# Evaluation of Pilocarpine in Multiple Neuronal Cell Types Using Microelectrode Array

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# Pilocarpine Background

- Pilocarpine is a muscarinic cholinergic receptor agonist (M3)
- It is used to induce a temporal lobe epilepsy model in rats
  - Dosed in rats at 400mg/kg
  - Seizures in rats in 45 minutes
  - 7 to 10 day latency period
  - Rats then develop epilepsy type symptoms
- Original seizure response originates in the hippocampus

# Current rat cortical model fails to identify pilocarpine effect

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## *In Vitro* Screening for Seizure Liability Using Microelectrode Array Technology

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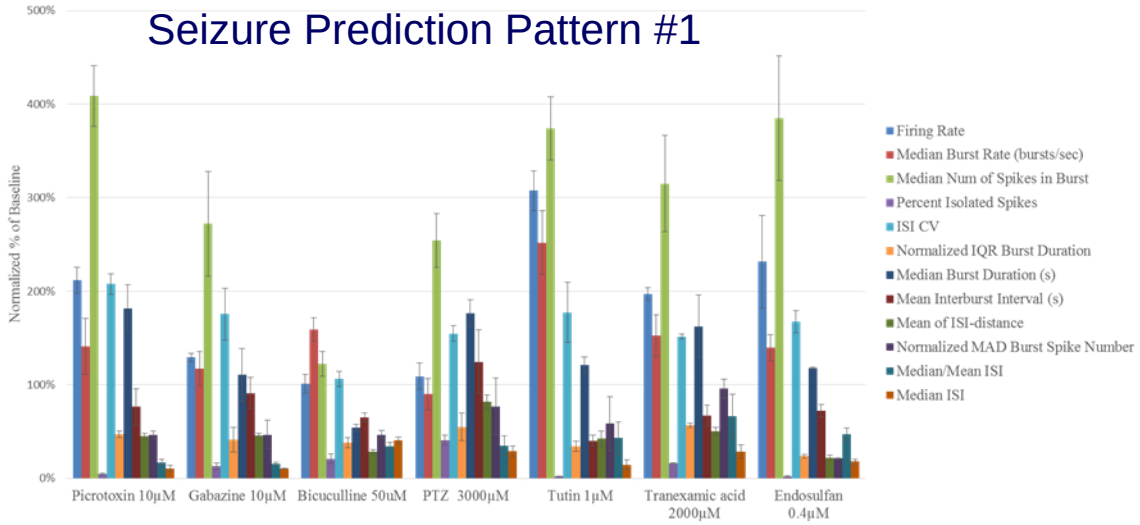
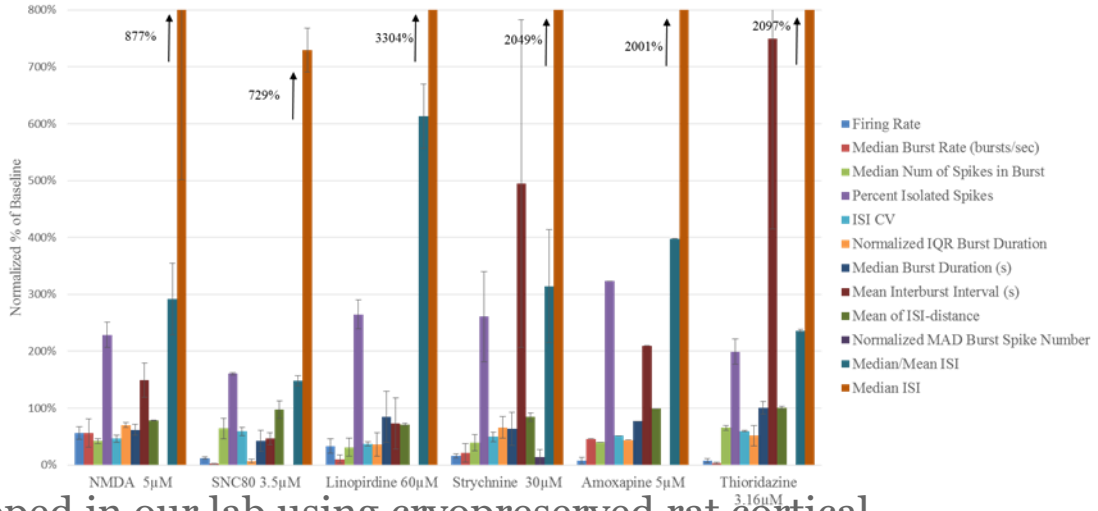
# Seizure Prediction Patterns in eCiphrNeuro

## Seizure Prediction Pattern #1




## Seizure Prediction Pattern #2




- eCiphrNeuro is a moderately high throughput assay developed in our lab using cryopreserved rat cortical neurons plated on 48-well microelectrode array plates to detect the neurotoxic and seizurogenic potential of test compounds
- After testing many compounds, we found that two distinct patterns of effect emerged that can accurately identify proconvulsant compounds (other patterns exist but are less common).
- In the course of running positive control compounds in this assay, we discovered that pilocarpine, used as a model for epilepsy in rats, did not have one of these phenotypic patterns.
- Disruptions in neural activity were observed, but these patterns did not emerge.

# Methods

- 48-well MEA plates were pre-coated with a 0.1% PEI solution and allowed to dry overnight. One hour before plating cells, the plates were treated with a laminin solution by dispensing a 10 or 15µL dot (depending on cell type) directly over the electrode grid and incubating at 37°C.
- Cryopreserved rat cortical neurons (QBM Cell Science), hippocampal neurons (QBM Cell Science) and CDI's iCell GlutaNeurons and Astrocytes were rapidly thawed and slowly diluted with the appropriate medium.
- After a gentle centrifugation step, the cells were resuspended at the appropriate density with the appropriate medium.
- The laminin droplet was aspirated and replaced with the same volume of the cell suspension at the appropriate density.
- The cells were incubated, humidified at 37°C in 5% CO2 for 2 hours before medium was added to the wells.
- Cells were maintained for 14-28 days by changing 50- 60% medium 3 times a week.
- Recordings were acquired on the Axion BioSystems' Maestro immediately before compound treatment (baseline) and 1 hour post treatment.
- Custom MATLAB scripts were used to analyze the spike trains for data generated for all of the cell types. Axion BioSystems' Neural Metric Tool was used to generate the raster plots.

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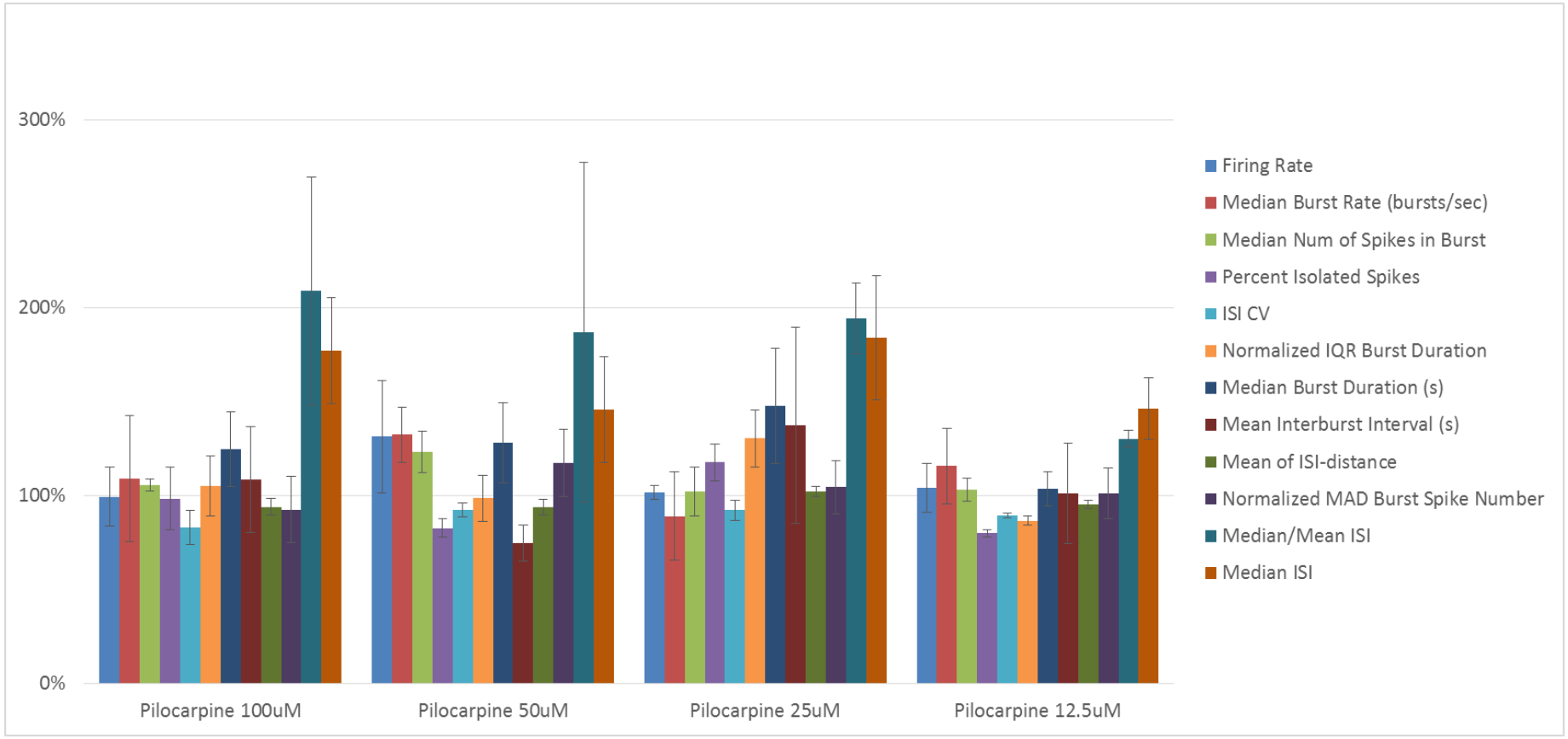
# Cryopreserved Rat Cortical Neurons

