Biochemical Markers of Neuron Growth

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1 Introduction

Alison Barth is a faculty member of the Carnegie Mellon Biology Department who is interested in the electrophysiology of the plasticity among neurons found in vertebrates. In other words, she deals with synaptic properties involved in neuronal growth, or decay, at the molecular level. In our project, we will be dealing specifically with glutamate and GABBA receptors, which are vesicles at the edge of the dendrite that receive excitatory and inhibitory chemicals respectively. These chemicals are known as neurotransmitters and are used to communicate between cells. Under the currently accepted schema, there are many subtypes of these receptors and research of this nature is attempting to learn more about their physical properties. We will be analyzing the measurements of various variables recorded from the action potential of the cells. This action potential is an electrical discharge from a depolarized cell after it has received enough of a stimulus, which then leads to the release of the neurotransmitter to the next cell. The study includes numerous cells found in the neocortex of mice and the ultimate goal is to enhance our knowledge as to the structural changes that are involved in sensory deprivation.

2 Data

In order to investigate the structural properties of the synaptic connections between cells we will be working with three levels of sensory deprivation in mice: control, spared and deprived. The mice are categorized by the number of their primary sensory organs (whiskers) remaining along with which neurons were examined. More specifically, the control group had no whiskers removed and the neurons that were examined are from the area of the brain that has been directly linked to a designated whisker. The spared group had all but one whisker removed and measurements were taken from the region of the brain that is mapped to this specific whisker. The deprived group also had all but one whisker removed, but the recordings are from neurons that are mapped to a whisker that has been removed. It must be noted that there is a period of time for the mouse to experience outside stimuli after the whiskers were removed. The targeted brain tissue is then harvested in a manner that allows it to survive for many hours after the animal is terminated. Therefore, for all intents and purposes, the brain is

still alive and functioning while these impulses are being received and so these synaptic connections are essentially forming in vivo.

The data for this project was given to us in the form of two Excel spreadsheets, one for excitatory post-synaptic currents (EPSC) and one for inhibitory post-synaptic currents (IPSC). This data has been recorded from a machine that registers the minute electrical impulses that occur when neurons communicate. Our main variables of interest are amplitude, rise time, decay time and frequency. Rise time is defined as the amount of time (ms) that the impulse takes from its onset to reach its action potential. The decay time is the amount of time (ms) that the impulse takes to reset back from its peak to its resting voltage. Amplitude and frequency are defined just as they would be in any other scientific setting. Also, these synaptic events were recorded from 10-20 cells per sensory deprivation level, each cell ranging from 100 to 1500 events.

3 Questions

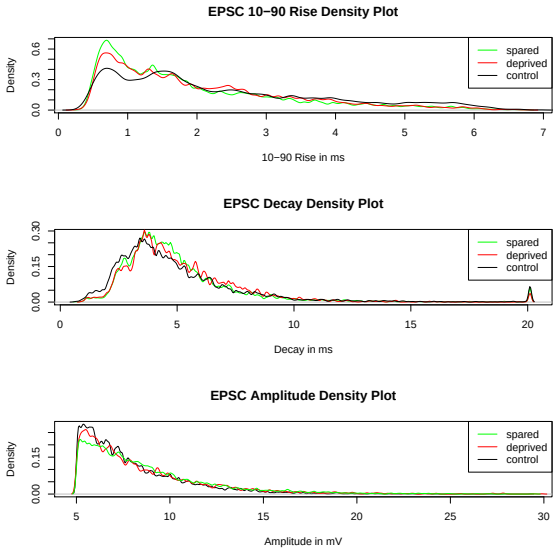

Figure 1: Kernel Density Estimation for EPSC

Our ultimate goal for this project is to use post-synaptic kinetics to get a better understanding of the neuronal connection process. Alison hypoth-

