Experiment 1:

The goal of this experiment was to evaluate the impact of SCN2A deficiency on neuronal activity in hCS and HStrS by patch-clamp recording. At least 10 cells would be analyzed in 4-10 organoids. The neurons would be generated from CRISP to create mutations (wild type (WT), clones (HET), homo (HOM)). 3 groups total, 4-10 clones of each of the 3 groups.

In the first experiment she will look at 10 cells in 1 group, and the organoids will be the same (client hopes the organoids will be the same). Om total there were 27 neurons from WT group, 26 from HET group with 8 organoids total. Ideally the distributions of each of the organoids should be similar. Currently she has 10 neurons x 2 groups will test them. She will do so by injecting current into the neurons to measure the firing rate (of peaks in time frame) of the organoids at the current level. If stimulation or current is low this leads to a low firing rate. If stimulation is high, then the firing will be high.

If neuron is old, healthy, and able. More stimulation will kill the neuron through the firing. Sometimes the neuron isn't mature enough and will not have the endurance to handle the firing. Client is currently clumping all the neuron data together. But she would ideally like to separate the data to understand which neurons are healthy or not.

Regarding the effect size, there is a comparison step being made with current injection and AP number with the WT (n=27) and HET (n=26) organoids. At a low current injection there is little difference, but at high current there is a higher difference. The curves of the organoids show the average for each neuron we could measure the rate of increase of neurons or average number of firings with neurons and statistically test that they are different for the effect size.

Experiment 2:

Determine overall neural activity of hCS and hStrS with SCN2A deficiency by MEA recording. Essentially it is similar to experiment 1, except this will all be done through a machine. The procedure will place the neurons in a machine and will tell if the neurons are different or not.

For this experiment there will be 16 organoids, and the MEA will test if the organoids are different through averaging the 16 measurements per organoid. The interpretation of the results is that a larger number indicates a higher firing rate.

There will still be 2 groups with 8-10 organoids per group for this experiment and t test will be used for the test.

The main question in this experiment is determining are voltage spikes, electrodes, and firing rates are different across the 2 groups.

Experiment 3:

The main goal was to examine cellular subcellular structural alteration in HCS and hStrS by immunostaining. Since it is based on images from immunostaining, the client wants to know if the marker is an axon or is longer or shorter from different genotype. This will be done through 3 measurements.

- A: Calculating length of xo initial segment.

- B&C: Analyzing pre and post snap and measuring the difference of the snap by measuring organoids and 3 areas within the organoids.1 measurement will be done at the organoid level, and 1 at neuronal level.

Other Experiments:

Since time ran out, the client could not talk in detail about the other experiments other than a brief overview, however she shared this information about the remainder of her study.

In the paper they talked about the challenges of studying behavior with mouse models, as such experiments 8 to 10 will have the most noise associated with them. Each of these experiments had 10 to 20 samples. Additionally, the assembloid experiments could also have a lot of errors as well, however they were not yet completed.

- Experiments 6-8 could also have a lot of errors, however they have not been completed yet.