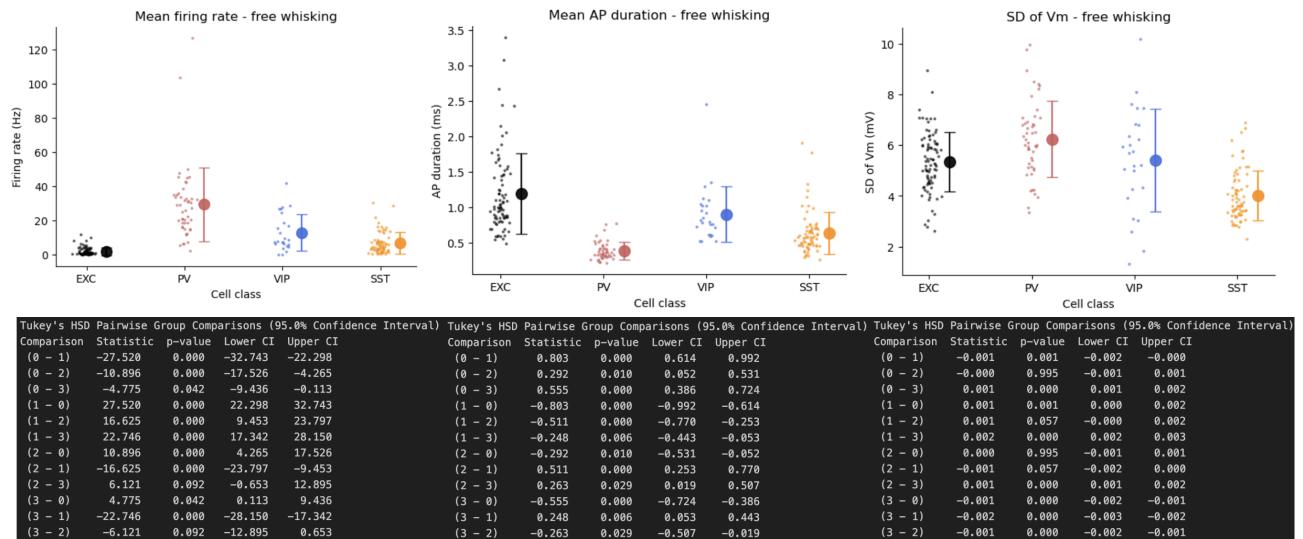


Figure 2: 1. Cell Class features, 2. Tukey's HSD Pairwise Group Comparisons (left to right) Mean firing rate, mean action potential duration, standard deviation of membrane potential, FFT, Mean membrane potential.

The most important or relevant feature for classifying or distinguishing between inhibitory and excitatory neurons appears to be the mean firing rate. This feature seems to separate the two categories from this data sample to some extent. In general, inhibitory cells tend to have a lower firing rate than excitatory cells. By referring to the p-values from Tukey's HSD pairwise Group comparison test we can notice that the excitatory cell type has a statistically significant difference with the other cell type, with a bigger (so less significant) p-value for the SST cell type. However, this is not always the case, as a subset of inhibitory neurons also fires at low rates. Therefore, additional features should be considered to better separate the two classes. For example, AP duration is generally much longer in excitatory cells than in inhibitory ones. We notice the same range of Pairwise group comparisons between excitatory and inhibitory cells with a bigger p-value this time for VIP cells.

When considering the subclasses, it becomes more challenging to discriminate between them. However, similar to excitatory neurons, PV-inhibitory neurons tend to have a shorter mean AP duration than other inhibitory subclasses (Tukey's HSD Pairwise Group Comparisons PV-VIP : 0.000, PV-SST :

0.006). This feature, combined with their higher firing rate (Turkey's HSD Pairwise Group Comparisons PV-VIP : 0.000, PV-SST : 0.000), could be used to cluster them and distinguish this subclass. The other inhibitory subclasses can be differentiated using the standard deviation of the membrane potential, where it is "clearly" seen that most somatostatin cells cluster around a lower variance, while other cells exhibit higher variance. This classification approach would help separate somatostatin cells from VIP-inhibitory neurons (all p-value = 0.000).

1.3 Question 3

Based on the analyses performed in Part 2 and 3, summarize what happens at whisking onset time and active-contact onset time for the different cell classes. Justify your answers with some graphs.

