Evaluation of Pilocarpine in Multiple Neuronal Cell Types Using Microelectrode Array

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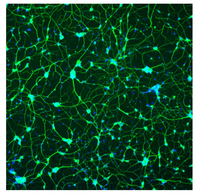

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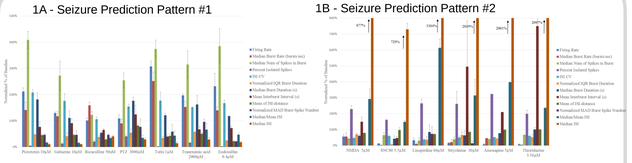
Abstract

Pilocarpine is a muscarinic receptor agonist that was developed to treat dry mouth, due to its ability to stimulate the salivary glands, and glaucoma, due to its ability to reduce the production of fluid in the eye. Pilocarpine is also used as a rat model for epilepsy where it induces status epilepticus at a relatively high dose (380 mg/kg) within 10-30 minutes, and after a prolonged duration of status epilepticus, brain injury and neuronal loss occur producing an epileptic phenotype. We tested pilocarpine in an in vitro model for seizure liability using a microelectrode array platform and cryopreserved rat cortical neurons and determined that this drug, up to a concentration of 31.6µM, did not produce a response which was indicative of a seizure causing compound. We postulated that using a different neuronal cell type might be more sensitive to detecting in vitro seizurogenic response. The in vivo model of pilocarpine-induced epilepsy is initiated in the hippocampal region of the brain, therefore we chose to test pilocarpine in cryopreserved rat hippocampal neurons. Although there were effects on the spontaneous spike trains post dose at all concentrations, the results did not produce a typical seizurogenic response. There were significant increases in isolated spikes for all concentrations and a slight decrease in synchrony. Overall, however, these effects do not fall into the criteria for a seizurogenic response. The third cell type we tested was a co-culture of human iPSC-derived glutamatergic neurons and astrocytes. This model was significantly affected with pilocarpine treatment at concentrations down to 3µM. Activity rates were significantly reduced, burst structure was significantly deteriorated and network activity was disrupted with a loss of synchronous activity. These results would suggest a possible seizurogenic or at least highly disruptive neural response. In conclusion, three neuronal cell types were used to assess in vitro effects after treatment with pilocarpine. Two of the cell types, cryopreserved rat cortical neurons and rat hippocampal neurons, did not indicate a seizurogenic response. The co-culture of human iPSC-derived glutamatergic neurons and astrocytes, however, was adversely effected by treatment with pilocarpine, indicating a probable CNS liability.

1A - Seizure Prediction Pattern #1

1B - Seizure Prediction Pattern #2



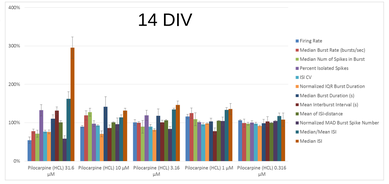
Figures 1A and B. eCiphrNeuro is a moderately high throughput assay developed in our lab using cryopreserved rat cortical neurons plated on 48-well microelectrode array plates to detect the neurotoxic and seizurogenic potential of test compounds (Bradley et al., 2018). After testing many compounds, we 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

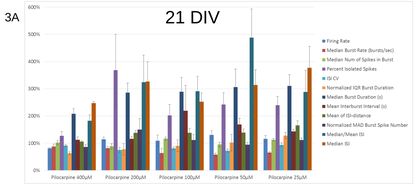
- 48-well MEA plates were pre-coated with a 0.1% PEI solution and allowed to dry overnight. One hour before plating cells, the plates were treated with a laminin solution by dispensing a 10 or 15µL dot (depending on cell type) directly over the electrode grid and incubating at 37°C.
- Cryopreserved rat 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% CO_2 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.

Results: Cryopreserved Rat Cortical Neurons

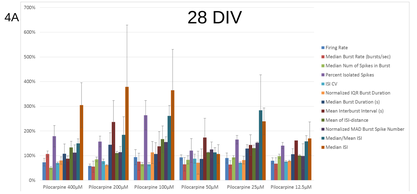
2A



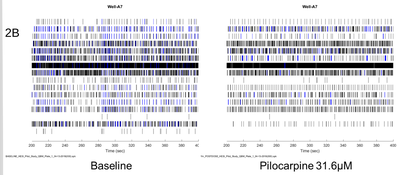
3A



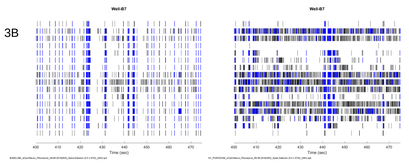
4A



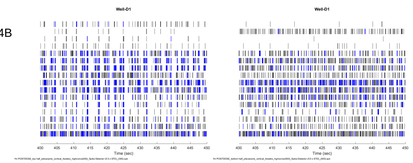
2B



3B



4B



Baseline Pilocarpine 31.6µM

Figures 2A and B. Initial and follow-up testing with pilocarpine in cryopreserved rat cortical neurons at 14 DIV and up to concentrations of 100µM did not have a phenotypic seizurogenic response in this assay. 2A is a graph of pilocarpine dose response with a top concentration of 31.6µM. There is a slight disruption in burst organization and a decrease in overall firing and burst rates as seen in both the graph and raster plots.

