# Neural Signal Analysis Assignment

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## Introduction

In this study, it was investigated how neuronal activity modified after an injury to primary motor cortex. An ischemic injury was induced in the caudal forelimb area of anaesthetized Long Evans rats through microinjections of Endothelin-1 (ET1). To study it, intracortical microelectrode arrays were inserted in the ipsilesional rostral forelimb area (P1) to record spike activity and to trigger intracortical micro-stimulations in the somatosensory cortex (P2). Activity from both P2 and P1 was recorded.

The assignment is based on the analysis of in vivo data from six rats (three lesioned, ET1, and three controlled, SHAM).
The recorded activity is divided in:

- before and after the injury;
- basal and mapping.

The purpose of this analysis was to evaluate if and how the connectivity changed after the injury. The focus was how the effective connectivity (between the stimulated channels and the others) changes.
It represents a direct (causal) link between a stimulus in a certain point of the network and the response caused by it in another point. This information is provided by the PSTH (its area): it can in fact be seen as a representative of the strength of the connection between one neuron and another. So, if a neuron A is stimulated, if the neuron B responds with a high number of spikes, then it means that they are strongly connected, otherwise they are not.
Using the PSTH an assumption is being made, which is that the information is quantified in terms of number of spikes. To quantify the effective connectivity change between the stimulated channel and a whole region (P1 or P2, both recorded using 16 electrodes), it has been computed the difference between pre and post lesion PSTH area for each channel. To do this analysis, only mapping data have been considered.

## 1. Processing and Analysis

*Artifacts study and Pre-processing*

Firstly, the study of the stimulation artifacts was done. They were present in all channels both in P1 and P2 recordings even if the stimulation was only in a P2 channel.
Blanking the artifacts and the relative transient periods (before or after filtering), wasn't an optimal solution because it removed information that could be useful for the analysis, also it introduces some problem relative to spikes detection at the discontinuity region. So, it was decided to not eliminate the artifacts, therefore the only pre-processing technique used was filtering.
It has been used a second order Butterworth pass band filter with frequency range between 300 Hz and 3000Hz to extrapolate the Spike activity of the neural network. Then it was used a simple thresholding function to detect the artifacts (Th=500, distance between peaks=1s).

*Spike Detection*

After that, Spike detection was performed. There were two main problems that could invalidate it:

- The artifacts create problem in the (spike detection) threshold computation.
- The transient period gave not possibility to understand if the spiking activity detected during it was due to real neural activity or was a result of the filtering.

After trying a Simple Hard Threshold algorithm and a Hard Threshold algorithm, it was chosen the Hard Differential Threshold algorithm.

To calculate the threshold, it was considered the standard deviation (σ) of the data from the last artifact to the end of the recording. In this way it was solved the problem due to the presence of the artifacts, in fact the threshold has been calculated in a period of the signal without artifacts.
The parameters applied were: Sliding Window length = 50 samples (0.2 ms), Th = 7*σ.
Now Spike Trains are obtained.

*PSTH*
Operating with Spike Trains it was possible to create the Post Stimulus Time Histogram (PSTH). Parameters chosen were Window length = 400 ms and Bin size = 10 ms
It was noticed that in the first bin (in one case also in the second) the spiking rate was very high with respect to the others because of the transient period. Indeed, the first bin was not considered in the creation of the graphs.
In this way the information provided in the first ms after the stimulation was not examined because it can invalidate our results. This was the reason why the PSTH analysis was focused more on the late response that allow us to obtain more reliable results.

*Area*
To quantify changes in effective connectivity, the differences between pre and post lesion PSTH were calculated for all channels. Then the results relative to each single area are plotted in a histogram.
It has been chosen different bin sizes to calculate the histograms in order to have a similar number of bins for each case.

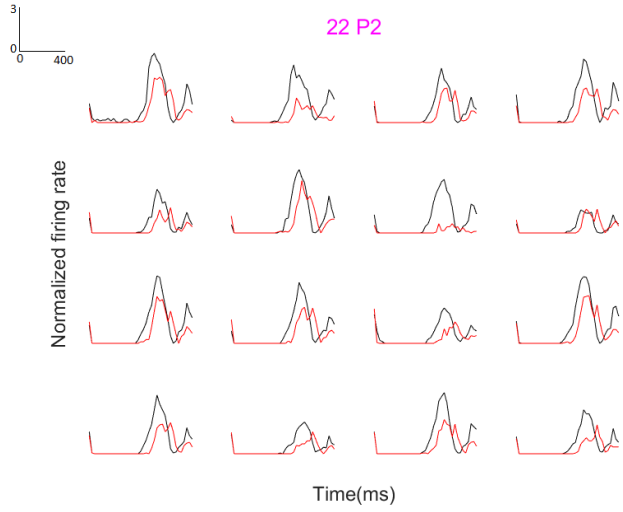
$$\frac{Area\ Psth\ pre\ lesion - Area\ PSTH\ post\ lesion}{Area\ PSTH\ pre\ lesion} \cdot 100$$