Each class of cell type in the C2 column of the barrel cortex plays a distinct role in processing and integrating sensory information from the whiskers, and they participate in different circuits depending on whether the whiskers are actively sensing without object contact (free whisking) or actively contacting objects.

A simple measure to assess basic cell behavior is the membrane potential. At the whisking onset, if there is an increase in membrane potential (ΔV_m), the cell will be depolarized and thus either activated or more likely to be activated (depending on the AP threshold) during the sensory event. Conversely, cells showing a decrease in membrane potential are inhibited due to hyperpolarization. From Figure 3, we can observe that PV and VIP cells are more depolarized at whisking onset, while SST cells are more hyperpolarized, and all of these changes are statistically significant, though only a few millivolts. Excitatory cells show small depolarisation, but the change in membrane potential is not significant enough to draw a definitive conclusion. This may be due to more complex electrical behavior, delays, or other dependencies, which would require further investigation. Additionally, we notice that the variance is high across the population, suggesting sparse and uncoordinated firing.

Examining the time course of the membrane potential average across the population as a function of time confirms this trend (fig: 3). PV and VIP cells show a small increase in membrane potential around the whisking onset, while SST cells exhibit a decrease at time = 0. Excitatory cells have a more complex pattern: their membrane potential increases a few milliseconds before the onset, decreases in the first second, and then recovers quickly to its initial value before rising again and maintaining an increase of a few millivolts.

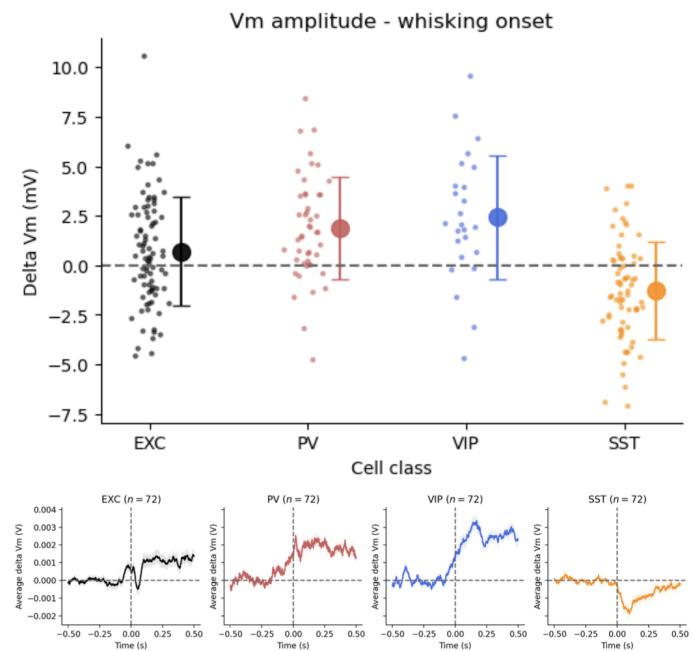


Figure 3: (top) Delta V_m (post-pre free whisking), p-values: EXC:0.0397, PV: 3.164×10^{-6} , VIP: 0.0007, SST: 9.371×10^{-5} , (bottom) Average delta V_m time course around free whisking onset