# Initial Experimental Results – Rat Cortical Neurons – 14 DIV

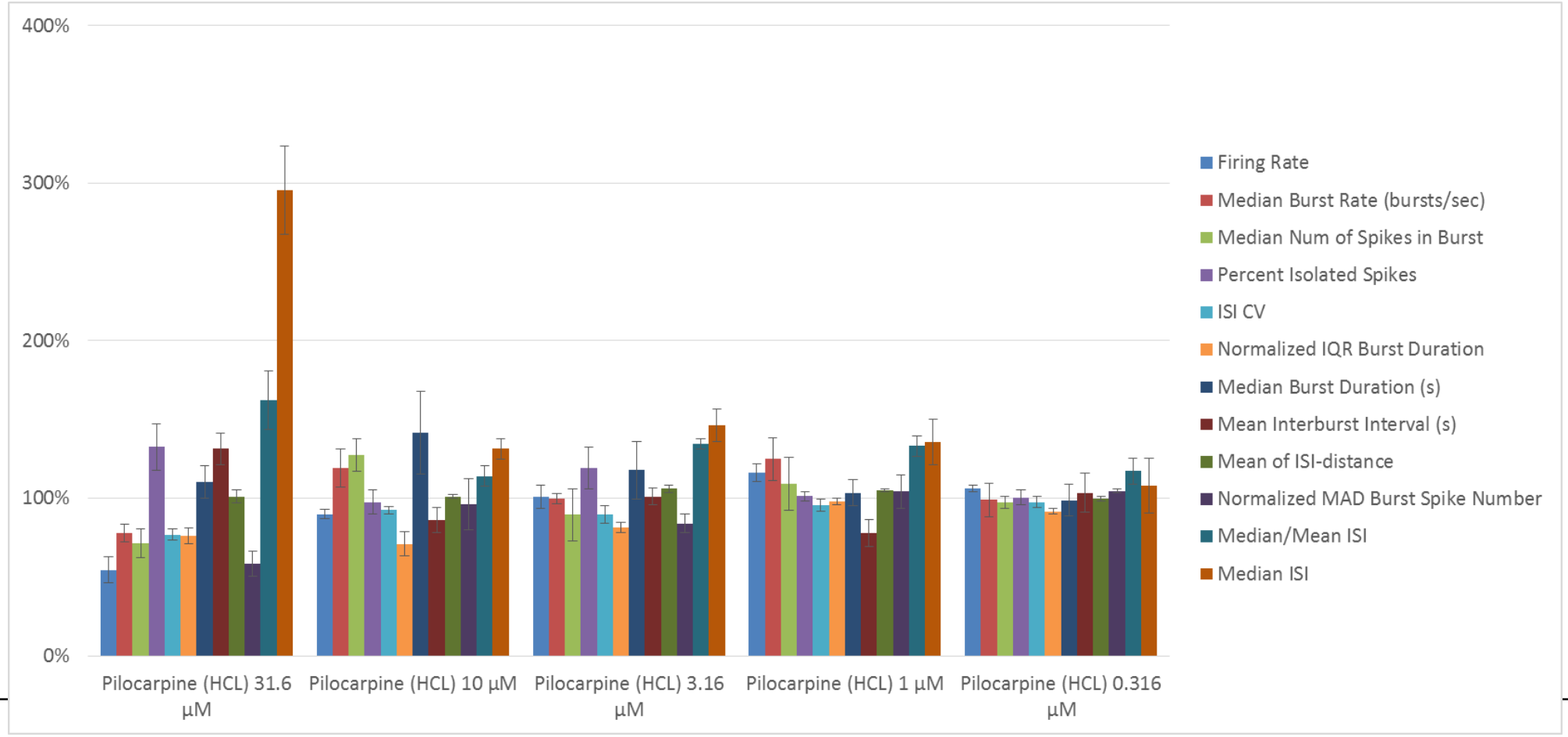
No seizurogenic or neurotoxic implications




Top conc. = 100µM
2-fold dilution

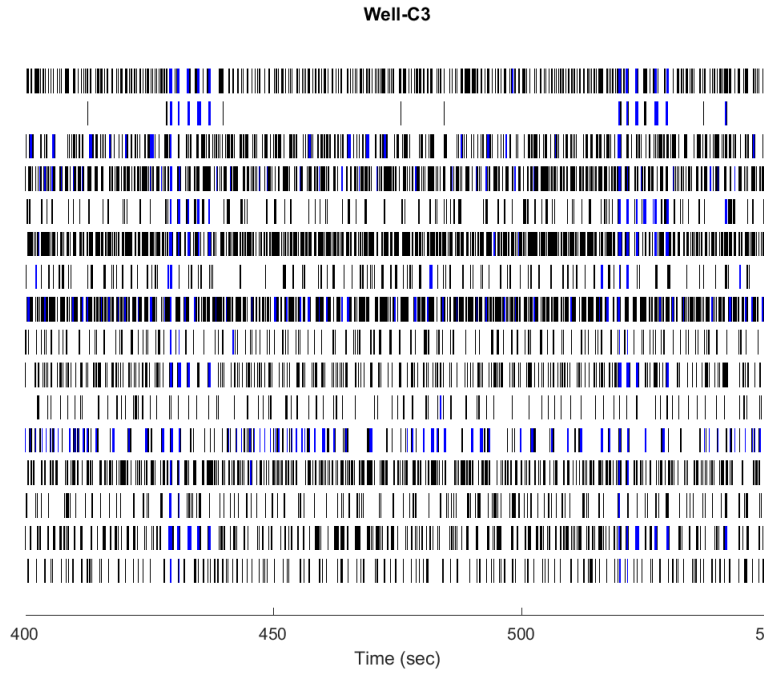
# Follow-up Testing– Rat Cortical Neurons – 14 DIV

