The Differences Between Various Memory-Improvement Treatments on Neurons using Multielectrode Array

The code repository can be found here.

Introduction

First, we would like to generally describe the process of forming a memory. Please note that in the following document, "neurons" will only refer to those found in the brain.

Different groups of neurons are responsible for different thoughts or perceptions. A 'group' (called a synapse) is formed when two neurons create a connection by transferring electric signals between one another. Active connections tend to get stronger, whereas inactive ones get weaker and can eventually disappear entirely. Retrieving a memory is the reactivation of the specific synapse that was formed when the experience first happened.

The main goal of prof. Knafo research is to find a drug that is proven to improve memory. Neurons are bred in a well with electrodes in a laboratory. Each well is given one of two treatments. A few of them are the control group and are not given a treatment at all. The electrodes monitor and document every connection made in each of the wells. When a neuron sends an electric signal to another neuron, an electric potential difference (voltage) is created and recorded by the electrodes.

The problem we are tackling is the last step of the process: we would like to determine whether the drugs (AP5, FGL) are efficient or not, and if so, which of the two is more efficient.

Our approach is to find the Pearson's correlation between different electrodes on a single well. Then, we will perform an ANOVA test to determine if the change in correlation was significant. Finally, we will use Tukey's multiple comparison method to determine which treatment is the most efficient.

Data Overview

We have extended the amount of data we used for this project since the proposal was submitted and it now includes 24 data files (.csv), produced automatically by the multielectrode array. Each file was recorded at different episodes of the experiment: before treatment (1 file, starts with bl); first hours following the treatment (5 files, starts with ac); overnight after the treatment (18 files, starts with on). The `README.md` file was updated accordingly.

Each of them contains 3 main features:

- Time (s) [Float]: Seconds passed from the start of the recording until a signal was sent.
- Electrode [String]: The electrode on which the neuron that sent the signal is placed. This column contains the well's ID and the electrode's ID, separated by a lower dash. like the following format: [Well name]_[Electrode name]
- Amplitude (mV) [Float]: The electronic signals strength (voltage).

There is a total of 24 wells, each containing 16 electrodes. Neurons activity was recorded at selected times during a period of 24 hours, for 11 minutes each.

The data also contains some additional information, irrelevant to the problem we are tackling. Farther information about the above can be found in the `README.md` file in the `Data` folder in this project repository.

Methods and Results

At first, we attempted to find the answer to our research question using a KNN model. Our hypotheses were that each treatment may encourage a change in the neurons' firing rate. We calculated said change depending on treatment, but model accuracy was low. It could not accurately predict the type of treatment given.

We concluded that the change in firing rate is not a way to measure the treatment's efficiency. An electrode might send signals consistently but with no response.

We decided to try a different approach. We calculated every pair of electrodes' correlation within a single well using Pearson's correlation method. The correlation is stronger in electrodes that sent a signal during adjacent times. We repeated this process for every well at each time period.

To determine whether a significant change in the correlation occurred due to the administration of the treatment, distance, or a combination of the two, we performed numerous ANOVA tests. Our null hypotheses were:

1. There is no difference in correlation in different treatments nor at different distances.
2. The interaction between distance and treatment has no effect over the correlation.

For p-value < 0.05, a null hypothesis is rejected.

We will compare three models to determine which variables, and in which combinations, have an effect over the correlation, and see whether the treatment given impacts the correlation.

The models are:

1. Additive two-way ANOVA: assumes no interaction between the distance and treatment.
2. Two-way ANOVA with interaction: assumes interaction between distance and treatment.
3. Two-way ANOVA with treatment variable: assumes interaction between distance and treatment and that time might cause a change in the correlation.

The additional null hypotheses for the last model are:

3. There is no difference in correlation at different time periods.
4. The interaction between treatment and time period has no effect over the correlation.

We compared the models using the AIC's method. The method showed that the third model is best-fitted to our data. The model results are presented below(tbl1):

tbl1	Df	Sum Sq	Mean Sq	F value	Pr(>F)
treatment	2	3.005	1.5025	475.77	<2e-16
distance	9	7.595	0.8439	267.21	<2e-16
time	2	1.367	0.6835	216.41	<2e-16
treatment:distance	18	1.338	0.0744	23.55	<2e-16
treatment:time	4	1.529	0.3821	121.00	<2e-16

With these results we can reject all 4 null hypotheses at once.