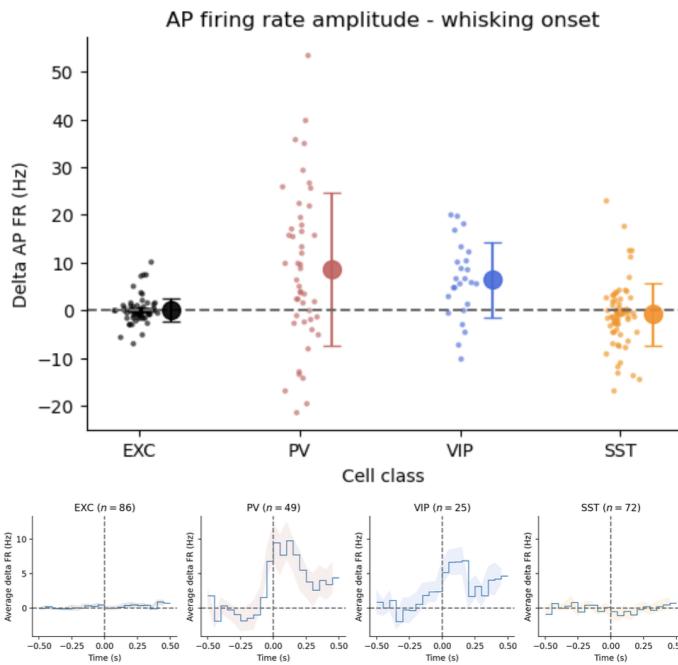


Figure 4: (top) Delta FR (post-pre free whisking), p-values: EXC:0.6661 , PV: 1.109×10^{-3} , VIP: 1.374×10^{-3} , SST: 0.1323, (bottom) Average delta FR around free whisking onset.

no clear trend is observable (not statistically significant). Delayed by about 1 second after the onset. In terms of variance, PV cells have a stable delta Vm across the population, indicating a consistent and coordinated response to the stimulus.

We complement this analysis with the firing rate (fig:6). Interestingly, excitatory cells do not increase their firing rate after the active-contact onset; they maintain the same rate. PV cells, however, drastically increase their firing rate and sustain a high rate for the first second after the onset. VIP cells' firing rate does not show a significant response, and SST cells exhibit a small increase that is not statistically significant.

Finally, when we correlate changes in firing rate with changes in membrane potential for the Active touch task (fig: 7), we observe a strong correlation across the population, especially for PV, VIP, and SST cells (significant at different levels, but all > 0.05). Excitatory cells show a lower correlation ($r = 0.5$). This suggests that membrane potential changes do not significantly affect the firing rate of excitatory cells, whereas inhibitory cells can modulate their firing rates in response to input, and they

A second analysis focuses on the firing rate (fig : 4). Excitatory and SST cells do not show significant changes in firing rate. In contrast, PV and VIP cells both increase their firing rate a few milliseconds before whisking onset, firing at around 10 Hz for several seconds.

Next, we perform the same analysis for active-contact onset. When we examine the mean delta Vm at the onset (fig:5) we see that excitatory cells react by increasing their membrane potential, with a mean delta Vm around 2.5 mV. PV cells also show an increase, but VIP and SST cells show no significant changes. The detailed time course (fig:5) confirms that excitatory and PV cells show a small, rapid depolarization (delta Vm ~4mV in the first second), with a slow decrease in excitatory cells and a fast decrease in PV cells. VIP cell profiles do not provide much insight, as they exhibit high variance, and while there is a peak 1 second after the onset,

SST cells show a 2 mV increase but are delayed by about 1 second after the onset. In terms of variance, PV cells have a stable delta Vm across the population, indicating a consistent and coordinated response to the stimulus.

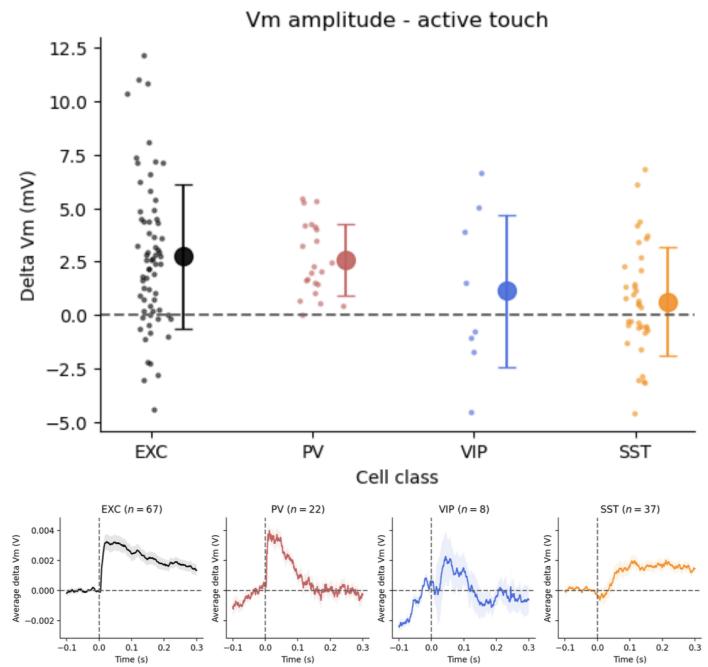


Figure 5: (top) Delta Vm (post - pre active touch onset), p-value EXC: 4.448×10^{-8} , PV: 4.768×10^{-7} , VIP: 0.546, SST: 0.182, (bottom) Average delta Vm time course around active touch onset

possess the appropriate electrical properties to fire at high rates in response to strong stimuli. For Free whisking, all the inhibitory cell types show this correlation between the firing rate and the membrane potential, especially the VIP cells.

