Uncovering New Knowledge About Nerve Growth Using Clustering and Thresholding: Distinguishing Responders From Nonresponders

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Introduction
At a spinal cord injury site, the axons in the area are often severed. This disrupts the communication between the neurons in the brain and motor neurons. The axons do not grow back by themselves, so treatments are being developed to overcome the environmental and intrinsic inhibitions of growth the neurons face. In these experiments we are working with cortical neuron cells in vitro, so the treatments focus on activating the neurons’ growth program, which is an issue concerning the intrinsic state of the neuron.

T Treating Severed Nerve

Transcription Factors
One of the treatment methods currently being researched is using viruses to transfet cells with different transcription factors, which alter gene expression. We look at many transcription factors, as well as combinations of them, and see how each treatment affects neurite growth in vitro.

Effective vs Ineffective Treatments
Effective treatments (e.g., image on right) alter neurite length, neurite branching and neurite numbers. Ineffective treatments (e.g., image on left) show little growth.

Previous Analysis
In the past, the analysis of data collected from these experiments has involved only a few of the many (around 30) features collected. This presents us with information that certain treatments do a better job than others at increasing the average values for some of the important cell features, but not much beyond that.

Data Collection / Normalization
Each experimental plate includes the different treatments that are being analyzed, as well as a control group, and a no virus group. The no virus group is used to filter out non-transfected cells in the other treatment groups. Then, we normalize each remaining point to the median feature values of the control group, where the new values are each point's median absolute deviation from the control, otherwise known as robust z-score.

Clustering Attempts
The goal with clustering was to see if any of the clusters are dominated by certain treatments, or at the very least, if the control group would be easily separated from the rest of the more effective treatments. Each of the clusters had a fairly even distribution of each treatment in it, even though it seemed to be doing a good job of placing similar phenotypes together. We wondered if using too many features in our clustering was an issue, so we reduce the dimensions of each point with principal component analysis. However, this did not yield better clustering results than the complete data.

Clustering Separated Cells by Phenotype

Conclusion
The response of rat cortical neurons to different treatments was found to be more complicated than a simple shift in the average neurite growth. The results show that different treatments will vary the percentage of cells that respond to the treatment once transfected. We can also see that there is a correlation between the percentage of responders in each group, and the average values for neurite growth for all transflect cells in a group. It is more than likely that the average values are a result of the percentage of responders in a given group.

Summary
The goal of this project was to perform a more in-depth analysis of data involving transcription factors’ effects on neurite growth in vitro. After failing to observe distinct phenotypes of cells in each treatment group, we were able to differentiate responders and nonresponders in each treatment group, and noted a correlation between the percentage of responders in each group and the values for average neurite growth per group gathered in previous analysis.

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