Assessing the Differential Sensitivities of a Microelectrode Array Assay and a Neurite Outgrowth Assay for Detecting CNS Liabilities Using a Set of Neurotoxic and Seizurogenic Compounds

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Abstract

Domoic acid is a neurotoxin first associated with the poisoning of 107 individuals and three subsequent deaths on Prince Edward Island in 1987 after these individuals consumed mussels containing this toxin. Due to its neurologic adverse effects, including memory loss, the illness was called amnesic shellfish poisoning. The toxin has since been attributed to the diatom, Nitzschia pungens f. multiseries, which was ingested by the mussels during normal filter feeding. In our lab, we use domoic acid as a tool compound when testing neurons on a microelectrode array (MEA) platform (Axion Biosystems Maestro). Domoic acid completely eliminates all spontaneous spike activity when treating neurons at concentrations ≥1 µM. When tested in a neurite outgrowth assay using a high content imager (ThermoFisher Scientific CellInsight CX7), domoic acid does not affect cell health or neurite outgrowth when tested up to 25 µM for 72 hours. We tested various neurotoxins and seizurogenic compounds on the MEA platform and in the neurite outgrowth assay to compare results and assay sensitivities. The antipsychotic haloperidol demonstrated seizurogenic liabilities at 1 µM on the MEA but had an IC_{50} of 14.6 µM for valid neuron count and 11.7 µM for neurite outgrowth when tested in an HCS assay. $GABA_A$ antagonist, picrotoxin, has a seizurogenic MEA profile as low as 1 µM but does not effect cell health and neurite outgrowth at concentrations up to 50 µM. Chlorpromazine, on the other hand, has a seizurogenic profile and effects cell health and neurite outgrowth at similar concentrations (~5 µM). With the results from domoic acid, haloperidol, picrotoxin, chlorpromazine and 9 additional control compounds, we have determined that the MEA platform is more sensitive for detecting electrophysiological CNS liabilities than the neurite outgrowth assay. Alternatively, compounds which affect neurons by non-electrophysiological cytotoxic effects can be detected more sensitively with the HCS neurite outgrowth assay than the acute MEA assay. Therefore, an effective overall strategy would be to test compounds for safety in both assays.

Methods

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Figure 1: Axion Biosystems Maestro High Throughput Microelectrode Array Platform.
- 768 stimulating and recording channels
- SBS-Compliant Multiwell MEA plates
- Accommodates 12, 48 and 96 wells
- Fully integrated heater with software controls
- Automated electrode characterization & diagnostics

Microelectrode array evaluation

- 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 15µL dot directly over the electrode grid and incubating at 37°C.
- Cryopreserved rat cortical neurons (QBM Cell Science) were rapidly thawed and slowly diluted with Neurobasal medium supplemented with B27, L-glutamine and penicillin-streptomycin
- After a gentle centrifugation step, the cells were resuspended at the appropriate density to achieve 75,000 cells per well
- 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 14-18 days by changing 60% medium 3 times a week
- Microelectrode Array 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 each recording

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Figure 2: CellInsight CX7.
- Ideal for multiplex biology, including time-lapse and live-cell imaging
- Provides multiple outputs that would take several assays to obtain on traditional systems
- Solid state high-intensity 7-color LED light engine
- Flexibility of widefield or confocal imaging in all 7 wave-lengths
- High-resolution fluorescence microscopy images.

Cell staining and imaging

- 384-well clear bottom tissue culture plates were treated with a laminin solution one hour prior to plating cells
- Cryopreserved rat cortical neurons (QBM Cell Science) were rapidly thawed and slowly diluted with Neurobasal medium supplemented with B27, L-glutamine and penicillin-streptomycin.
- After a gentle centrifugation step, the cells were resuspended and plated at 10,000 cells per well.
- The neurons were incubated for 1 hour prior to treatment with test articles and control compounds.
- At the end of the 72 hour treatment period, the cells were fixed, permeabilized and stained with the following reagents:
  - Hoechst 33342
  - Anti-Beta III Tubulin Antibody, Alexa Fluor® 488 Conjugate | AB15708A4
- Cell health and neurite outgrowth were assessed utilizing an optimized neuronal profiling bioapplication applied to images obtained on an CellInsight CX7

Compound Test Set

Compound	Description
Domoic Acid	Neurotoxin that effects glutamate signaling. Attributed to the diatom, Nitzschia pungens f. multiseries.
Haloperidol	Antipsychotic (butyrophenone)
Picrotoxin	$GABA_A$ receptor antagonist & proconvulsant, used primarily as a research tool
Chlorpromazine	Antipsychotic (phenothiazine)
Clozapine	Antipsychotic. Serotonin antagonist.
Strychnine	Neurotoxic poison found in the seeds of Strychnos nux-vomica. Glycine receptor antagonist that causes convulsions and seizures.
Fluoxetine	Antidepressant of the selective serotonin reuptake inhibitor (SSRI) class
Sertraline	Antidepressant of the selective serotonin reuptake inhibitor (SSRI) class
GABA	Gamma aminobutyric acid. Inhibitory neurotransmitter.
Tetrodotoxin	Neurotoxin that blocks voltage gated sodium channels. Primarily associated with puffer fish of the order Tetraordontiformes.
Vincristine	Chemotherapeutic. Antimicrotubular antineoplastic agent.
Paclitaxel	Chemotherapeutic. Cytoskeletal drug that targets tubulin.
Nocodazole	Chemotherapeutic. Antimicrotubular antineoplastic agent.