Overall, when the whiskers encounter an object, excitatory cells in the C2 column of the barrel cortex react to stimuli by modulating their membrane potential but do not significantly change their firing rate. In contrast, inhibitory cells, particularly PV cells, are much more activated during active touch, as evidenced by their firing rates. PV cells are known to provide feedforward inhibition to surrounding excitatory neurons, thereby sharpening the temporal and spatial dimensions of the sensory signal. VIP neurons play a role in inhibiting SST neurons to release excitatory cells from their inhibition. This interaction is apparent in the data: when VIP neurons are more activated (higher delta Vm), SST neurons are inhibited (lower delta Vm), and vice versa when VIP activation is reduced.

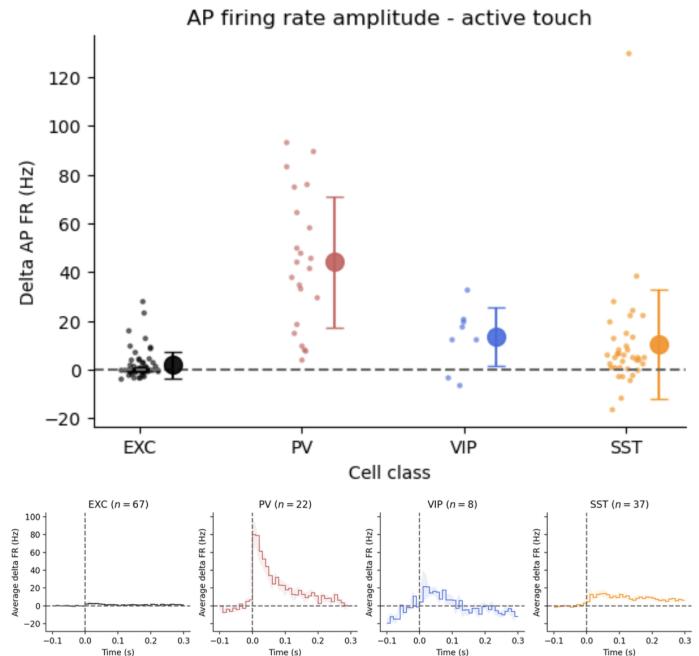


Figure 6: (top) Delta FR (post - pre active touch onset), p-value EXC: 1.547×10^{-2} , PV: 4.768×10^{-7} , VIP: 3.906×10^{-2} , SST: 9.156×10^{-5} , (bottom) Average delta FR around free active touch onset