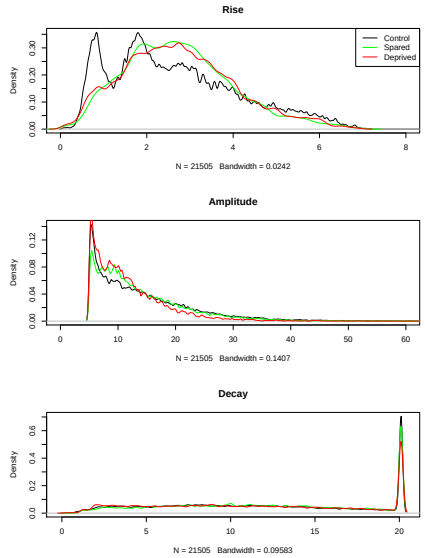

Figure 2: Kernel Density Estimation for IPSC

esizes that the three levels of sensory depravation should exhibit differences in kinetics. She feels that neurons could be divided into two distinct groups that are based on their rise times: fast and slow. Based on the graphs shown above, our exploratory data analysis confirmed Alison's intuition to an extent. It is interesting to note that the control group was the only one to display such characteristics for the IPSC's, but each of the treatment levels displayed multiple modes for the EPSC's. Further analysis must be done to determine the cause of such a phenomenon. We are currently developing an EM algorithm that will attempt to identify different populations within the groups of neurons, each sharing similar rise times. Once identified, we would then use a mixture model to a fit distribution to each group. If this approach proves to be a successful, then we can use this classification model to study the behavior of the other variables in relation to the rise time. In other words, we may be able to use the rise time as a proximal indicator to the voltmeter, which would then allow us to better understand the true effect of amplitude. The amplitude of a post-synaptic current shrinks as it travels through the dendrites and because of this, witnessing a small amplitude may not be a function of the synaptic structure, but merely a result of the distance that the impulse had to travel.

Apart from rise time, we are currently working with Professor Barth to try to build a scientific model for the other three aforementioned variables. In order to do this our first priority is to determine if there are significant differences between the three levels. If they do exist we can use this information to further develop hypotheses regarding the synaptic structure. For instance one hypothesis is that amplitude can be regarded as an indication of the strength of connectivity between two cells.

4 Results

In order to test whether each of the variables was significantly different at the three treatment levels we employed multiple ANOVA tests. Because we are testing multiple variables, it is reasonable to use a Bonferonni correction in order to curtail any possible inflated Type I error. Thus, for an overall $\alpha = .05$ level test, we should only reject the F- test when our p-values are less than $\alpha_i \approx .02$ for each of the three dependent variables.

Level	Rise	Decay	Amplitude
Control	(2.21,2.51)	(4.74,5.40)	(7.52,8.36)
Deprived	(1.74,1.96)	(4.52,5.19)	(8.15,9.19)
Spared	(1.91,2.17)	(4.71,5.60)	(7.81,9.03)
p-value	.000	.482	.085

Table 1: blocks include 95% confidence intervals for the mean of EPSC using t distribution

Level	Rise	Decay	Amplitude
Control	(2.59,2.77)	(11.22,12.70)	(13.03,14.62)
Deprived	(2.69,3.00)	(10.40,12.94)	(10.76,12.05)
Spared	(2.62,2.90)	(10.76,12.45)	(11.34,15.07)
p-value	.130	.829	.001

Table 2: blocks include 95% confidence intervals for the mean of IPSC using t distribution

Therefore, based on the ANOVA, we can conclude that at least one of the three levels on rise time is significantly different than the rest for EPSC's.

By this same reasoning, we can conclude that at least one of the treatments on amplitude is significantly different than the rest for the IPSC's. In order to verify which of the levels are different than the rest, we calculated 95% confidence intervals for each cell, as noted by the tables. As you may see, within each column of both tables there is overlap between the confidence intervals, except for two places. In the EPSC table, there is no overlap between the deprived rise times and the other two rise times. Also, there is no overlap between the confidence intervals for deprived amplitude heights among the IPSC's and the other amplitudes. The combination of the ANOVA and confidence intervals seem to suggest that these two are significantly different than the rest of the levels on their respective variables.

5 Conclusions/Discussion

Our analysis of the kinetic post-synaptic data has been aimed at determining the significance of the differences between the different levels of sensory deprivation. Our progress, however, has been slowed due to difficulties with the data. We did not have access to the frequency data until recently. Thus its analysis will begin upon the completion of this report. Another tripping point is the decay time data for IPSC cells. Observing its kernel density plot, one can see a very significant difference from the other plots. The problem is that only times up to 20 (mS) were recorded, anything longer was truncated down to 20. While this phenomenon can be seen in other plots, between 70 and 75 percent of the IPSC decay time data is beyond the cut off, rendering any analysis using this data unreliable. The lab technician who collected the data is currently recalibrating the machine in order to reprocess the raw spike train data with a proper cut off point.

Looking to the future we still have many questions to answer. In addition to having our data problems solved, we need to analyze an additional factor variable, fosGFP. fosGFP is a green flourescent protein that binds to very active neurons. We are currently unclear as to whether the fosGFP cells are chemically special, or if they are neurons that are of special significance to processing whisker input. The status of our cells as either fosGFP or not fosGFP was indicated, however it was only recently stressed as being important by Professor Barth. Thus, we are unsure as to the effect they may be having when mixed in with the other "non-green" cells.

