MEA Acute pre-processing notes

# Things would be good to discuss with lab

* I get the experiment.date and the run\_type determination from the time in the Neural stats compiler header. However, Seline told me this clock is not always right.
  + Is the date generally reliable?
  + Would you update the clock mid-run? (i.e., can I trust the run\_type determination based on this?)

# Checklist for lab folks to do before send off data

* Make sure that the culture dates in folder names are present and correct (I know sometimes you may plan to do a culture on one day, then it gets pushed back. So please make sure that the culture dates are correct).
  + I will pull the culture dates from 8-digit numbers in the folder names
  + If there is variability in the cultures for files within a given group, communicate that.
* Make sure that raw LDH files contains the word “LDH” in file name

# Approach for this document/the run\_me

* I’m thinking more text to explain the “Why” is better than fool proof code. Because the code is going to have to change.

# Abbreviations

* NSC = Neural Statistics Compiler
* CTB = Cell Titer Blue. Same assay as Alamar Blue
* AB = Alamar Blue. Same assay as Cell Titer Blue

# Things I might need to debug

* I’m currently pulling all meta data from only the CTB and LDH columns. If I ever discover in the lab notebook that the dosing arrangement is different in the CTB/LDH from the MEA plate… would need to create some script to read from the sheet by plate
  + I imagine this could happen if there was a mix up when transferring the contents from the MEA plate to the LDH/CTB plates

# Tips

* If having errors in extractAllData, or actually in fileToLongdat, can wrap line 31 to create “Add.dat” in a tryCatch, with pulling up browser on error, so that you can determine which file is giving an issue
* Whenever you encounter an error, debug() and debugonce() are your friend!!

# Known issues

## Extrapolation of treatment labels for MEA parameters from cytotoxicity data

Currently, the scripts are set to extract the meta data for the LDH and AB data only. Then, in level 4, the meta data for the LDH and AB assays is extrapolated to the data rows for the other parameters by well.

This should work UNLESS there is a discrepancy between the actual treatment in an MEA plate and an LDH or AB plate. (This could happen if there was a mix-up when transferring media from the MEA plate to the LDH or AB plate). Thus, it would be better to get the meta data for the MEA plates from a separate source from the meta data for the LDH and AB plates.

For example, the meta data could be taken from the Calculations files, sheet “Plate X”, from the “Dosing Plate” sheet, or a sheet similar to the “Log” sheets in the NFA could be created. In any case, communicate with the lab technicians so that they know which sheets the scripts read from, and which sheets should be updated if there was a change in the treatments and/or concentrations.

## Definition of apid – particularly for AB/LDH assays

**Current definition of apid is the experiment date (not the assay plate ID). So all 3 physical MEA plates tested on the same day will have the same apid.**

**This choice was dumb..**

**This choice might be an issue for the cytotoxicity assays, where the exact amount of time that a reagent is present may vary from plate to plate and thus may be important for normalization.**

## Normalization of LDH data

* Going to document in the run\_me, how need to chat with Kathleen

# Well quality

* Note experiment.date vs culture.date in the well quality tables… not sure how to standardize, but for now, do what is best.

# Overall notes

* The “assay plate ID” is currently set to the EXPERIMENT DATE (rather than the MEA plate ID). This was done such that the bval would be calculated based on the median of all DMSO + cndx 1 & 2 wells on all 3 plates run on a given experiment date (rather than just 1 plate). (Note from Amy: I’m not sure why I made this choice, seems like 1 plate would be enough. But, there are only 3 DMSO control wells on each plate, so I would not normalize to only the DMSO controls on a single plate).

# Level 1

Read in data from neural statistics compiler files and do preliminary checks and data cleaning.

* Analysis duration
  + This should be 2400 seconds (or 40 minutes). If the recording duration is significantly different than 2400 seconds, then you may want to assess whether this recording is still usable.
    - The definition of “significantly different than 2400 seconds” is up for interpretation. Previously, I have used a coarse filter of 500 seconds above or below 2400 to flag recordings.
    - Note that some endpoints are summed across the recording (e.g. number of spikes, number of bursts), so these endpoints are definitely affected by the analysis\_duration.
* Analysis start
  + I think this corresponds to the start time of the data relative for the beginning of the .RAW recording that is used to calculate the endpoint values with the NSC.
  + This field is taken from the neural statistics compiler header. If the analysis start is significantly different than 0, check if there were any abnormalities in the amount of rest time before the recording started (there should be 20 minutes usually). If so, flag the recording for follow-up analysis to determine if the data is still usable.

# Level 2

Calculate the percent change in activity from baseline to treated recordings in each well.

# Level 3

Prepare the cytotoxicity data.

* Identify the raw data files to read

# Level 4

* Check if all compounds dissolved in DMSO, or is variable (if variable, will want to create a column that documents… but it’s kind of optional?)
  + Also may need to determine how to normalize, if can collapse controls…
* Determine dose units for all compounds (uM or ug/mL, or other?). Ultimately, need to convert all to uM