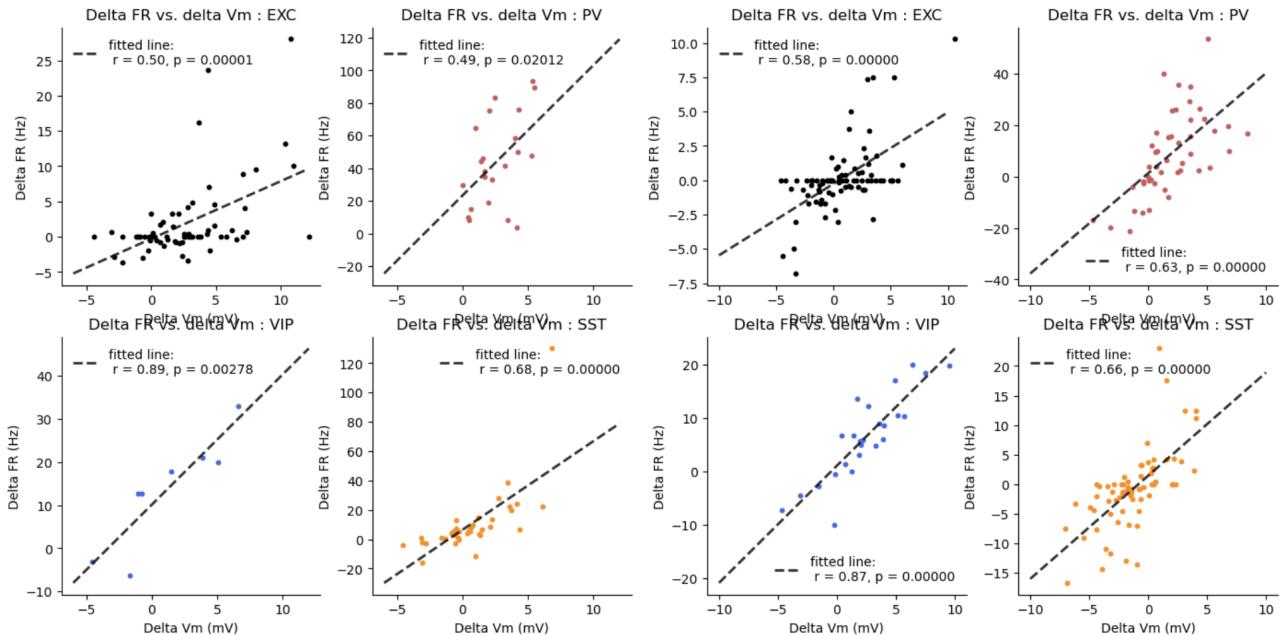


Figure 7: FR correlation with Vm for each cell class. (right) Active touch (left) Free whisking

Based on what you have learned in the course, what could be a possible mechanism explaining the change in membrane potential of somatostatin (SST) expressing GABAergic neurons at whisking onset?

Somatostatin-expressing GABAergic neurons are inhibitory neurons in the neocortex. They primarily innervate the distal dendritic regions of pyramidal neurons in layer 1 of the neocortex. These neurons receive facilitating excitatory synaptic input from nearby pyramidal cells and inhibition from VIP neurons.

At the onset of active free whisking, the somatosensory cortex receives sensory information via ascending sensory pathways, leading to excitatory postsynaptic potentials (EPSPs) in the excitatory neurons of the barrel cortex. Additionally, the motor cortex sends long-range glutamatergic projections that innervate layers 1, 5, and 6, allowing for the integration of sensory information in the context of motor action (active sensing). During free whisking, somatostatin-expressing GABAergic neurons are hyperpolarized due to the action of VIP and stop inhibiting layer 1, allowing sufficient excitatory activity to occur. However, during active contact, these neurons depolarize with a delay, become activated, and inhibit the excitatory cells, thus sharpening the sensory signal in time.

These neurons likely play a critical role in the integration of sensory and motor information, helping to refine and sharpen the sensory signal in association with motor commands.

2 Project

Explain in a few lines what is the question you want to address, what is the rationale and what is your hypothesis. Explain briefly what analyses you have done to answer your question and how you have proceeded. Present your results with some graphs and explanations.

With the data provided, we decided to try to build a classifier that could determine the cell type of a given cell between the 4 categories: excitatory cell, PV cell, SST cell, and VIP cell. We decided to train it on the raw data provided as well as the processed data extracted from the notebook (electrophysiological data from the free wishing and the active touching tasks). Our hypothesis to justify this choice is that electrophysiological data, dynamic and not dynamic, could help to classify the cells. Indeed we show previously that the cell behavior changes depending on the task.

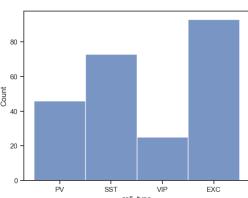


Figure 9: Cell type distribution across the extended data set

From the raw data: Cell Depth, Cell Layer, Cell Targeted Brain Area are anatomical features that could help to determine the cell type and Cell type as our labels.

From the properties of cortical neurons during quiet wakefulness notebook that extracted the suprathreshold/subthreshold activity and cell class and keeps: The firing rate, AP threshold, AP duration, Mean Vm, STD Vm. These are the principal electrophysiological metrics to characterize the basic electric properties of the cell.

Then we took from notebooks 2 and 3 the task-dependent electric features of the cells. We kept for both tasks, Free wishing, and Active touch. whisker protraction (WP) amplitude pre and post, Membrane potential (Vm) amplitude pre and post, and Action Potential (AP) firing rate frequency pre and post.

