Development Of A Novel *in Vitro* Method to Study Effects of Volatile Compounds on Neural Activity

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Introduction

Background:

- Activity of neurons on microelectrode arrays (MEAs) can assess neurotoxicity.
  - This works well for compounds soluble in aqueous solutions.
- Volatile compounds cannot be easily suspended in solution, precluding testing in vitro.

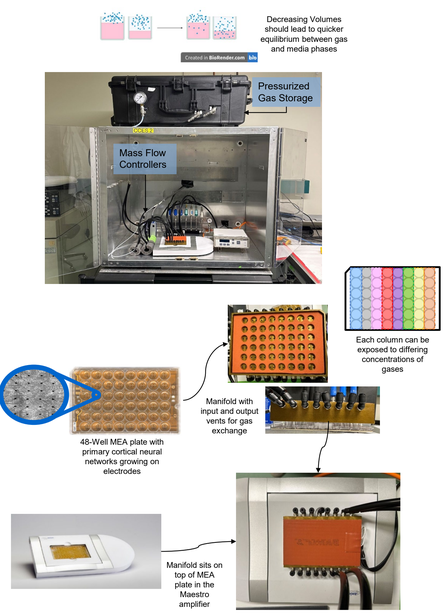
Goal: Develop an *in vitro* method to test neural activity after exposure to volatile compounds.

Approach:

Develop and Test a novel delivery manifold for volatile compounds:

- Optimize the media volume.
- Determine the ideal cell density.
- Confirm that any turbulence caused by the air passing over the cells will not cause artifacts in electrical recordings.
- Determine if well position affects activity.
- Test system with toluene (positive control) to confirm the test method works.

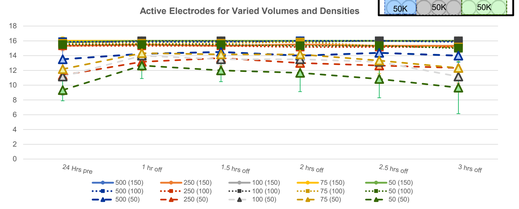
Methods

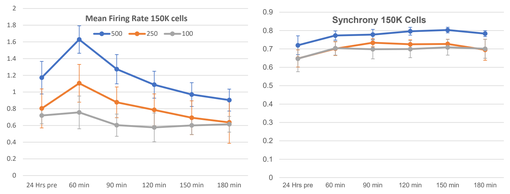


Initial Trials Indicate 100uL of Media and 150K cells per Well are Stable over a 2 Hour Period

Varied Densities Design:

- Day 0: Activity of the cells was recorded 24 hours pre-experiment.
- Day 1: Media was removed. Cells were left to acclimate for 1 hour, then the activity was measured, then the media was replaced, and the cells were recorded again after an hour of recovery.

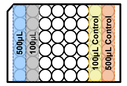


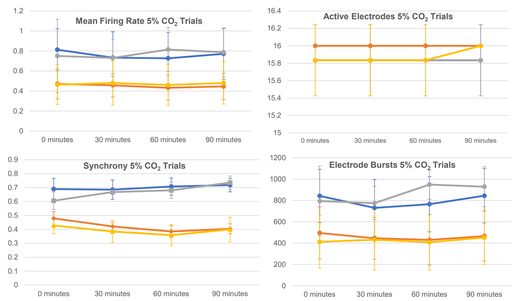


Trials with Humidified 5% CO_2 Indicate Turbulence does not Impact Electrical Activity in Either Media Volume

Experimental Design:

- Media was removed from the cells and the MEA was placed on the Maestro.
- Heated (37ºC) and Humidified (50%) 5% CO_2 was put over the cells.
- The activity of the plates was recorded every 30 minutes for 15 minutes.