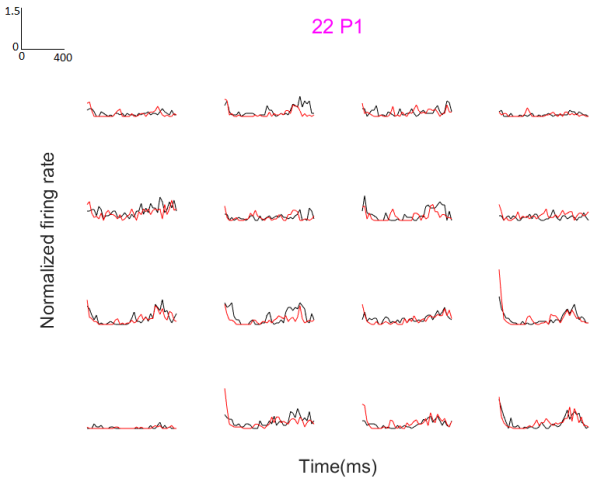
*Choice of the subjects*
It was performed the analysis on all the six subjects, but in the end only the results of three of them (rat_16 and rat_17 from lesioned ones and rat_22 from controlled ones) were considered, because the others didn't provide interesting results.

## 2. Results

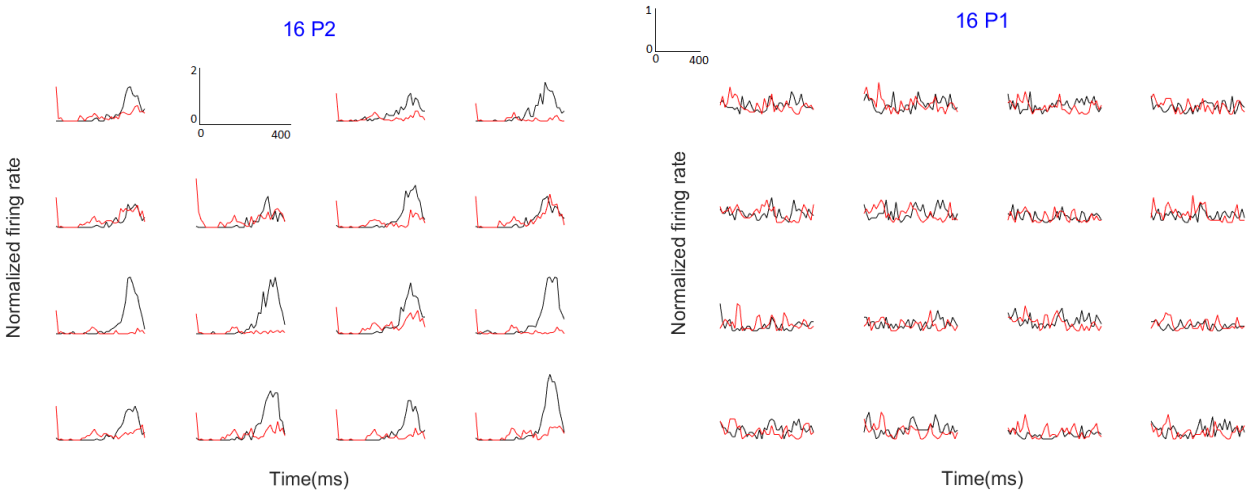
There are 16 channels for each area. The stimulation is provided only in P2; two of these channels are used to stimulate so they can not be used to record the neural response. Stimulation channels were not known for the rat_22, while for rat_16 the first set of stimulations came from channel 1 and the second set from channel 12. For rat_17 come from channel 0 and channel 12.
The study concern only the first set of stimulation. A visual comparison of the results between the two stimulations is provided in the last page.

The Post Stimulus Time Histogram is useful to characterize neural spike trains in response to a stimulus. In the following plots are displayed the 32 channels (16 for P1 and 16 for P2) in pre (black line) and post lesion (red line) condition.
It can be observed on the x-axis both part of the early response (first 50 ms), which includes the activation of the neurons, and the delayed response (from 50 to 400 ms), which represents the reverberating response of the network.
Furthermore particular attention has to be done on the plots scale, because in P2 the signal is always higher than P1.