We first try to implement a simple KNN model on those data without any step of dimension reduction. After optimizing our hyperparameters we obtain a F1 score of 0.92 for the training and 0.73 for the testing. We computed the diffusion matrix to look at our model's performance in figure 8. The model performs well and classifies most of the cells but seems to have trouble with the SST cells. Notice that

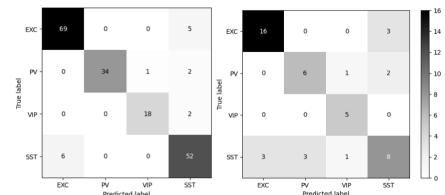


Figure 8: Training (right) and Testing (left) performance of the baseline KNN classifier, Confusion Matrix

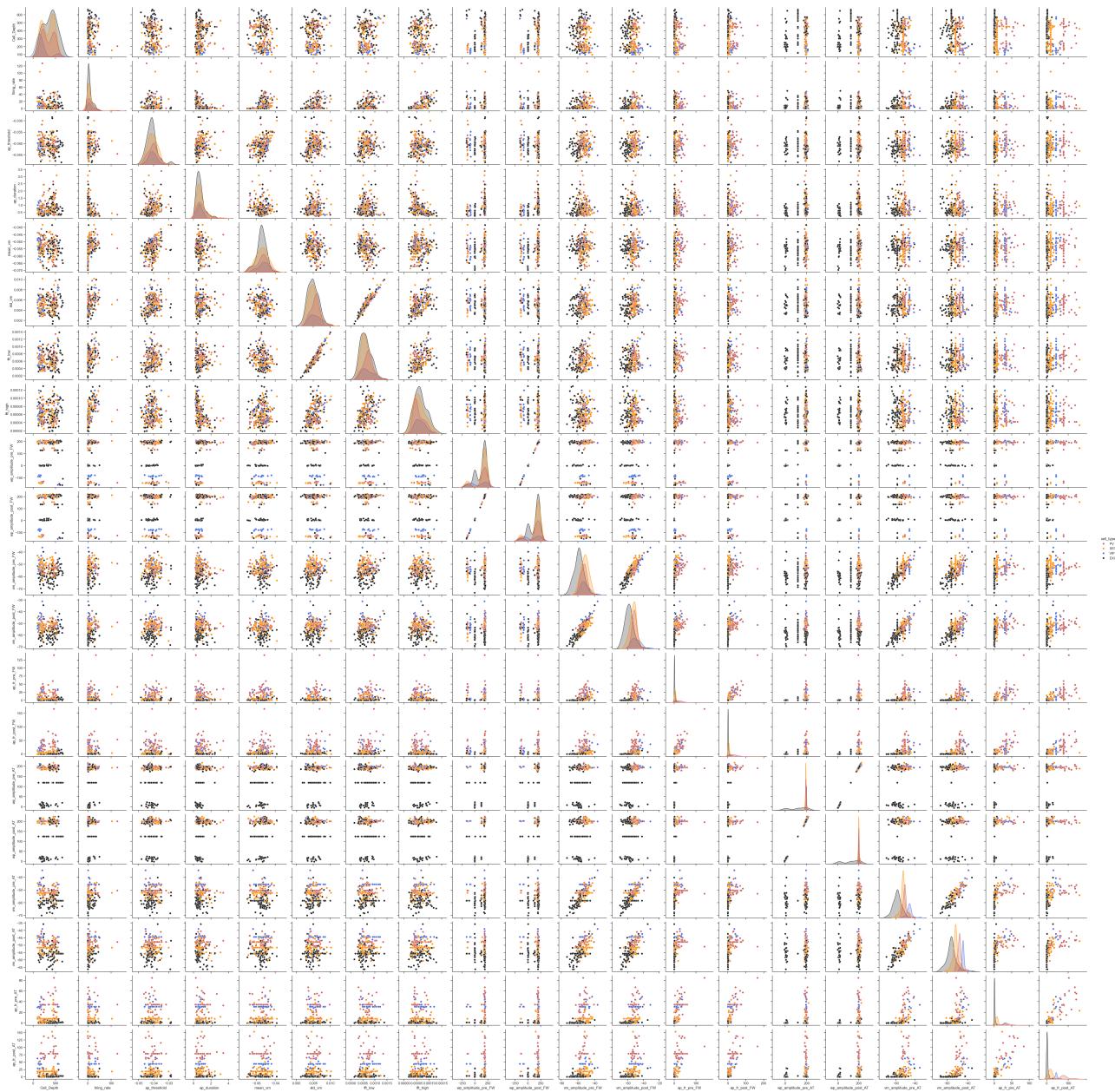


Figure 10: Pair plots of the data features

we fixed the seed to have the worst result, the algorithm performing already quite well, we wanted to try to improve the hardest situation, the one where the performance was the worst.