Baseline Pilocarpine 200µM

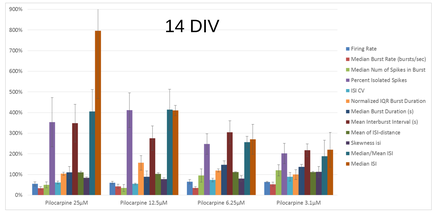
Figures 3A and B. Follow-up testing with pilocarpine in cryopreserved rat cortical neurons at 21 DIV and up to concentrations of 400µM. The baseline at 21 DIV shows a more synchronous neural network. The effects of pilocarpine at all concentration tested shows a substantial and deleterious effect on burst organization and a decline in synchrony. Further analysis and characterization of this effect will be done in future experiments.

Baseline Pilocarpine 400µM

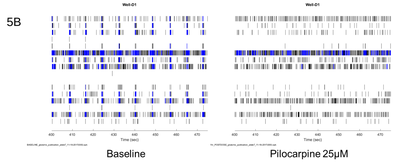
Figures 4A and B. Follow-up testing with pilocarpine in rat cortical neurons at 28 DIV and up to concentration of 400uM. Although testing higher concentrations at 28 DIV caused deleterious effects on firing, bursting and synchrony endpoints, the baseline recordings showed a decrease in the robustness of the neural network as compared to the 21 DIV data. Therefore, additional testing will not be done at timepoints past 21 DIV.

Results: iCell GlutaNeurons and Astrocytes Co-culture

5A



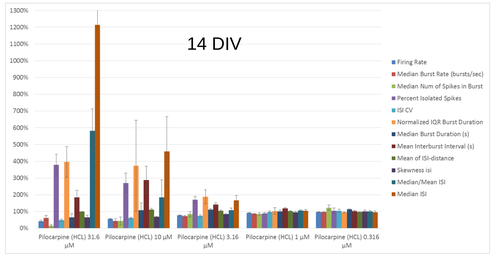
5B



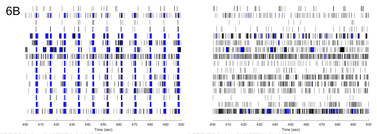
Baseline Pilocarpine 25µM

Figures 5A and B. Pilocarpine tested in CDI iCell GlutaNeurons and Astrocytes co-culture at 14 DIV up to 25µM. The response to pilocarpine in the GlutaNeurons/Astrocytes co-culture was more robust than in the rat cortical neurons at 14 DIV. Firing rates are substantially reduced, burst organization is almost completely disrupted and network synchrony is deleteriously impacted. This effect is observed to be in a dose response manner. This co-culture appears to be more sensitive to pilocarpine at 14 DIV than the rat cortical neurons and the rat hippocampal neurons at 14 DIV.

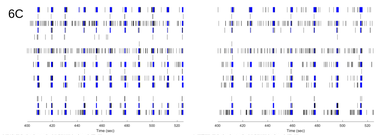
6A



6B



6C

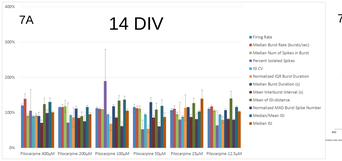


Baseline Pilocarpine 31.6µM Baseline Pilocarpine 0.316µM

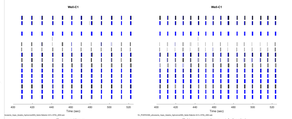
Figures 6A and B. Follow-up testing with pilocarpine in CDI iCell GlutaNeurons and Astrocytes co-culture at 14 DIV. Top dose was 31.6µM with half-log dilutions down to 0.316µM. At 31.6µM, there is a complete disruption in burst organization and neural network organization with decreases in firing and burst rates. This effect is observed to be in a dose response manner with no effect seen at the lower concentrations of 1 and 0.316µM. This can be observed in the raster plots of the spike trains in 6B and C.

Results: Cryopreserved Rat Hippocampal Neurons

7A

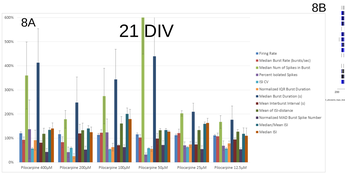


7B

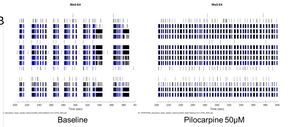


Baseline Pilocarpine 400µM

8A

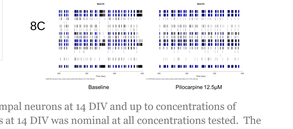


8B



Baseline Pilocarpine 50µM

8C



Baseline Pilocarpine 12.5µM

Figures 7A and B. Pilocarpine tested in cryopreserved rat hippocampal neurons at 14 DIV and up to concentrations of 400µM. The effects of pilocarpine on the rat hippocampal neurons at 14 DIV was nominal at all concentrations tested. The analysis of the spike trains with customized MATLAB scripts pick up some minor fluctuations (7A), but when observing the raster plots (7B), very little differences from baseline to 1 hour post dose at 400µM emerge.

Figures 8A and B. Pilocarpine tested in cryopreserved rat hippocampal neurons at 21 DIV. Substantial effects caused by treatment with pilocarpine were observed at all concentrations. The pattern of bursting, which changes significantly from the 14 DIV phenotype, becomes much more regulated with the pilocarpine treatment. This is observed at all concentrations but does seem to attenuate slightly at the lower concentrations. A regularization of the burst patterns can still be observed at 12.5µM (8C).

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

References

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