Lastly, we used the Tukey's Honestly-Significant-Difference (TukeyHSD) test to examine the correlation difference between the treatments (tbl2) and the correlation difference within the experiment start to its end(tbl3).

tbl2	diff	lwr	upr	p adj
Control-AP5	-0.0086913	-0.0207409	0.0033582	0.2081174
FGL-AP5	-0.1411841	-0.1532337	-0.1291346	0.0000000
FGL-Control	-0.1324928	-0.1445424	-0.1204432	0.0000000

The above table (tbl2) raises the following conclusions:

- There is a significant difference between the treatments.
- AP5 has no significant difference from the control group, while FGL has.

treatment	from	to	diff	lwr	upr	p adj
AP5	Before Treatment	Over Night	-0.0980532	-0.1548673	-0.0412392	0.0000038
Control	Before Treatment	Over Night	-0.0867393	-0.1435534	-0.0299253	0.0000859
FGL	Before Treatment	Over Night	-0.0141665	-0.0709805	0.0426476	0.9974680

tbl3

In addition, tbl3 indicates that by the end of the experiment:

- Both AP5 wells and the control wells correlation decreased significantly.
- FGL's wells correlation decreased insignificantly.

In comparison to the initial state of the three.

The following plots (fig.1) show the change in the correlation based on the distance of electrodes from one another at different time periods.

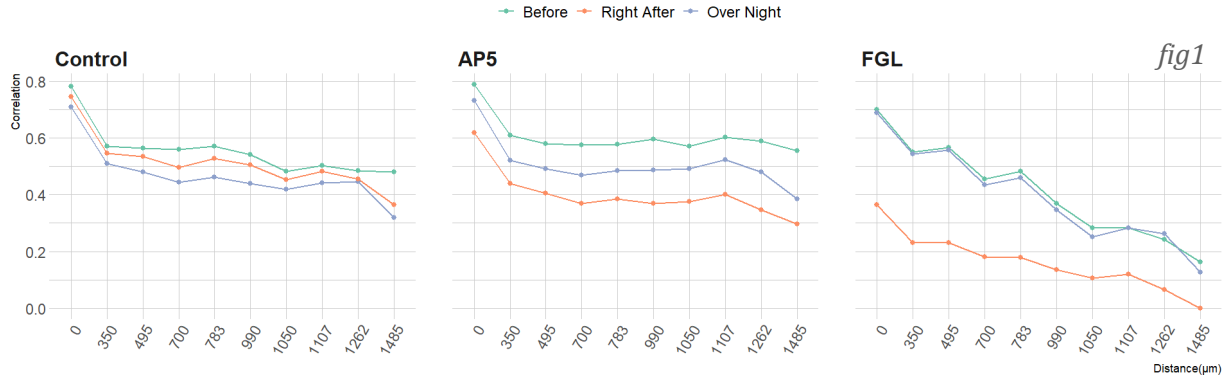

fig1

This is a clear visualization of FGL's efficiency in comparison to AP5 and the control group. The correlation in wells who received the FGL treatment decreased at the hours following the treatment, but bounced back overnight. On the other hand, the correlation in the rest of the wells for the same period are significantly lower than their initial state.

In conclusion, the FGL treatment was able to maintain the connections between the synapses. Therefore, it proved to be the more efficient memory-improving treatment of the two.

Limitations and Future Work

Prior work indicates there is more than one neuron per electrode. Finding the correlation between electrodes helps us understand the connections between the synapses. It cannot, however, teach us about the connections within the synapse itself, that may become stronger due to the treatment. Generalizing this solution beyond our sample will require a more accurate way to map the connections between neurons, so that we could further understand the treatments' impact.

With additional time and resources, first we would have consulted with prof. Knafo on how to identify a single neuron in a synapse and try to find the connections between the neurons themselves, rather than between synapses. Second, we would have tried to find and implement a more accurate and efficient method to evaluate the connections between the neurons. Finally, we would have extended the duration of the recording for more accurate results. With these additional steps, we would have been able to conclude the treatments' efficiency better, not only during the 24 hours following the treatment, but in the long-term as well.

Special thanks to prof. Shira Knafo, who kindly agreed to share the data from her research: "Understanding Neural Development with Multielectrode Array" with us.
The data was imperative to this project and proved to be invaluable during its making.