Indeed if we have a look at the data we can notice that the cell features already form distinguishable clusters, Which helps a lot the KNN to set decision boundaries (fig: 10).

The first observation is that our dataset is largely imbalanced. We already did data augmentation to make it bigger: we replaced the missing data with the mean value within the cell types. Thanks to that we reached a data set size of 189 samples. We can notice that the cell types are not equally represented, we have way fewer VIP cells, which could explain the model struggling more to represent them (fig: 9). We added weights or weight options for the hyperparameter optimization to balance the dataset for the following.

We decided to make a 2 step classification to first try to distinguish the excitatory cells from the inhibitory cells and then try to attribute the right label between inhibitory cells. This demarche comes from the intuition that the two steps of classification might not rely on the same set of features. If

we first exclude the excitatory cell, the second model should be more specific to inhibitory cells and better at their classification.

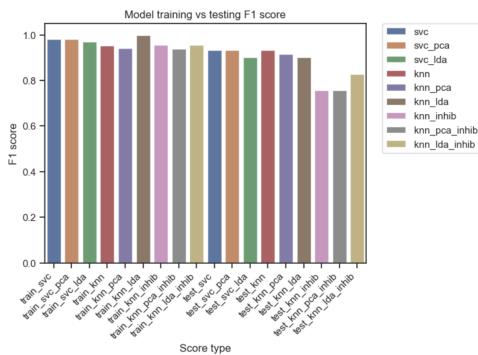


Figure 11: Model Performances on training and testing

Because we have 25 features, we decided to go for dimension reduction, we may have redundant features in the dataset. Indeed as we have seen in the pair plot (fig:9), we can guess correlations and dependency in general between features. We tried PCA and LDA decomposition. We kept 23 components to have 100 percent of the explained variance for the PCA for both steps of classification. **toexplain** This step is also important to see which features rely on our classification, and what are the main features that use the model. We will come back to it later.

We tried SVM and KNN models for the first step of classification (binary classification between excitatory and inhibitory cells) and then a KNN for the 2nd step to classify the inhibitory cells. All our models are optimized using a grid search algorithm. Summary of the hyperparameter is shown in tables 1, 2, 3. We split our dataset between training and testing, 70/30 ratio. And evaluated our model using the F1 score which is recommended for imbalanced datasets. The results from the training and testing can be found in the tables 1, 2, 3.

Table 1: Step1: Excitatory vs inhibitory classification using Support machine vector

dim reduction	C	gamma	kernel	F1 training	F1 testing
not	1	0.0001	linear	0.98	0.93
PCA	1	0.0001	linear	0.98	0.93
LDA	0.1	0.1	rbf	0.96	0.90

Table 2: Step1: Excitatory vs inhibitory classification using k-Nearest Neighbors

dim reduction	metric	n neighbors	weights	F1 training	F1 testing
not	manhattan	3	uniform	0.95	0.93
PCA	euclidean	10	uniform	0.94	0.92
LDA	euclidean	20	distance	1.0	0.90

Table 3: Step2: Inhibitory cell classification using k-Nearest Neighbors

dim reduction	metric	n neighbors	weights	F1 training	F1 testing
not	manhattan	3	uniform	0.96	0.76
PCA	euclidean	3	uniform	0.94	0.76
LDA	euclidean	10	uniform	0.96	0.83

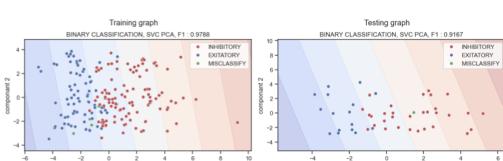


Figure 12: Best First step binary classifier

Let's now evaluate our models' performance and pick the best combinations of classifiers.