No seizurogenic or neurotoxic implications

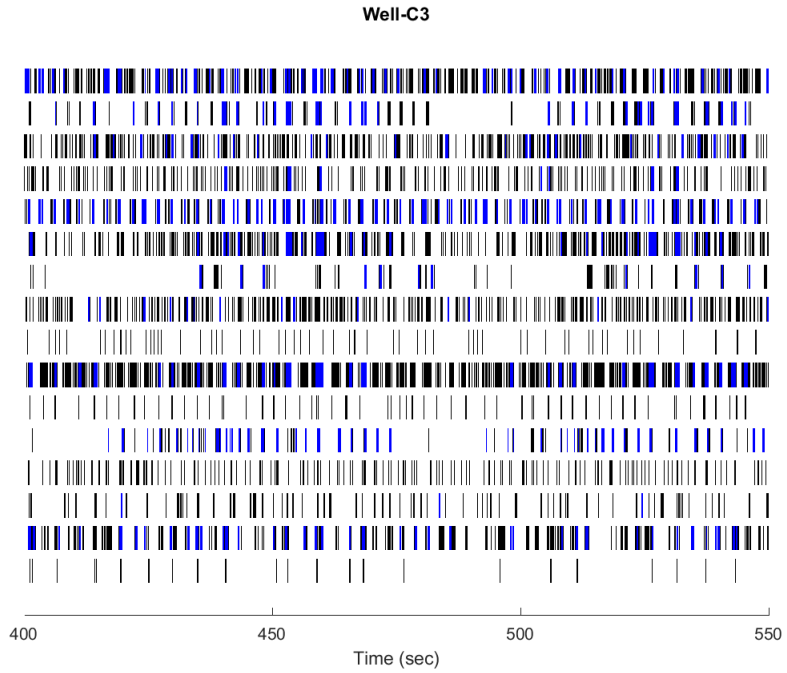



Top conc. = 31.6µM
½ log dilution

# Rat Cortical Neurons – 14 DIV

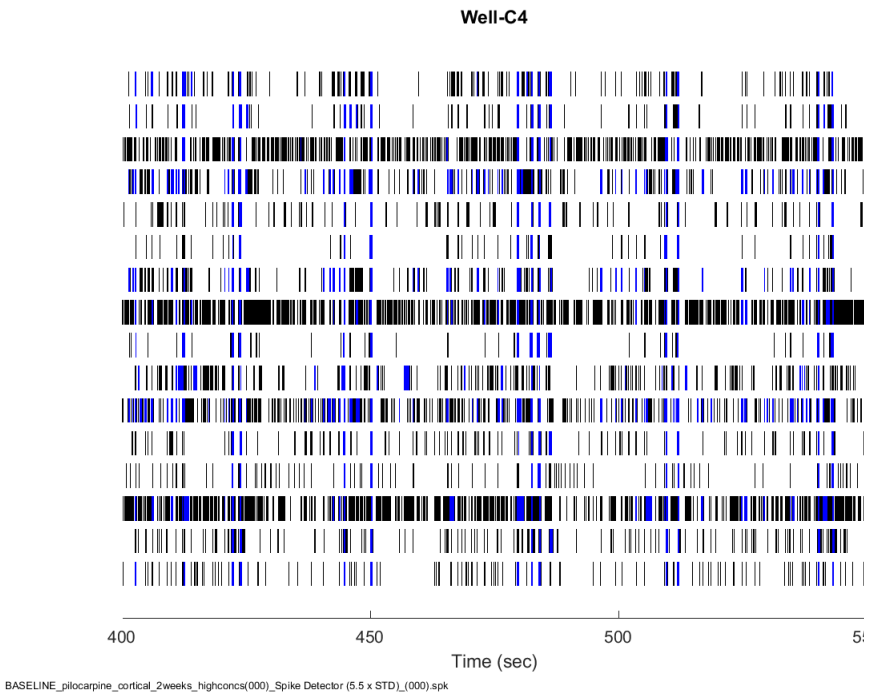



Baseline

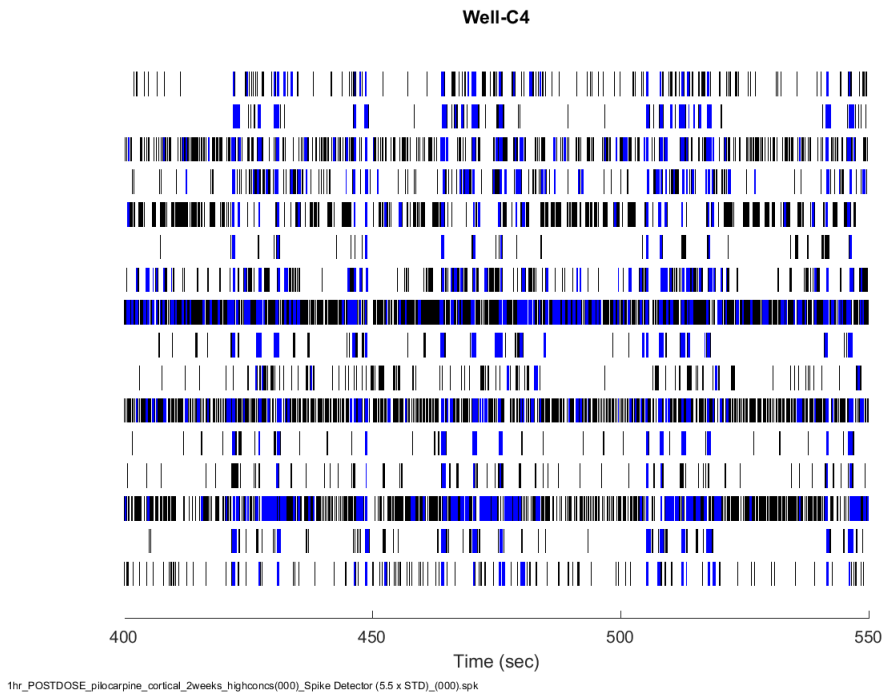



Pilocarpine 100µM

# Rat Cortical Neurons – 14 DIV




Baseline




Pilocarpine 50µM

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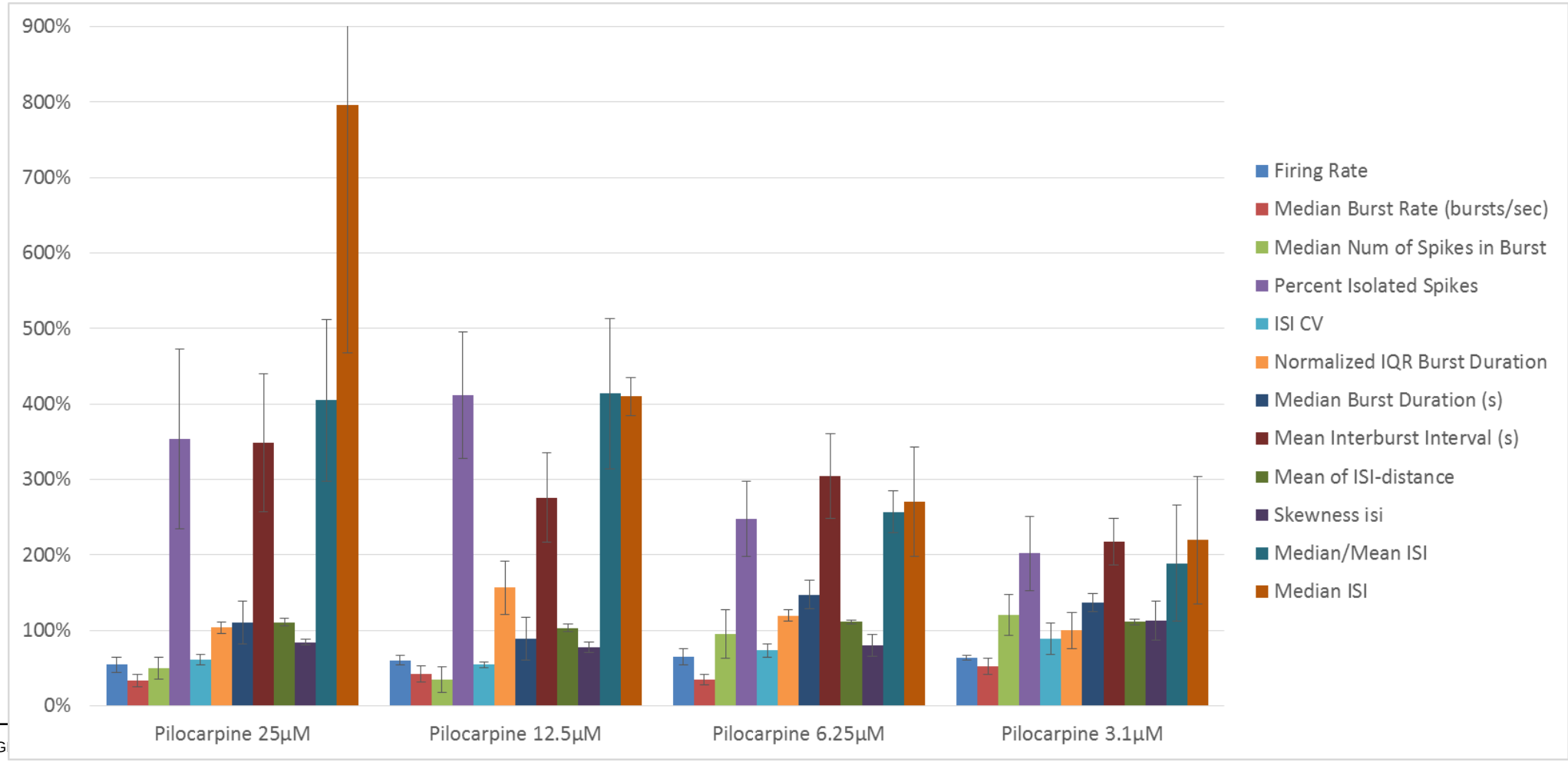
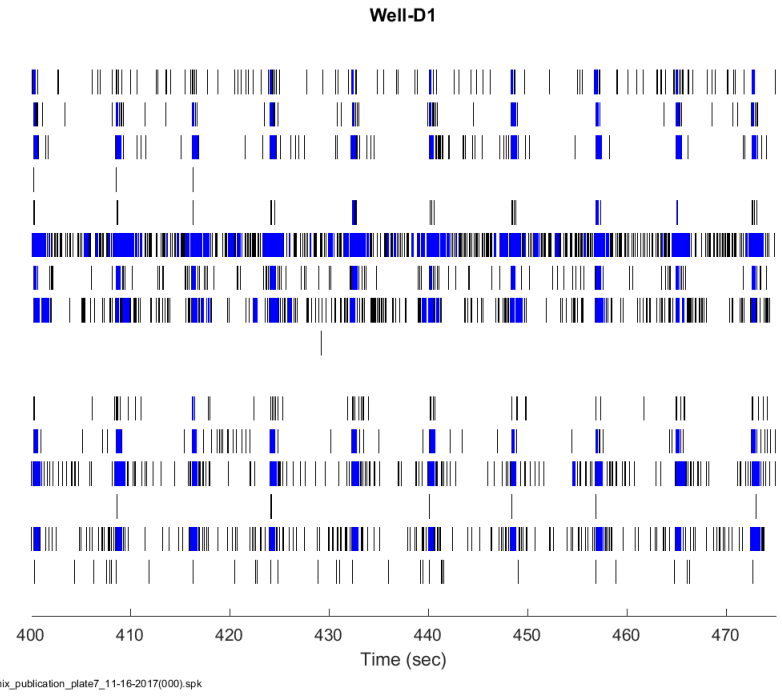
# CDI iCell GlutaNeurons and Astrocytes Co-culture

# CDI GlutaNeurons and Astrocytes Co-culture - 14 DIV




Top conc. = 25µM
2-fold dilution

# CDI GlutaNeurons and Astrocytes Co-culture - 14 DIV

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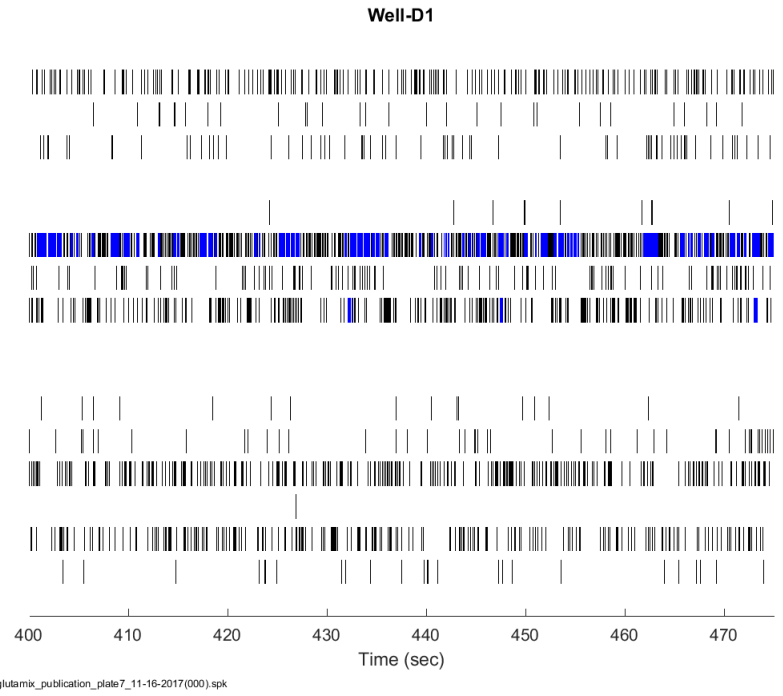
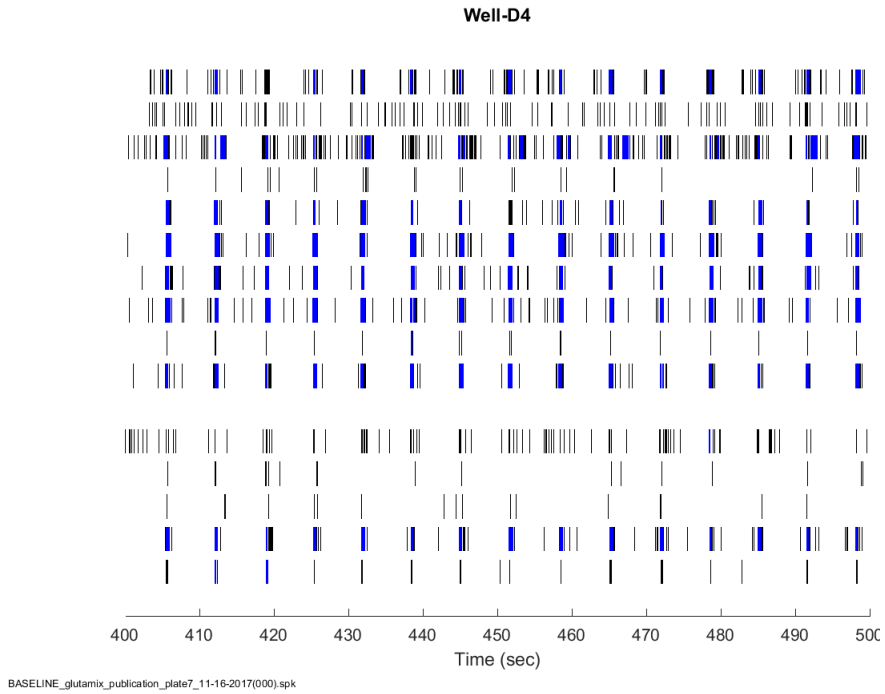
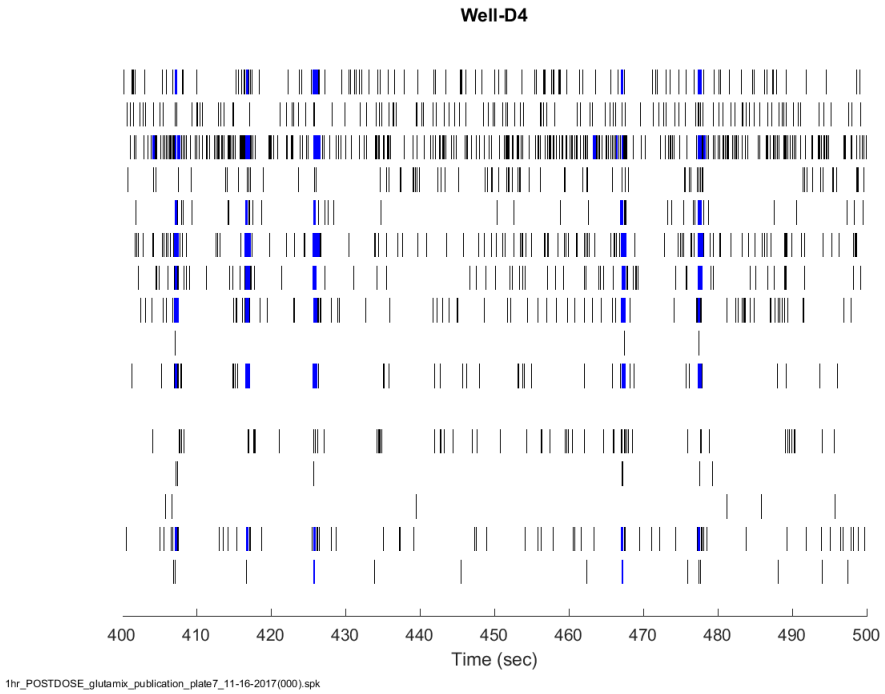