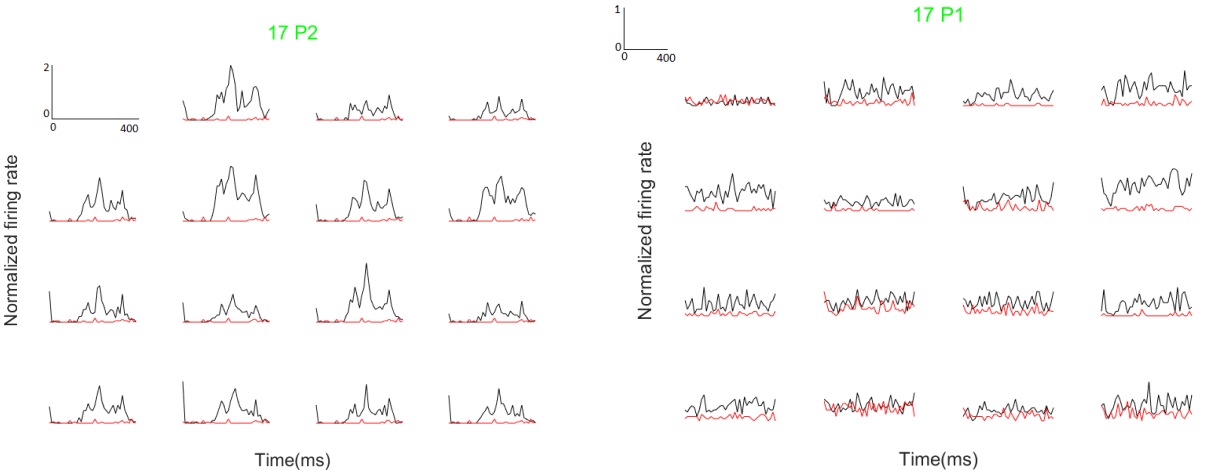


In the figures, it can be seen how an healty situation looks like: there aren't much changes between the areas, even if a decreasing in the channels relative to P2 area can be seen.



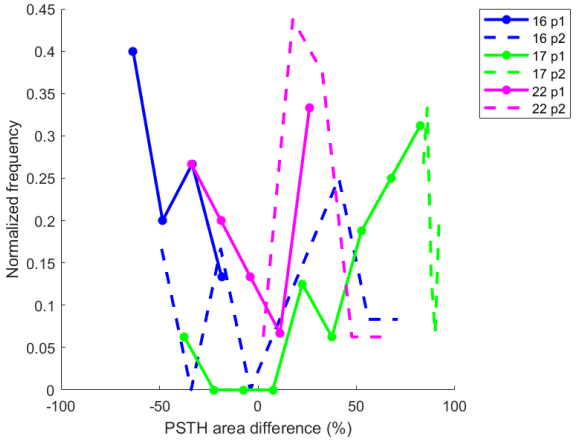

The first thing to notice is how the response to the stimulus in the P2 area decreases after the lesion in most of the channels. However, in the P1 area there aren't many changes. The PSTH areas increase a bit after the lesion. For the P2 PSTH it has been chosen to not consider the first 2 bins for the motivations explained before.




This subject acts differently from the previous one; there is a big loss of response also in the P1. The post lesion response of the P2 is mostly near to zero in all 16 channels. In both the zones the PSTH areas decrease after the lesion.

*PSTH area difference*

For each subject's zone (P1 of 16, P2 of 16, P1 of 17, P2 of 17, P1 of 22 and P2 of 22) was calculated the histogram of PSTH area difference after the lesion. It has been done considering the area difference for all the 16 channels.




The results show that the histograms relative to sham are centered around zero, this means that there is not a big difference between pre and post lesion. The ones relative to rat_16 and rat_17 are shifted towards right or left. Considering both the lesioned subjects it can be seen that P2 histograms are globally right-shifted, this means that PSTH area decreases after the lesion. For P1 in one case there is an increase (left-shift) and in the other a decrease (right-shift).

## 3. Discussion

We find out that in the 400 ms after (PSTH period) the stimulus in P1 there is a globally increase in spike activity instead there is a more evident decrease in P2, with respect to the control condition. More than a fact related to stimulation (and effective connectivity) this is a characteristic of the signal as it is reported in literature[1]. In fact, it has been studied that the ischemic lesion in the motor area led to an overall increase in spike activity within premotor area (P1) and a decrease in somatosensory area (P2) with respect to the control condition.

So, it can be hypothesized that there are 2 effects that lesion caused and that affect the PSTH: one is independent of the stimulation and is observed in all the signal, the other is related to stimulation. So one isn't related to the starting hypothesis instead the other can show a real change in effective connectivity.

The first can be quantified as a decrease in the area of PSTH relative to P2 and an increase in the one of P1, the second in a decreasing in both the zones.