We can see in figure 11 that all models fit the data and have a good performance at the end of the training. They perform, well on the testing and outperform the baseline model. The models that perform the best are SVC with PCA for the first step and figure 12 KNN

based on LDA for the second step (fig:13). We finally reached a testing performance of 0.83 (fig:13)

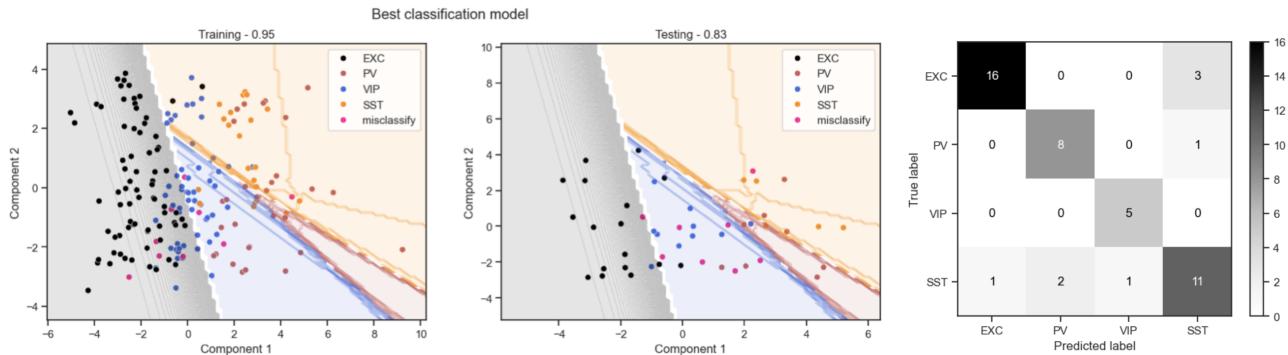


Figure 13: (right) Best classifier combination training plot, testing plot, (left) Testing confusion matrix associated

Now that we have developed a model that outperforms the baseline model, let's look closer into the components on which it relies. For both steps (binary classification and inhibitory classification), we kept 23 components of the PCA that explain 100 percent of the variance. We plot the first 10 components' composition of the initial features that explain 90 percent of the variance. For the first step, we see that all features are mostly taken into account. However, the explained variance is mostly composed of tasks and the membrane potential. The question is crucial to know if the tasks have been taken into account if the cells had behaved the same in the PCA would have removed one or two components.

The second component (15 percent of the explained variance) takes interestingly the anatomical position Layer 2 3 and 4.

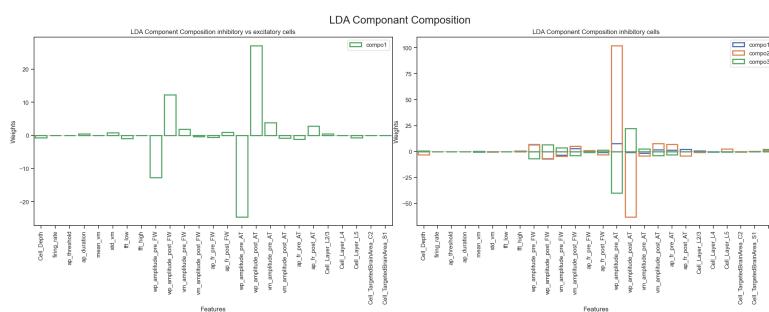


Figure 15: LDA component composition

the first component but not anymore the membrane potential amplitude. The second component



is very similar and we find as well very similar PC4, PC7 and PC9.

We did the same analysis for the LDA components. The first LDA component mainly takes WP amplitude pre- and post-free whisking event and active touch but a very small contribution to the Action potential firing rate frequency which could explain why it performs worse than the PCA. For the inhibitory classification, the LDA component relies a lot on the WP amplitude of the active touch and this is a small contribution of all the dynamic electrophysiological features.

NOTE: The fact that models rely on whisker Protraction Amplitude Pre- post might not be a good sign for our model. Because it's data from the experimental setup and not from the cellular response, we might have tried to remove it and see if we could improve the result. Indeed this feature could be informative if associated with a cellular response, we could have a WP-amplitude-dependent response of firing rate for example, and then the WP amplitude could be informative about how the cell type reacts but, taken separately it does convey information about the cell, we should be careful about how do we interpret it.