Pilocarpine 25µM

# CDI GlutaNeurons and Astrocytes Co-culture - 14 DIV

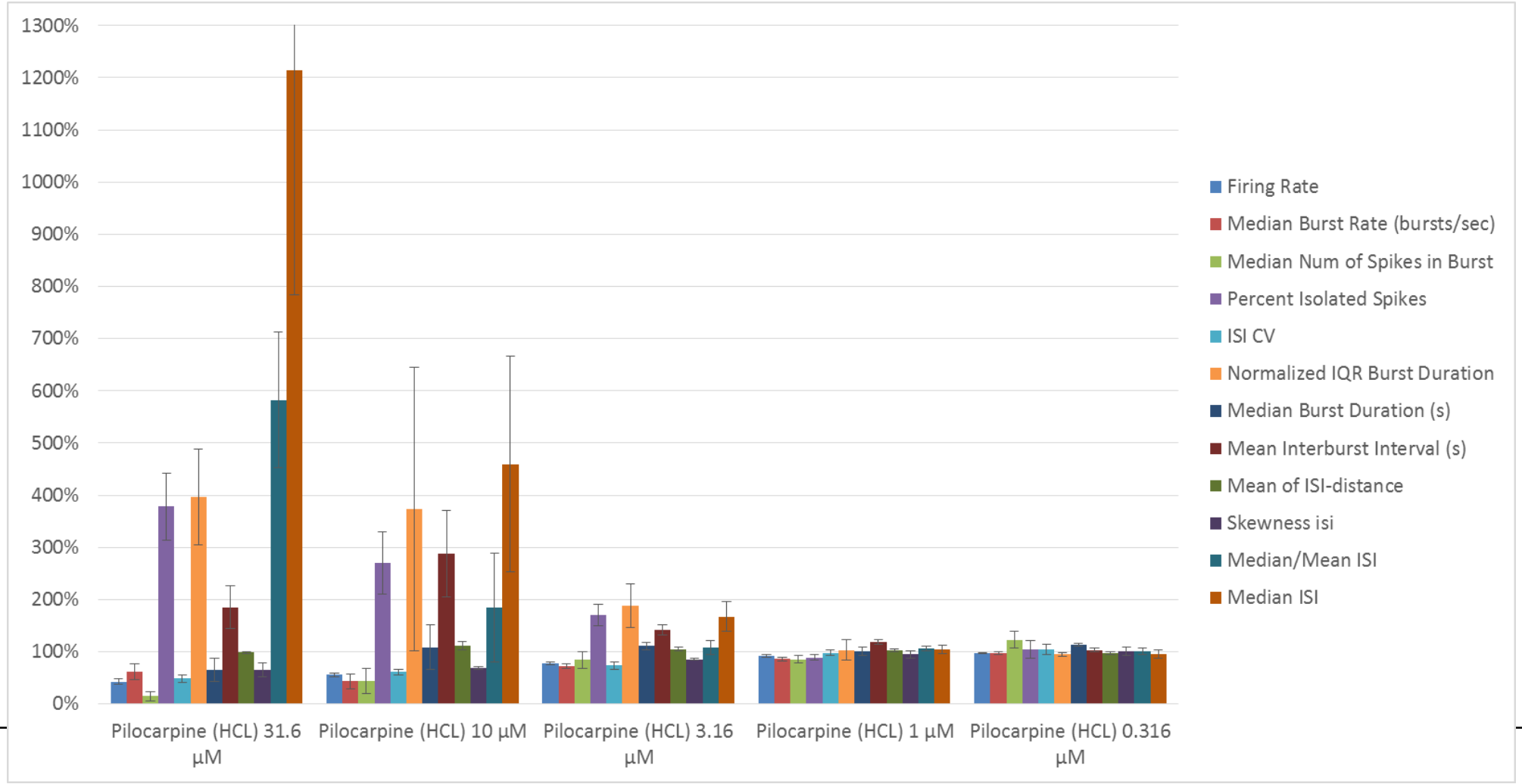



Baseline



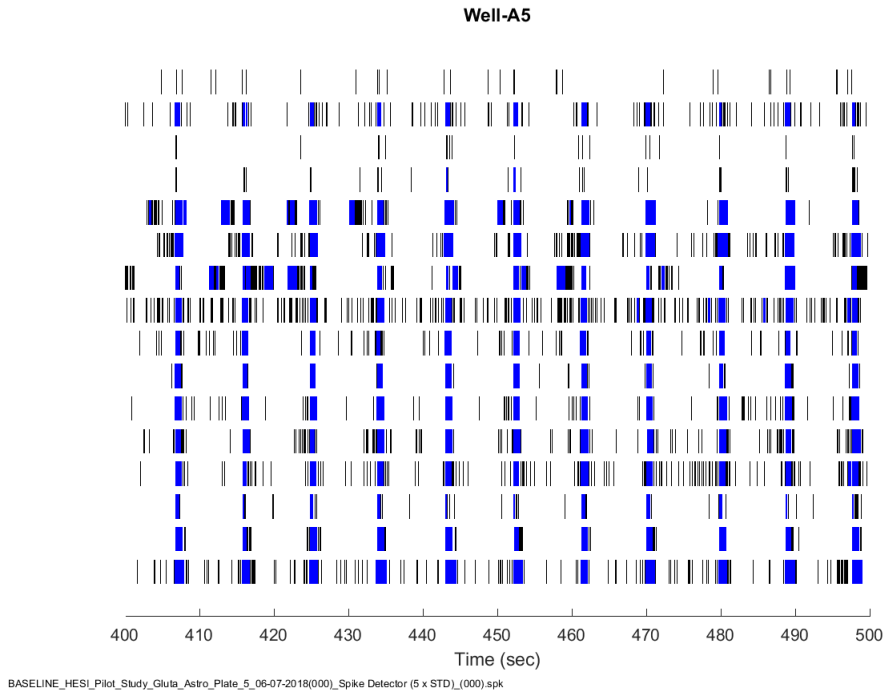

Pilocarpine 3.1µM

# Follow-up Testing– CDI GlutaNeurons and Astrocytes Co-culture - 14 DIV

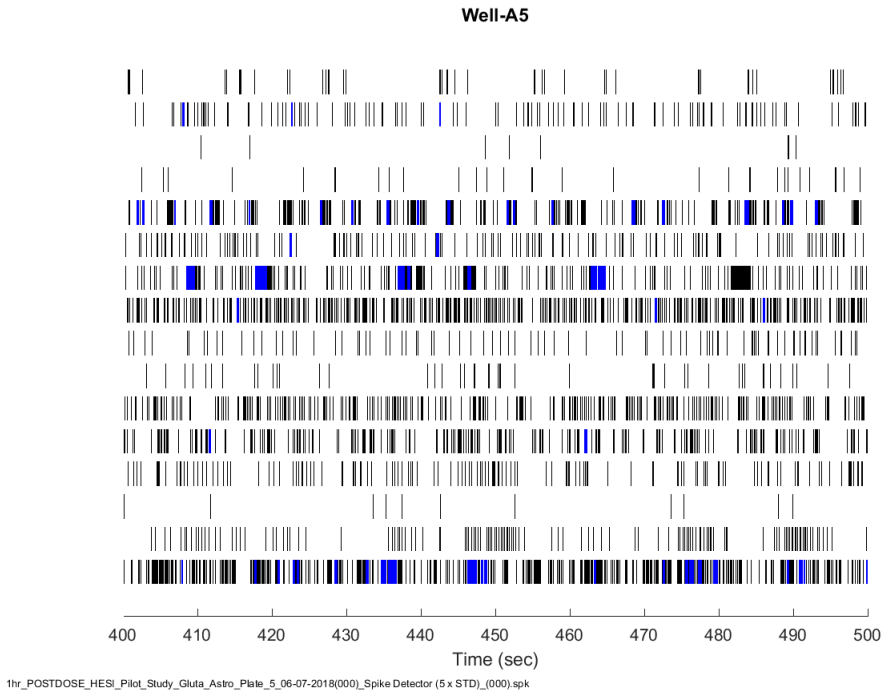



Top conc. = 31.6µM
½ log dilution

# CDI GlutaNeurons and Astrocytes Co-culture - 14 DIV

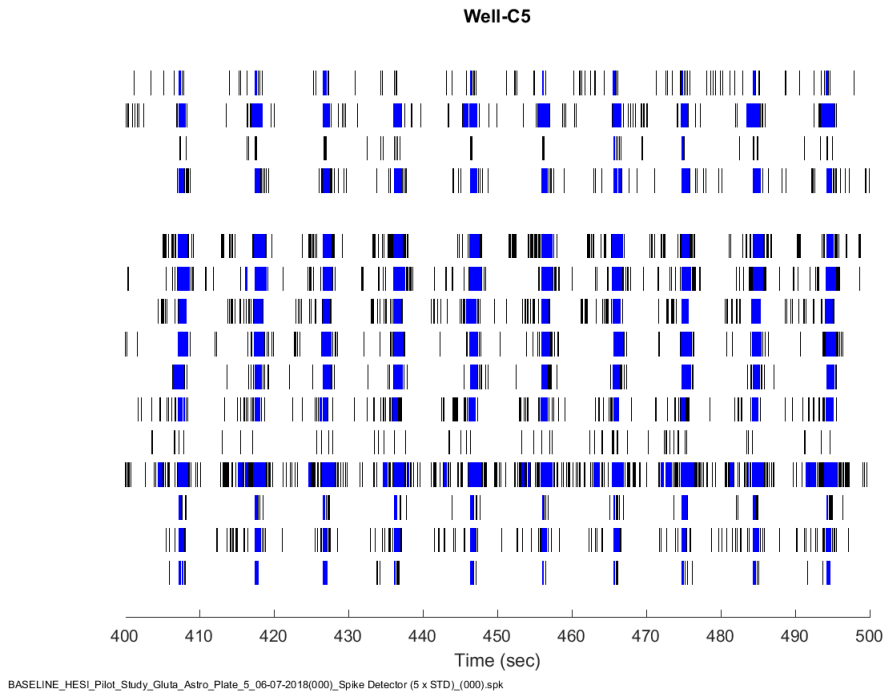



Baseline

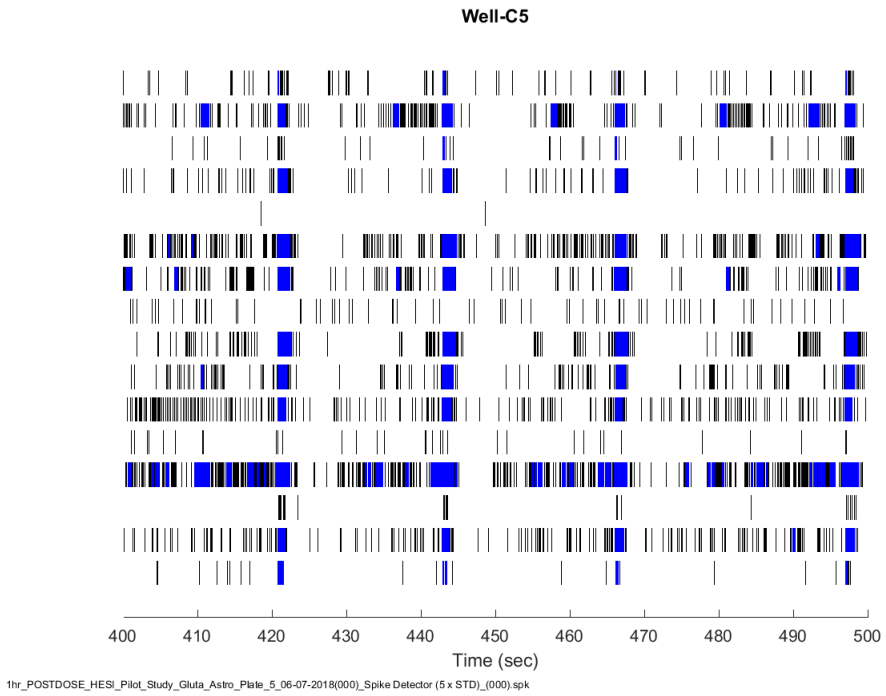