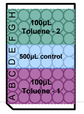


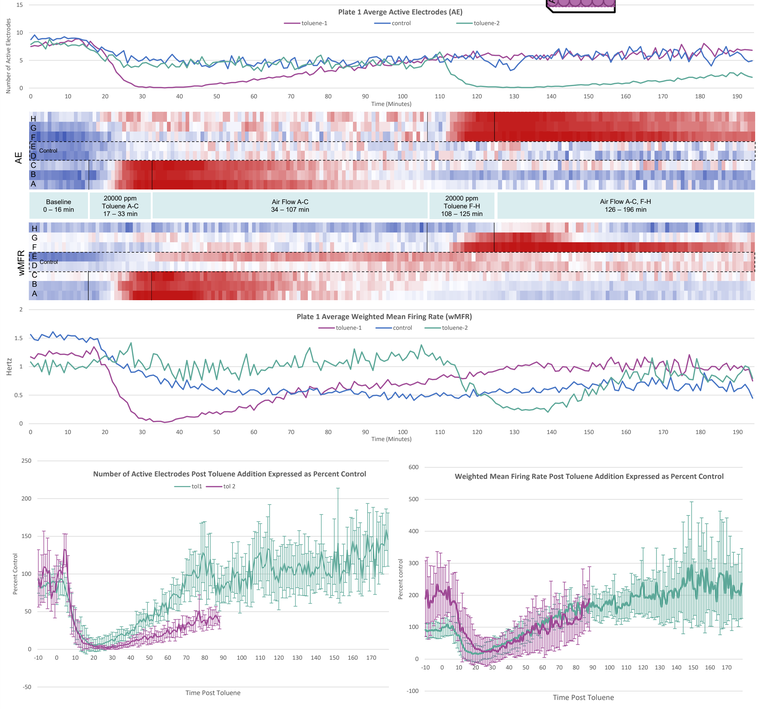


Acute Exposures With 37ºC and Humidified 20000 ppm Toluene Gas Decreased the Activity of the Neurons when Pulled over the Cells

Experimental Design:

- Media was removed from the cells and the MEA was placed on the Maestro.
- Cells activity was recorded for a 15-minute equilibration period.
- Heated (37ºC) and Humidified (50%) 20000 ppm toluene was put over the cells, in columns of three.
- When cell activity decreased substantially (based on visual inspection), toluene exposure was stopped, and room air was pulled over the cells. Then this was repeated with different columns.

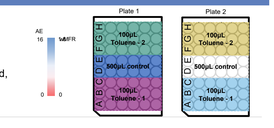


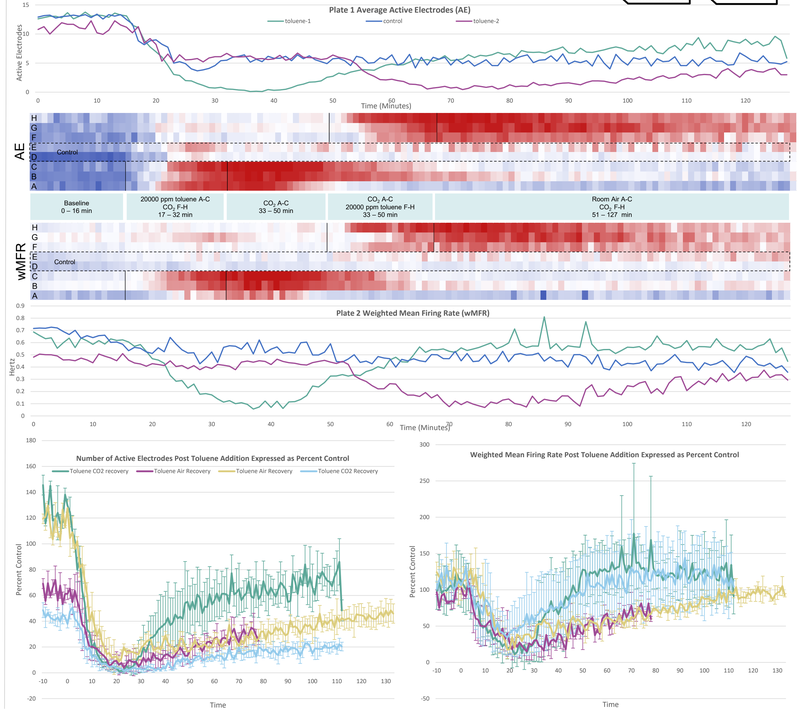


Acute Exposures With 37ºC and Humidified 20000 ppm Toluene Gas Decreased the Activity of the Neurons when Pulled over the Cells, Switching to 5% CO2 Expedites Recovery

Experimental Design:

- Media was removed from the cells and the MEA was placed on the Maestro.
- A baseline recording was completed for 15 minutes, then Heated (37ºC) and Humidified (50%) 20000ppm toluene and CO_2 was flowed over columns A-C and F-H respectively.
- When cell activity decreased substantially (based on visual inspection), toluene exposure was stopped, and CO_2 was pulled over columns A-C, while air from the room was pulled over columns F-H.
- Then the toluene exposure process was repeated for columns F-H.





Summary and Future Directions

Initial Findings:

- Network activity in Rat pup cortical cultures remains stable at 100µL of media for at least 2 hours in the Maestro, when plated at a density of 150K cells per well.
- The new manifold is able to distribute heated and humidified gases over top the media covered cells without severe turbulence effects.
- Exposure to 20000 ppm toluene does decrease the activity of the neurons when pushed over the cells in the new manifold system.
  - After toluene is pushed out of the system, electrical activity begins to recover.
- Alternating CO_2 and toluene in the manifold increases the rate of recovery for the toluene affected cells.

Next Steps:

- Expand Manifold Capacity:
  - Increase the number of mass flow controllers in the system to allow for multiple concentrations of gas to be tested concurrently.
  - Add the ability to switch quickly between toluene and CO_2 without reaching in and moving tubes.
- Compound Testing:
  - Characterize Toluene concentration response.
  - Test additional solvents.

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