Hello, my name is Carolyn Oh, I am currently in my third year at the University of Illinois at Urbana Champaign, and today I'll be talking about my work in Dr Jonathan Sweedler’s research group on the optimization of high throughput capillary electrophoresis-mass spectrometry analysis for mammalian single cells.

So, to begin with a breakdown of my title single cell analysis is a main focus in the group, and this is due to the fact that there is chemical heterogeneity in single cells, and this is vital for studying cell to cell signaling in neurons specifically for behavior and learning. The neurotransmitters that are involved in the cell to cell signaling have been linked to various behaviors and functions. In the group, a well-studied organism is *Aplysia californica* which is a species of sea slug, and this is due because of its very simple nervous system, as well as large neurons. And this allows for ease of single cell isolation. So to the right here in the upper right hand corner is an image of *Aplysia californica,* and below it is an illustration of its nervous system network.

So capillary electrophoresis-mass spectrometry, otherwise known as CE-MS. This is a method that I use for my for my research and it is very advantageous due to the fact that it has the ability to identify unknown compounds. It requires a small volume of samples. And it has a tolerance to salts, has a low limit of detection and limit of quantification, as well as high sensitivity. So how does it work? So, as you can see on the right hand side of the slide. There's a diagram of the CE-MS setup and analytes are separated in the capillary, the separation capillary based on their mass to charge ratio, and they migrate through the background electrolyte solution. Under the presence of an applied electric field that is from the high voltage power supply. And again the separation of analytes is based on their charge, size and shape. And in the bottom right hand corner of the slide, it is an image of our laboratory built manual CE system. However, one con of the manual CE system is that it does require a long time to set up, and to perform analyses. And this ranges anywhere from 40 minutes to over an hour.

So over the summer, a new device was introduced to the group that allows for high throughput CE-MS, and it's a microfluidic device that I will refer to as, and is called ZipChip. And this analyzes samples via CE. And again, it performs rapid separations and is capable of running standards, and samples in a few minutes. And this is a greater than or equal to, tenfold increase in analysis rate. So a run that would take 40 minutes for instance, now takes four minutes or even less than that. And lastly, low sample volume are required per analysis, meaning that if there's a sample where a very small volume is available, the ZipChip is capable of running this. So on the right hand side of the slide. The upper right hand corner shows the – shows what the ZipChip looks like and below it is the electrospray, which, which allows for the analytes to enter the inlet of the mass spectrometer as indicated on the right side of the photo.

So, the outline of my research project. My goal is to develop a protocol step for the analysis of single rat cerebellar neurons. This is an expansion of a method that was previously developed for *Aplysia californica* neurons that I will be expanding to mammalian single cells and again specifically rat cerebral neurons. Mammalian neurons are ten to a thousand times smaller in volume compared to those of *Aplysia* *californica*. However, there are salts that are present in the cells that lead to poor detection sensitivity, as well as a lower intensity of metabolites. I will be optimizing the sample preparation specifically a crucial desalting step by testing different solutions to determine which yields the most optimal results. And in this case I'll be looking for both a high intensity, as well as a high signal to noise ratio, meaning that there's low background noise and clear indications, and peaks that elute during the analysis run which I will explain in my later slides. On the right hand side of the slide is the workflow. So the first step is the collection of the neurons themselves. This moves on to the next step is then adding desalting solution to the cell samples. These are then placed into the centrifuge to collect all the sample at the bottom of the tube. And these tubes are then placed into a device called the SpeedVac which dries down the samples and evaporates off the desalting solution. And prior to analysis, the samples are reconstituted in the sample buffer.

Here is an overview of my experiment, so I have five metabolites of interest: lysine histidine, arginine, tyramine, and the neurotransmitter dopamine. I also have three controls. This being my desalting solutions, the sample buffer, and diluent, which is required in order to utilize the ZipChip. I also have combined standards at five varying concentrations at 5, 50, 100, 50, and 1,000 nanomolar. And these contain all my metabolites of interest, as well as an internal standard of quinine, which is used to normalize the data to. This is not found in any biological samples which is why it is used as the internal standard. I have a cluster of rat cerebellar cells, and these are stabilized with glycerol at minus 80 degree Celsius, and they come from three different rats, so three biological samples in total. And I will be running my standards first and then moving on to biological samples next.

So this is the workflow of sample preparation. I have six different desalting solutions, one through three being varying ratios of isopropyl alcohol to acetonitrile, or IPA to ACN, and four through six are ratios of ACN to water. In order to determine the effects of these desalting solutions, I spiked in a concentration of two millimolar NaCl to, to the standards, and for each desalting solution I prepared one aliquot of the standard. I tested a single concentration of the combined standard that I prepared and I specifically chose the 500 nanomolar concentration, as this is a mid range concentration that allows me to see the effects of the desalting solutions at both the higher end concentration of 1,000 nanomolar as well as the lower end concentrations like 10 nanomolar for instance. And these were run in triplicate for - in order to have reproducibility in the data.

So here is an example of an electropherogram of the data of one run of a 500 nanomolar standard treated with desalting solution two, which is 80% concentration IPA to ACN. And as you can see, the overall runtime is less than two minutes, which is very rapid – rapid separation. And you can see in the electropherogram here that all the peaks of interest, metabolites of interest, eluted and peaks were present, as well as quinine the internal standard. On this slide is the, displays the variations in intensity and signal to noise ratio, based on the desalting solutions used. Here on the bottom left is a reminder on what the composition of the desalting solutions were. And based on these results, it was clear that desalting solution one or 75% IPA to ACN was most optimal as this yielded the highest, both the highest intensity as well as signal to noise ratio and again this indicate - these data were plotted based on using the normalized intensity and signal to noise ratio.

So, overall, to conclude, the use of CE-MS was demonstrated through the ability to detect the amino acids of interest, as well as the neurotransmitter dopamine, and the desalting solution composed of 75% is isopropyl alcohol to acetonitrile, yielded the highest intensity, as well as signal to noise ratio. Therefore, this is the desalting solution that I will be applying to rat cerebellar neuron samples.

My future work includes applying this protocol to neurons of different regions of the rat brain, quantifying metabolites from the single rat neurons to determine the limit of detection and limit of quantification utilizing the ZipChip-MS system, as well as determining the minimum sample volume required to run a sample on the ZipChip itself.

Finally, last but not least acknowledgments, I would like to thank Professor Jonathan Sweedler for his support and allowing me to work in his group. Shannon Murphy, a graduate student I have been working with for the past two years now, and the Sweedler group in all. Last but not least, the ECI division of the ACS. Thank you for listening, and I am excited to read any questions or comments you may have for me. Thank you!