Pilocarpine 31.6µM

# CDI GlutaNeurons and Astrocytes Co-culture - 14 DIV

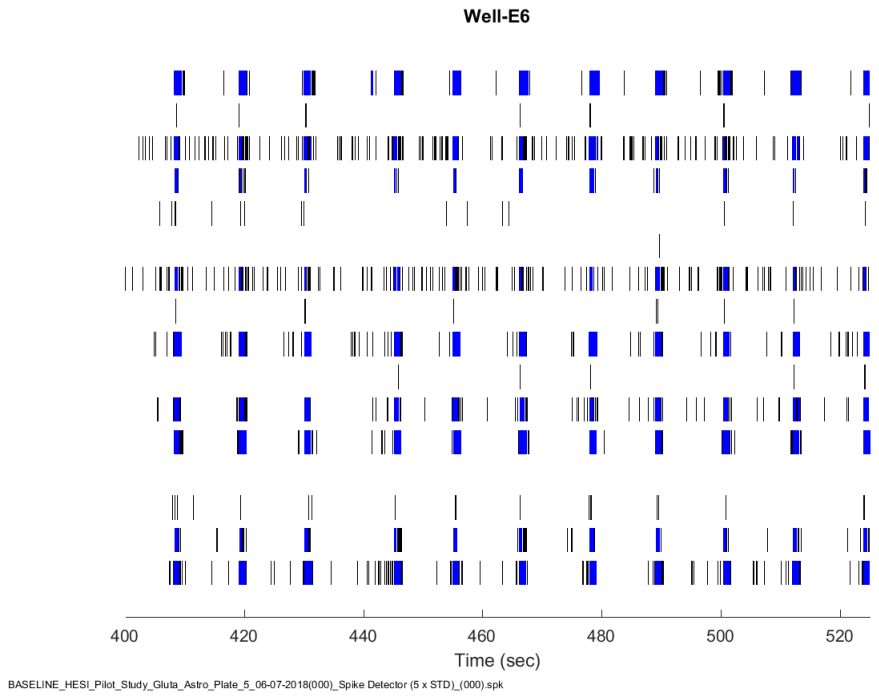



Baseline




Pilocarpine 3.16µM

# CDI GlutaNeurons and Astrocytes Co-culture - 14 DIV

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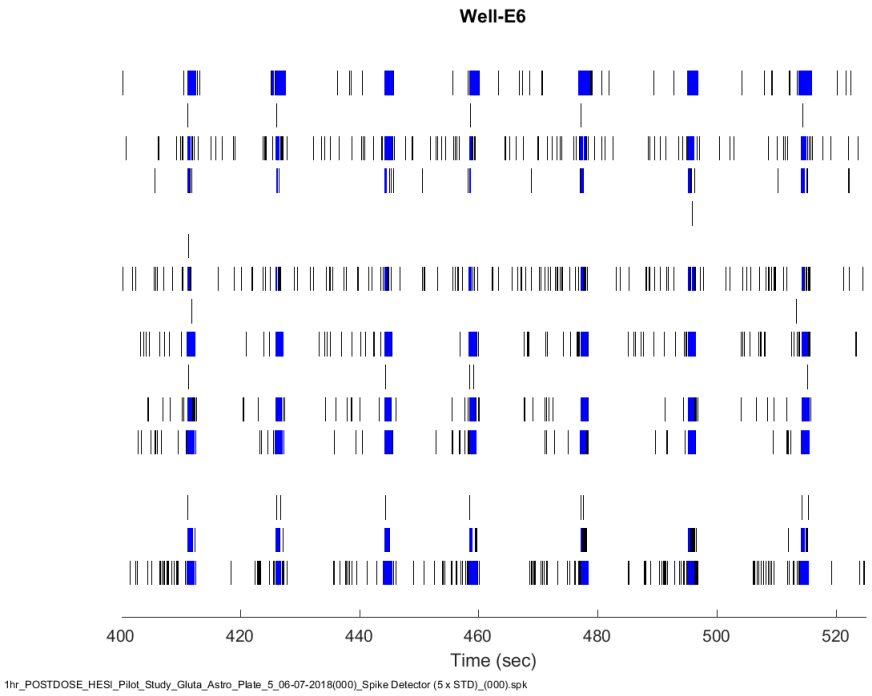


Pilocarpine 0.316µM

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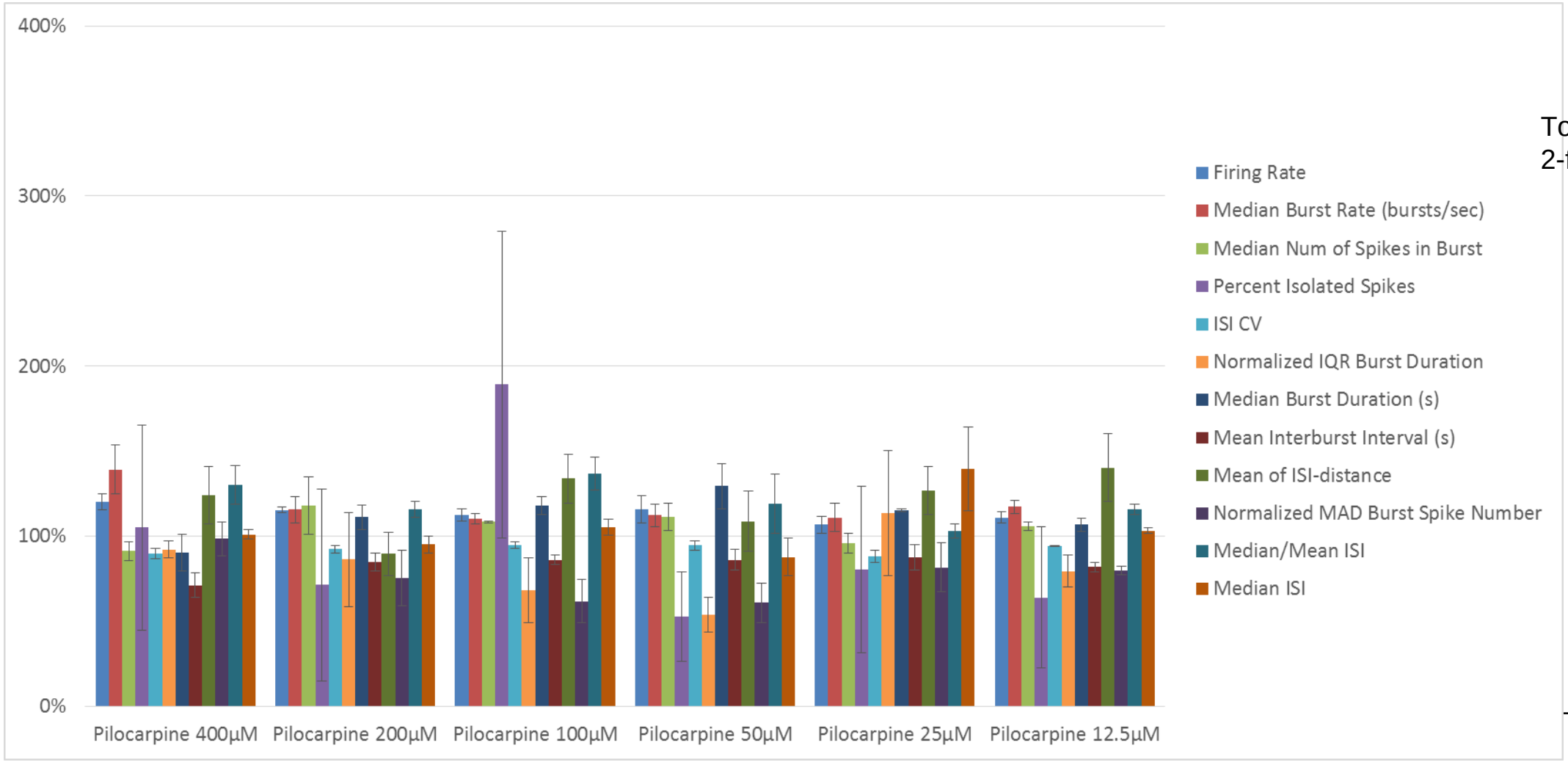
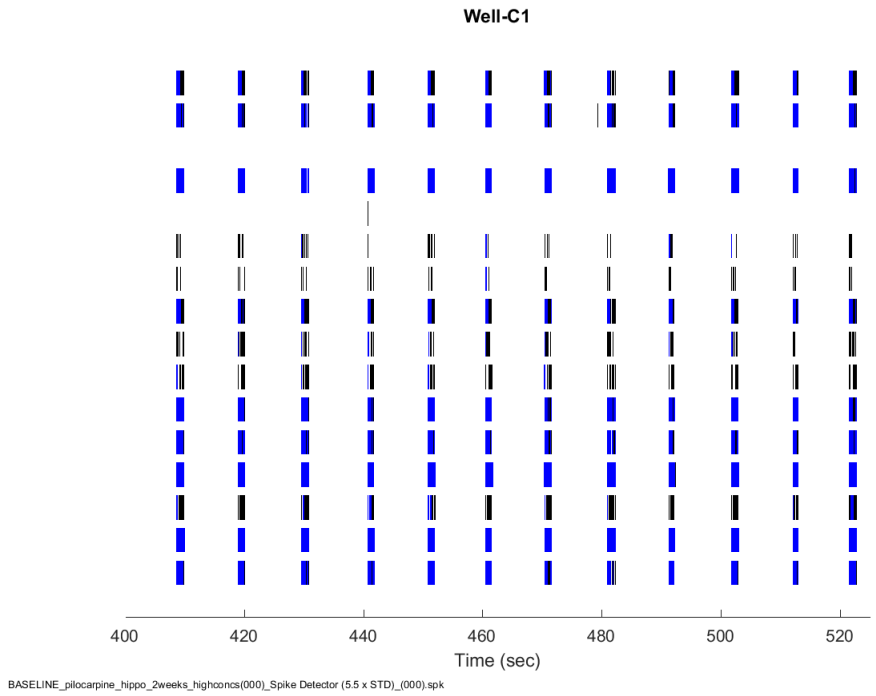
# Cryopreserved Rat Hippocampal Neurons