Another future endeavor of our project is to attempt to classify different

proteins that are attached to the glutamate receptors. Each receptor has two pairs of either the homomers: GluR1,GluR2,GluR3,GluR4 and/or any combination of two of them, which is known as a heteromer. Of particular interest to Alison is the differences between GluR1,GluR2 and the combination of GluR1/GluR2. She is especially interested in these specific combinations because of their sensitivity to the very kinetic properties that we are analyzing. Appendix B has an image of the kinetic properties of a synaptic event for each of the three combinations. One can easily verify that GluR1 has a very fast rise and decay time, GluR1/GluR2 has a fast rise time, but a slow decay time and GluR2 alone has a slow rise time and a slow decay time. It is also very interesting that the average amplitude for GluR2 homomers had to be rescaled to fit on the same plot as the other two.

The big picture Alison's lab is tackling is understanding plasticity in neurons. Plasticity is the strengthening of a neurons synaptic connections. This process is often termed learning in the neuroscience community as the neuron in question has improved the connection between itself and another neuron through the action of communicating with it. A start down this path can be made by quantifying the differences in kinetics between the three levels of deprivation as it forces cells in both the spared and deprived group to under go a change in input. The spared group, with its new found importance, is expected to under go plasticity. The deprived group, however, witnesses a decrease in importance, whether this is the reverse of plasticity is currently under investigation. By the end of the semester we expect to be able to reliable classify a cell by its kinetics as coming from one of the three groups, and with that knowledge begin to explain why the changes in the observed kinetics occur.

6 Appendix A

7 Appendix B

References

[1] Derkach, Victor and Oh, Michael, *Dominant role of the GluR2 subunit in regulation of AMPA receptors by CaMKII*, Brief Communications, Nature Neuroscience Vol. 8 No. 7, 29 May 2005, Supplementary Figure 3.

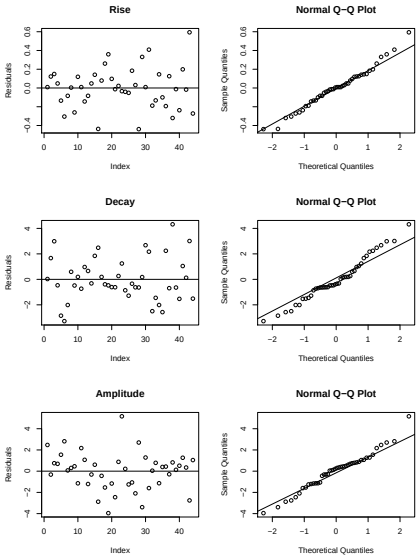

Figure 3: Residual Analysis for IPSC

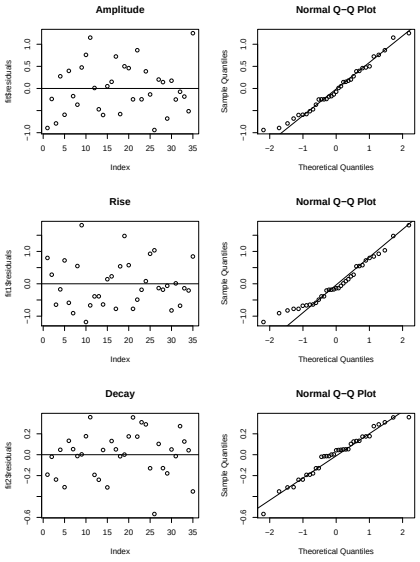

Figure 4: Residual Analysis for EPSC

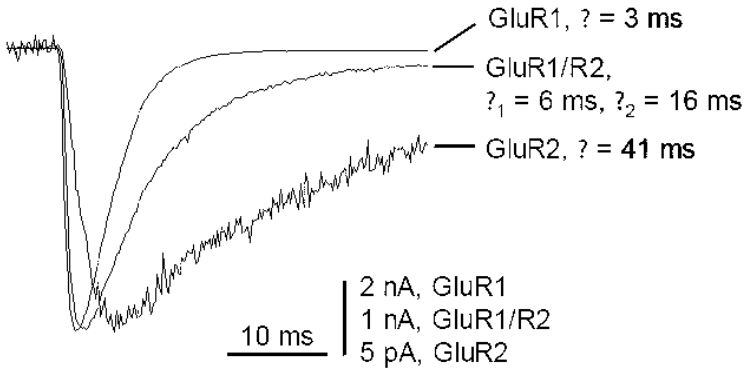

Figure 5: GluR1 and GluR2 homomers were two to three fold of magnitude smaller than the currents from GluR1/GluR2 heteromers, indicating a negligible contribution from GluR2 homomers