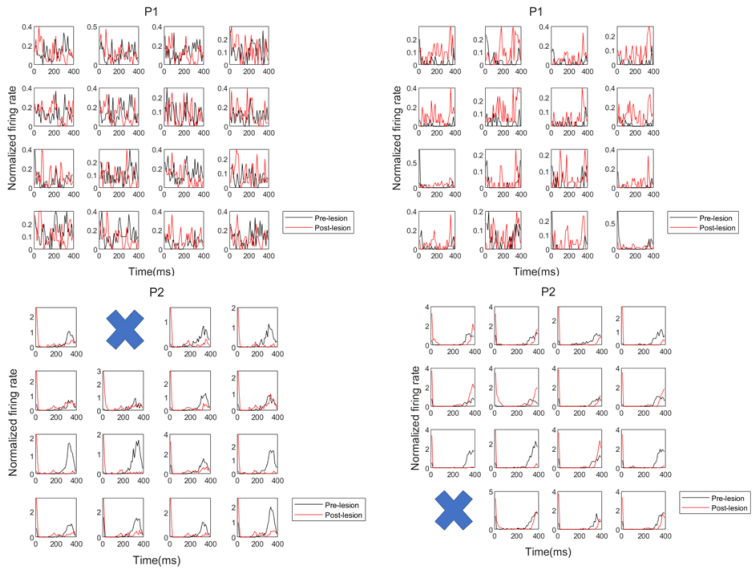
This can be the reason for which our results are so variable, we don't know which effect is more relevant for each subject. For this reason, instead of doing general hypothesis we choose to analyse single cases.

As expected, for rat_22, according to results, the effective connectivity does not change so much.

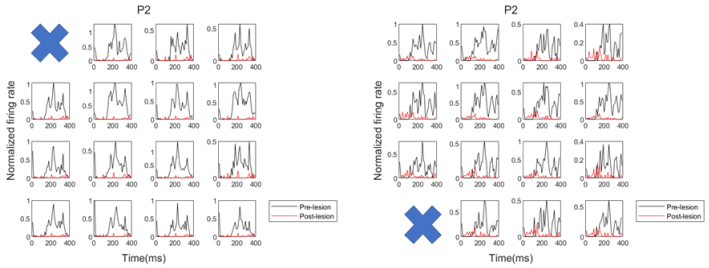
In rat_17 there is a general decrease in the psth in both areas. This can be related to a decrease in the effective connectivity especially for what concern P1. Instead for P2 we don't know if the decrease in the area is due to the global effect of the lesion or to a real decrease in effective connectivity.

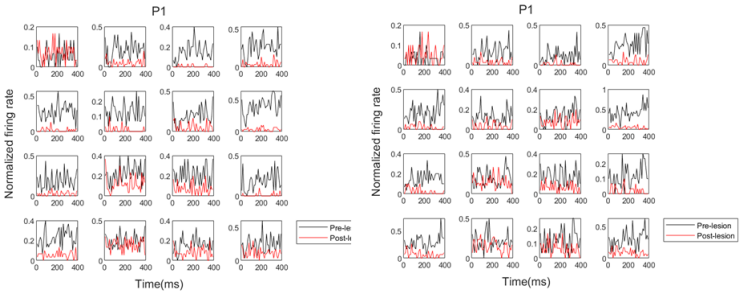
In rat_16 there is a shorter decrease in P2 and in P1 we have an increase. This increase can be caused by the global effect of the lesion.

[1] Carè et al. Bioelectronic Medicine (2022)

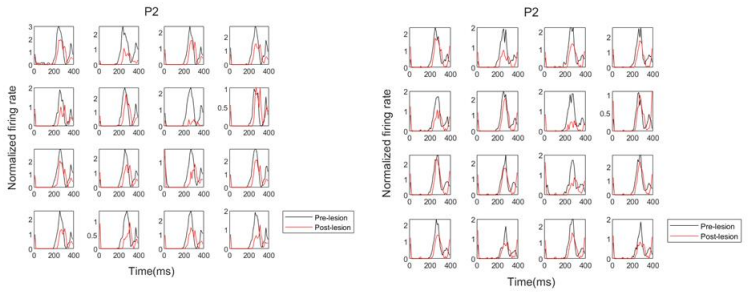


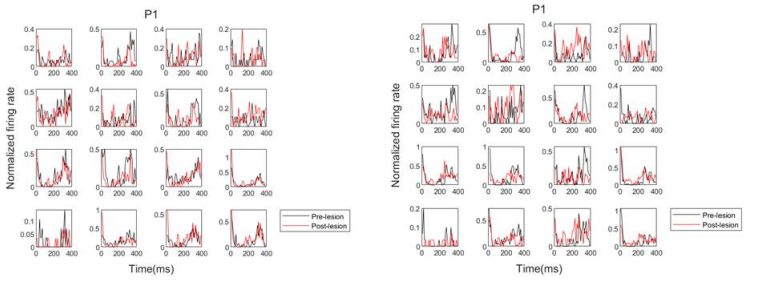

*rat_16*




*rat_17*




*rat_22*