# Cryopreserved Rat Hippocampal Cells - 14 DIV

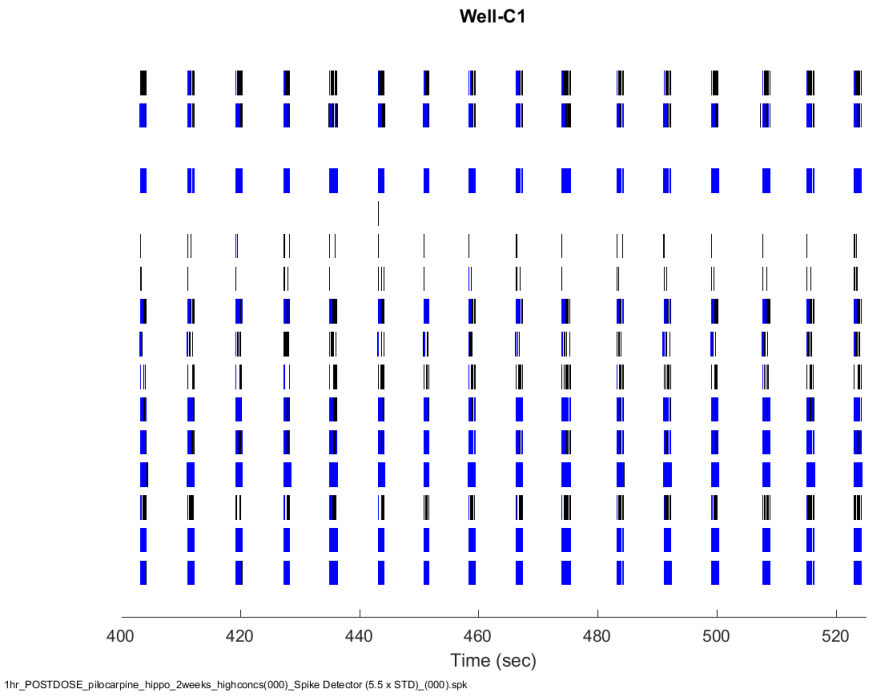



Top conc. = 400µM
2-fold dilution

# Cryopreserved Rat Hippocampal Cells - 14 DIV

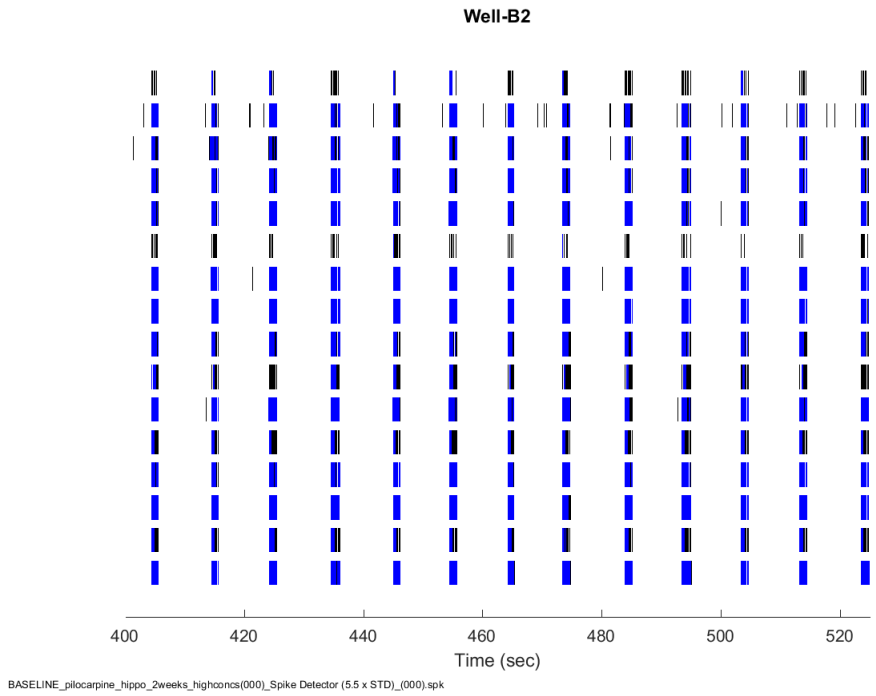



Baseline

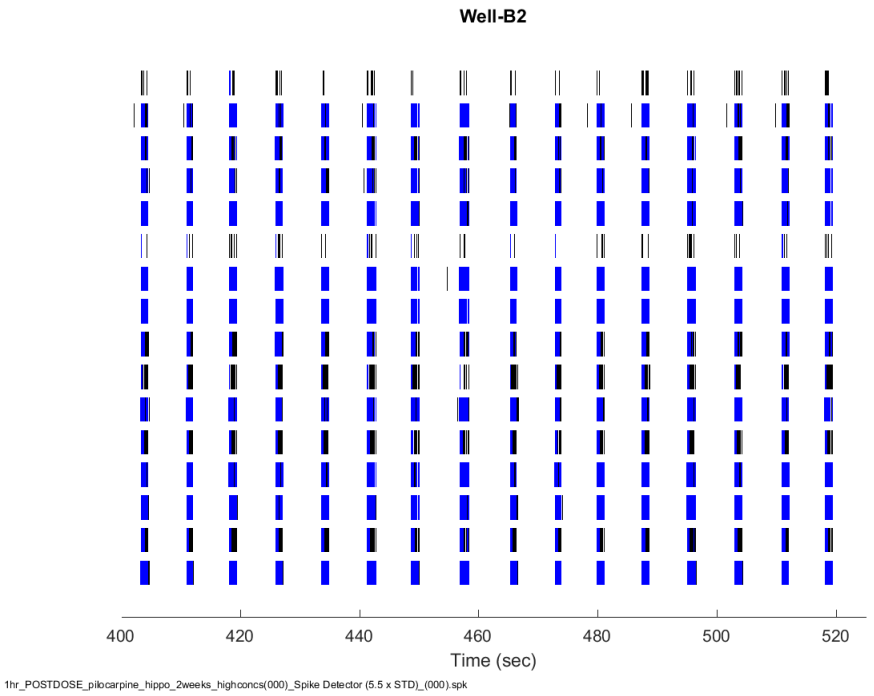



Pilocarpine 400µM

# Cryopreserved Rat Hippocampal Cells - 14 DIV




Baseline




Pilocarpine 200µM

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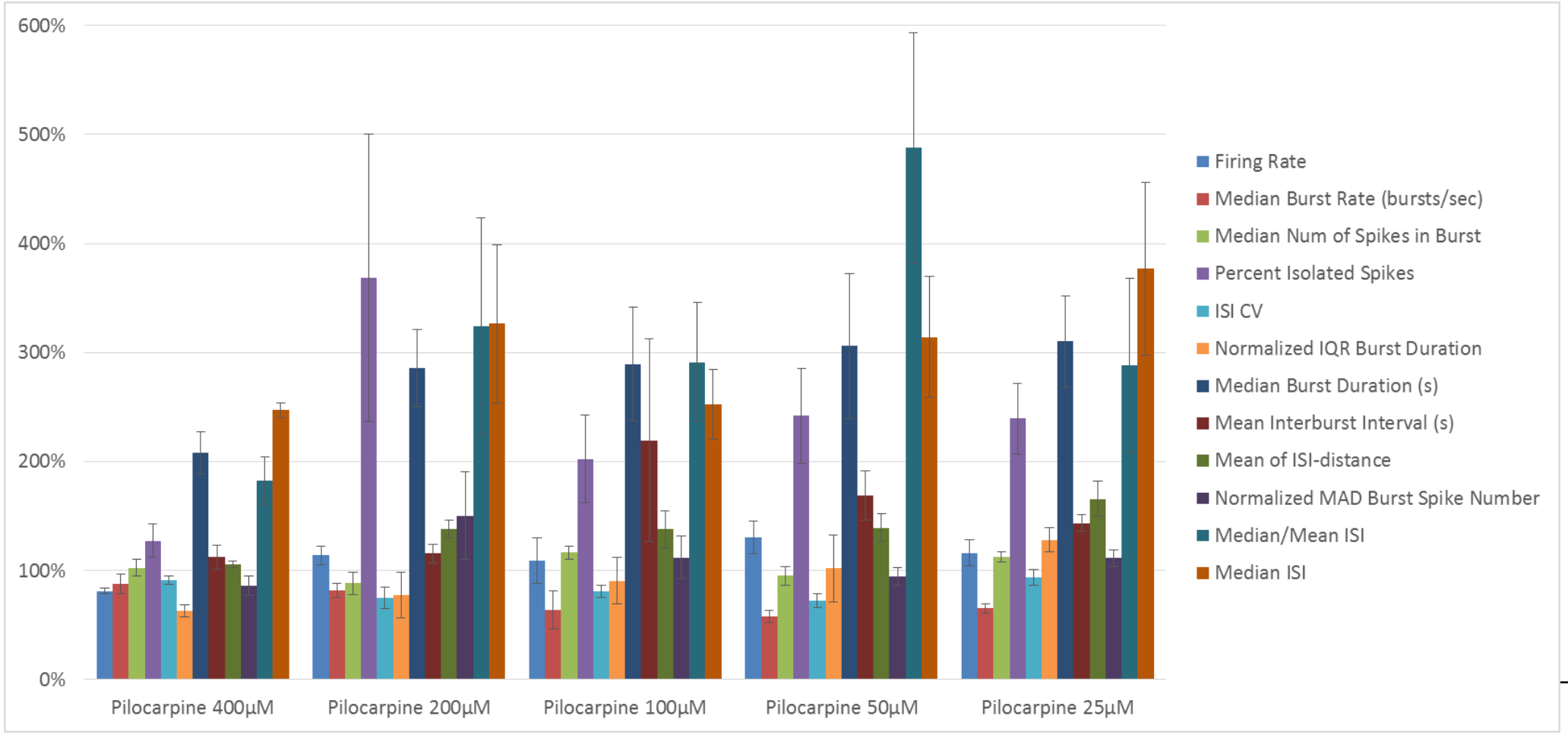
# Does the maturation of these rat neuronal models affect the responses?

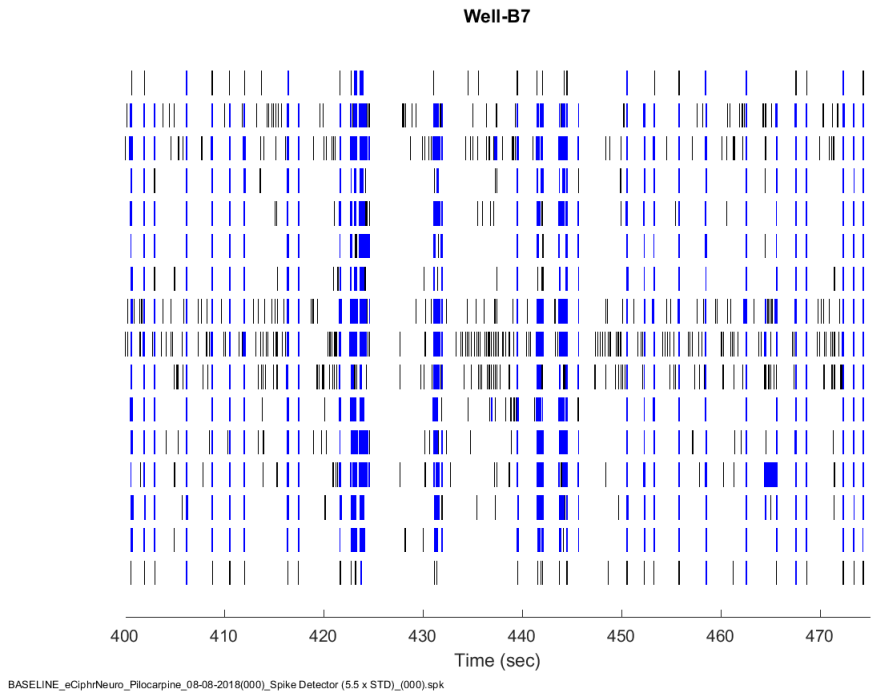
# Rat Cortical Neurons – 21 DIV

Intensified response…..

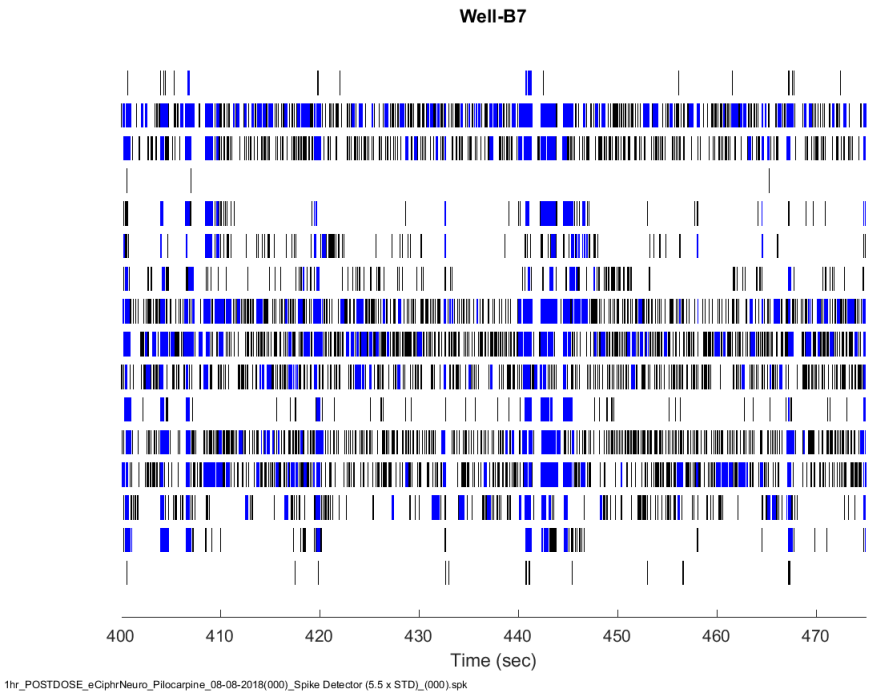



Top conc. = 400µM
2-fold dilution

# Rat Cortical Neurons – 21 DIV

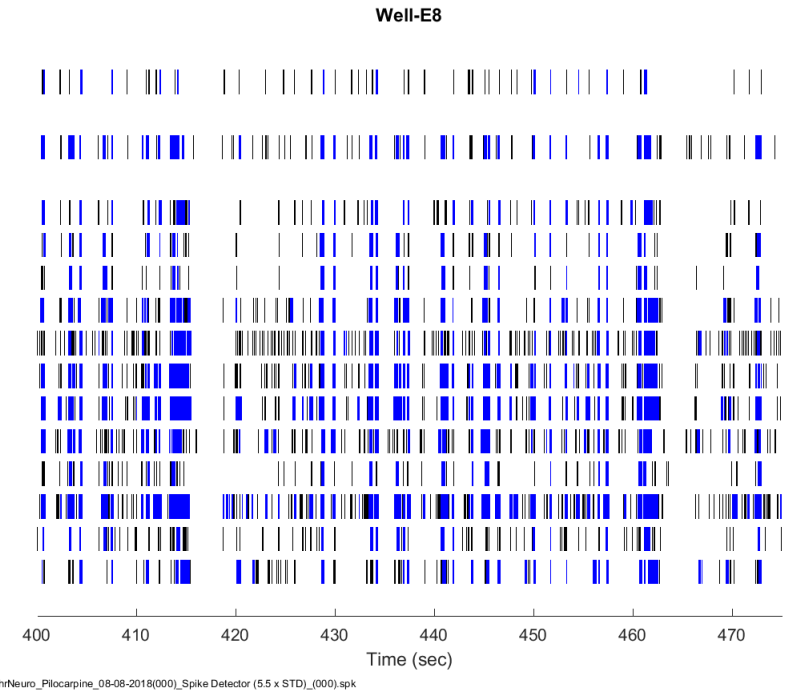



Baseline

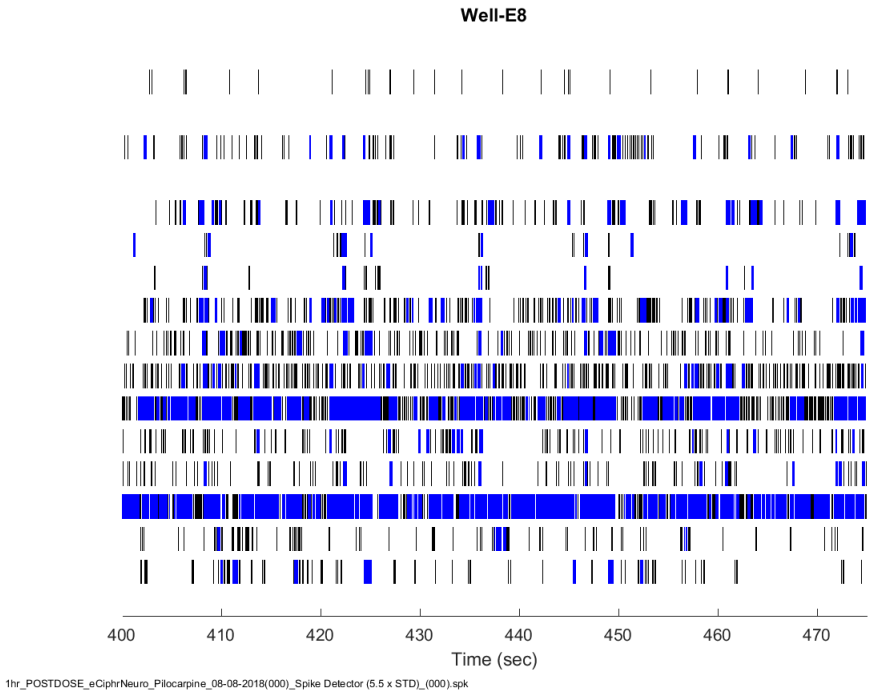



Pilocarpine 200µM

# Rat Cortical Neurons – 21 DIV



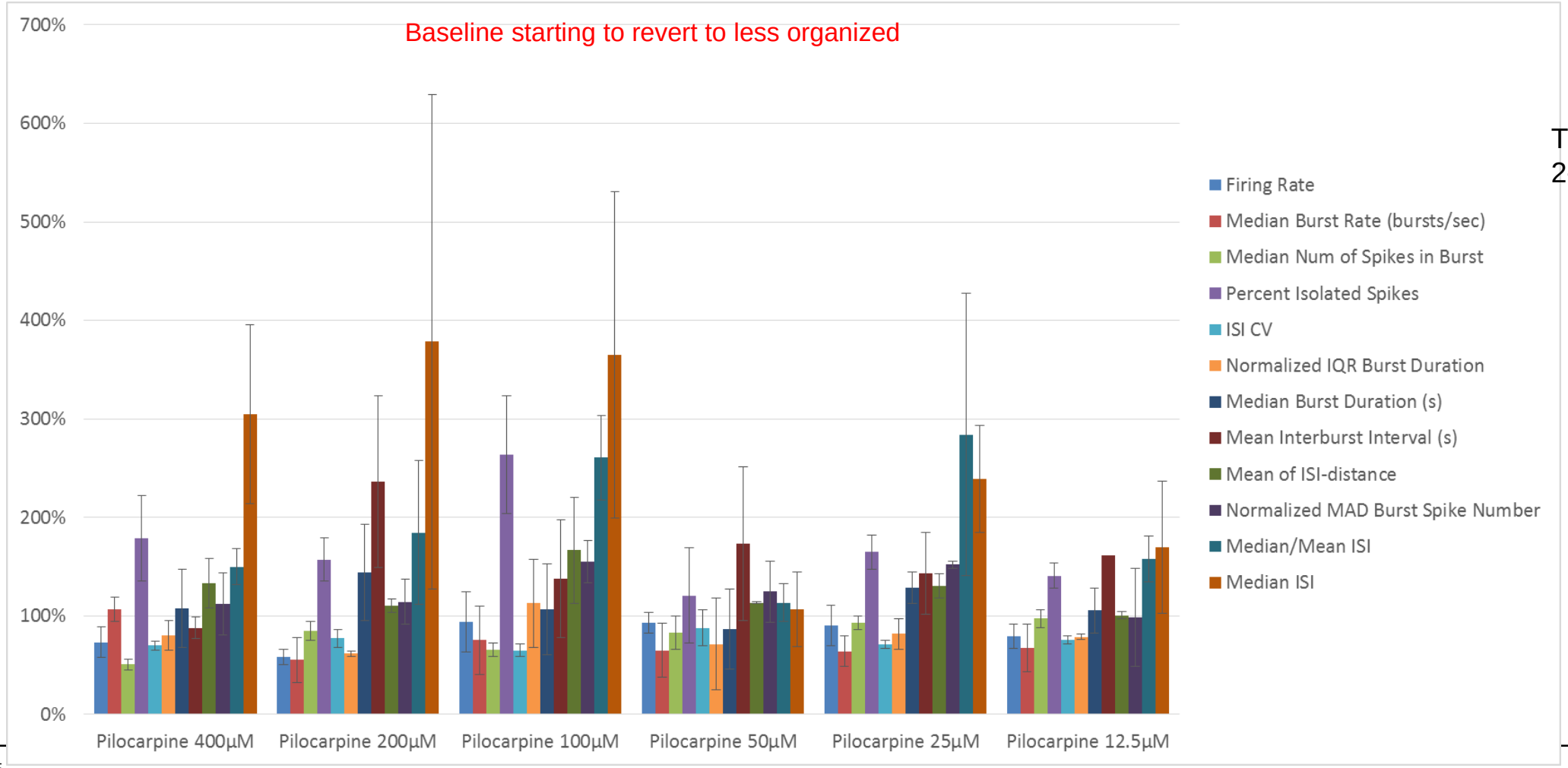

Baseline




Pilocarpine 25µM

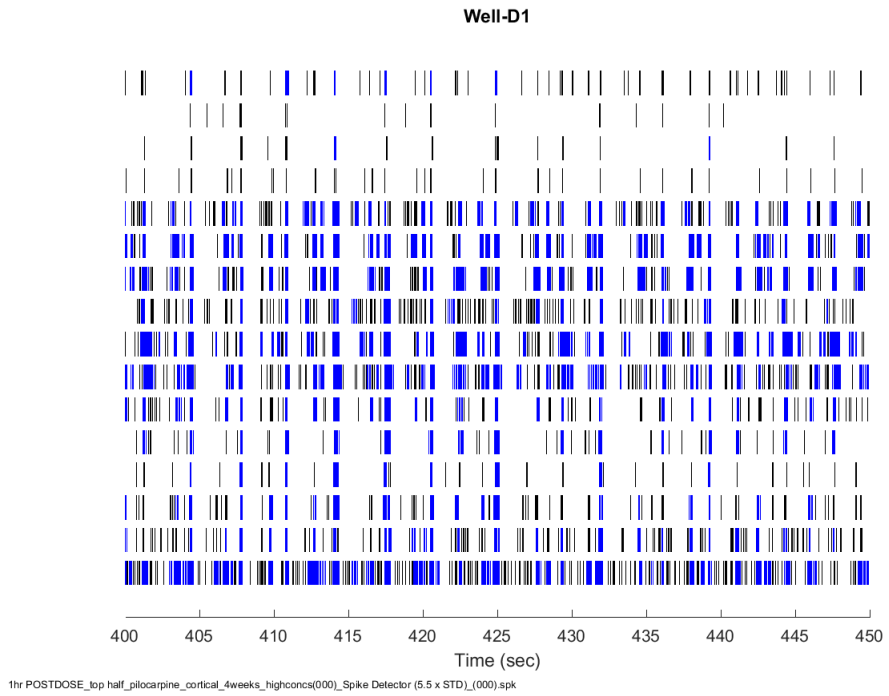
# Rat Cortical Neurons – 28 DIV

Baseline starting to revert to less organized

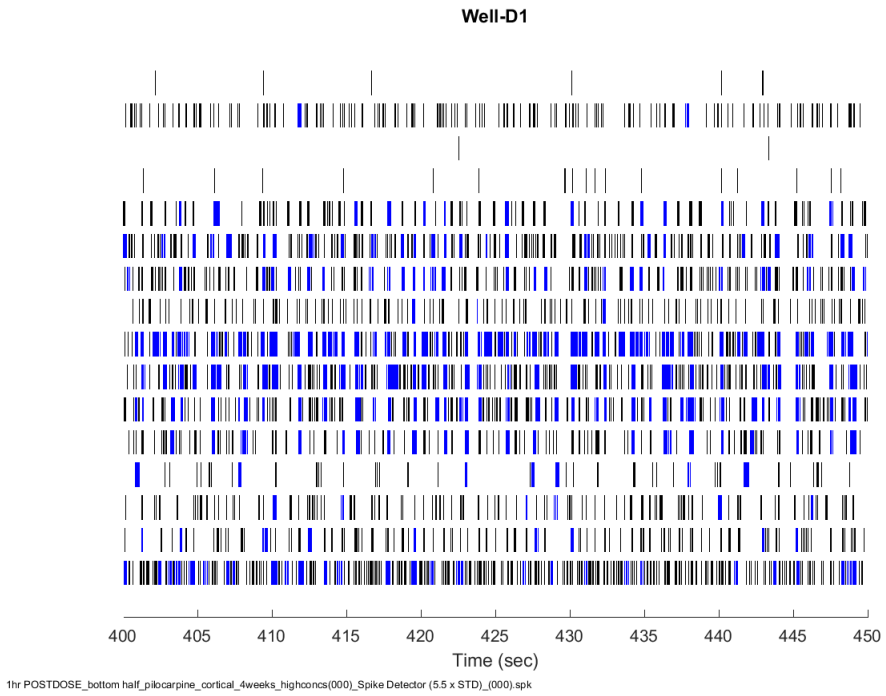



Top conc. = 400µM
2-fold dilution

# Follow-up Testing – Rat Cortical Neurons – 28 DIV

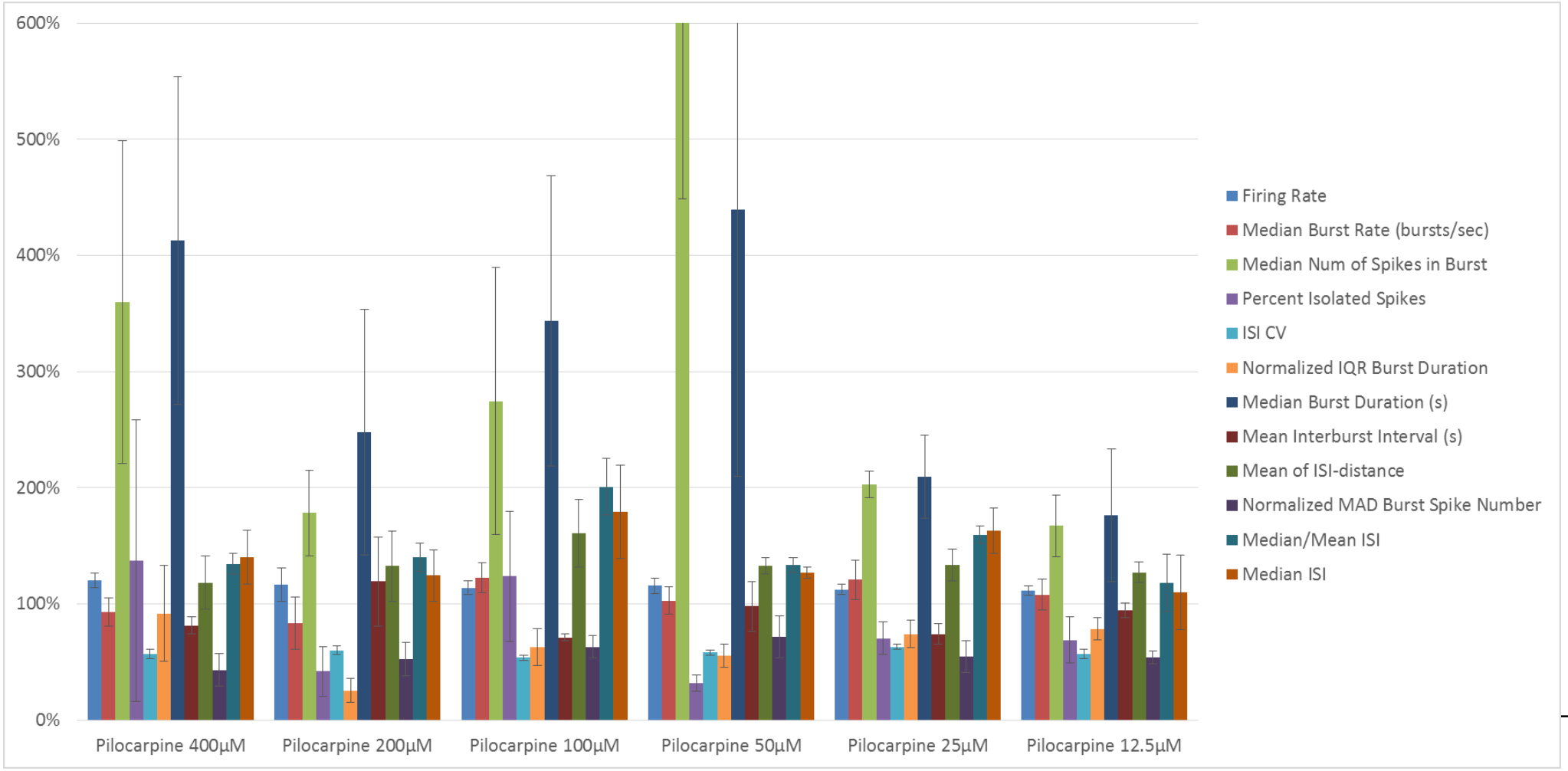



Baseline



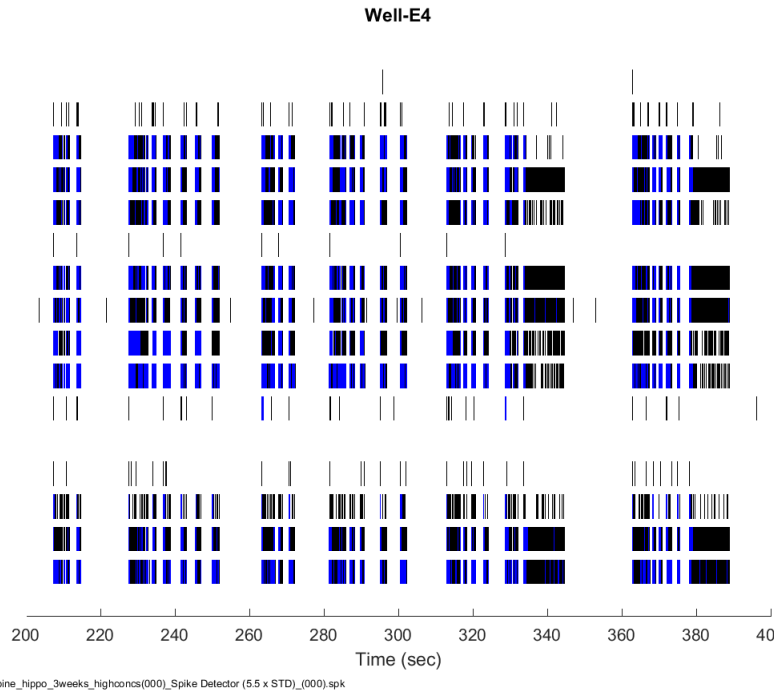

Pilocarpine 400µM

# Cryopreserved Rat Hippocampal Cells - 21 DIV




Top conc. = 400µM
2-fold dilution

# Cryopreserved Rat Hippocampal Cells - 21 DIV

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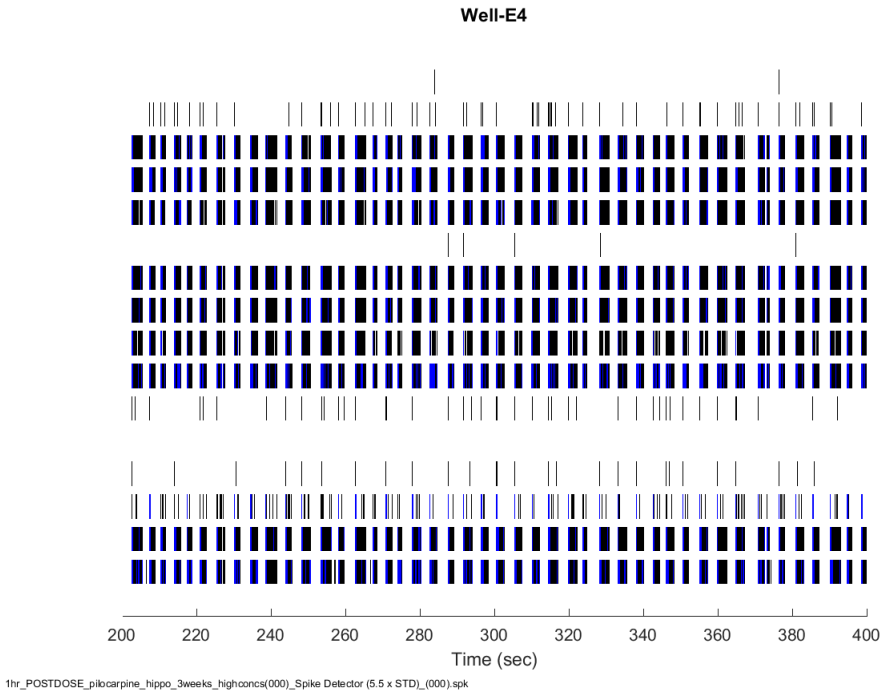
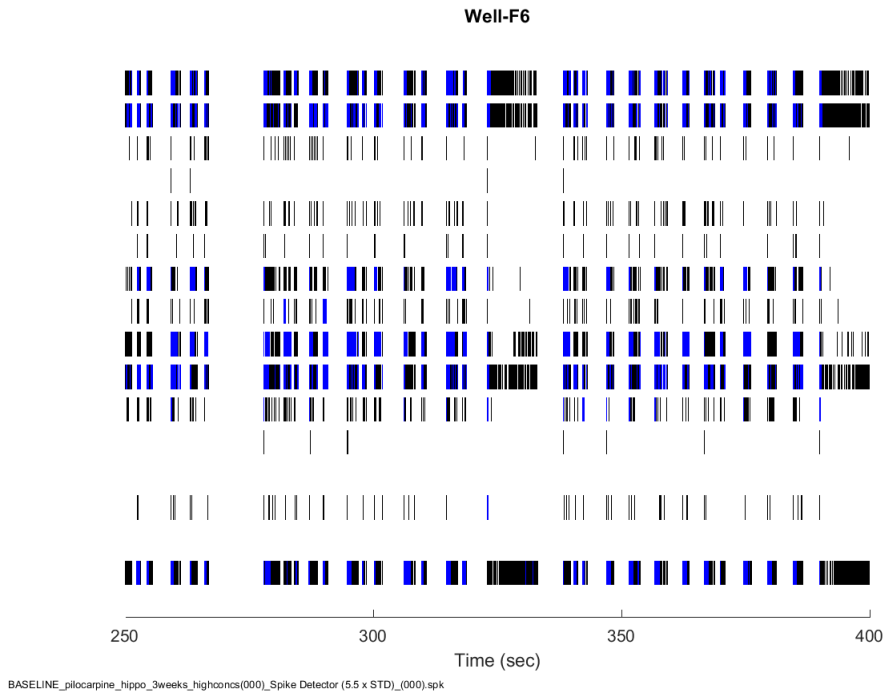
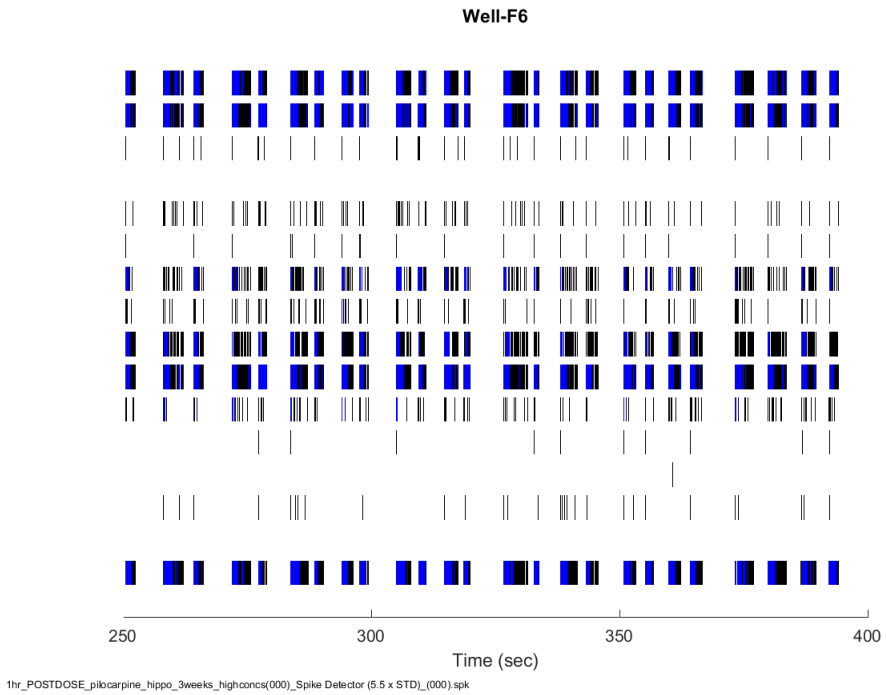



Pilocarpine 50µM

# Cryopreserved Rat Hippocampal Cells - 21 DIV




Baseline




Pilocarpine 12.5µM

# Conclusions

- Initial testing of pilocarpine with cryopreserved rat cortical neurons did not produce a seizurogenic phenotypic response at any of the concentrations tested (up to 100µM) at 14 DIV
  - Follow-up testing at higher concentrations (up to 400µM) at 14 DIV increased the effects on firing, bursting and synchrony endpoints but still did not produce a typical seizurogenic response
  - Follow-up testing at higher concentrations at 21 DIV increased the effects on firing, bursting and synchrony endpoints substantially. Further evaluation of endpoint responses as well as non-reported endpoints will be done
  - Although follow-up testing at higher concentrations at 28 DIV increased the effects on firing, bursting and synchrony endpoints, the baseline recordings showed a decrease in the robustness of the neural network as compared to the 21 DIV data. Therefore, additional testing will not be done at timepoints past 21 DIV
- Initial testing of pilocarpine with CDI iPSC-derived GlutaNeurons and Astrocytes co-culture (CDI) at 14 DIV demonstrated a substantial effect on firing, bursting and synchrony endpoints in a dose response manner
  - Follow-up testing verified these initial data
- Initial testing of pilocarpine with cryopreserved rat hippocampal neurons at 14 DIV did not produce a seizurogenic phenotypic response at any concentration tested and had little effect on any of the endpoints.
- Testing in hippocampal cells at 21 DIV substantially increased the effects on bursting characteristics and synchrony
- Follow up IF staining of the cells as well as expression analysis will be done to see if the muscarinic receptor is expressed at early time points

# Acknowledgements


- Jenifer Bradley