Results Table

Compound	MEA Minimum Effective Concentration (µM)	Valid Neuron Count IC_{50} (µM)	Neurite Outgrowth IC_{50} (µM)
Domoic Acid	1 µM	>25	>25
Haloperidol	0.316	14.6	11.7
Picrotoxin	1	>50	>50
Chlorpromazine	1	6.3	4.1
Clozapine	3.16	28	17
Strychnine	15	>100	>100
Fluoxetine	1	8.2	4
Sertraline	3.16	3.1	1.8
GABA	9.5	>100	>100
Tetrodotoxin	<0.03	>100	>100
Vincristine	>1	<0.05	<0.05
Paclitaxel	>10	50	<0.1
Nocodazole	≥1	1.2	0.04

Results: Microelectrode Array with Cryopreserved Rat Cortical Neurons

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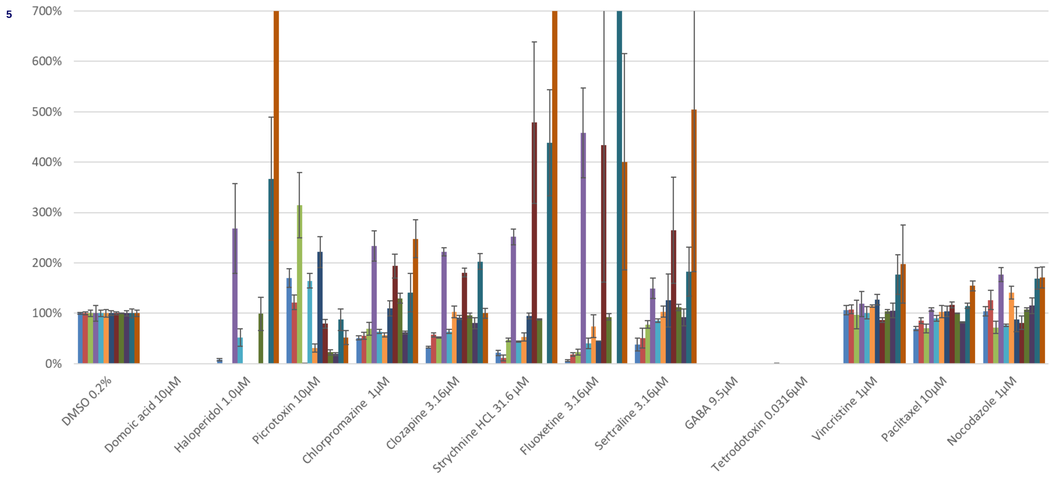

Figure 5: Graphical representation of MEA data for all 13 control compounds tested. The endpoints are representative of spike train activity, burst organization and network organization. Domoic acid, GABA and tetrodotoxin eliminate spike activity at the concentration depicted in the graph. Haloperidol, picrotoxin, chlorpromazine, clozapine, strychnine, fluoxetine and sertraline demonstrate CNS liabilities when tested on the MEA. Vincristine, paclitaxel and nocodazole do not have neurotoxic profiles when tested on the MEA. The vehicle control, DMSO 0.2% does not have an effect on spike train activity or organization in this assay.

Results: Neurite Outgrowth in Cryopreserved Rat Cortical Neurons

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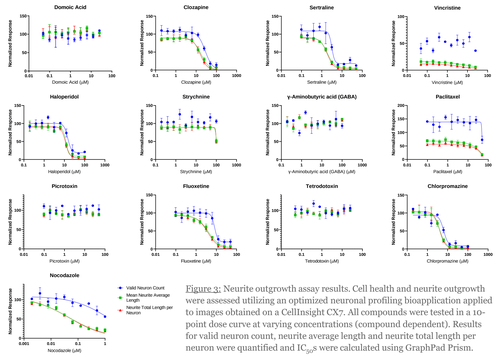

Figure 3: Neurite outgrowth assay results. Cell health and neurite outgrowth were assessed utilizing an optimized neuronal profiling bioapplication applied to images obtained on a CellInsight CX7. All compounds were tested in a 10-point dose curve at varying concentrations (compound dependent). Results for valid neuron count, neurite average length and neurite total length per neuron were quantified and IC_{50}s were calculated using GraphPad Prism.

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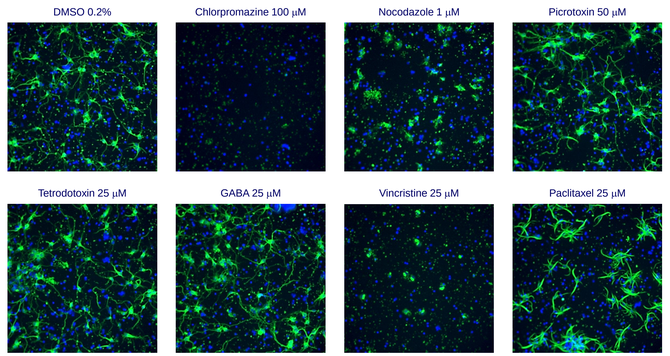

Figure 4: Neurite outgrowth assay images. Images for neurite outgrowth obtained with a CellInsight CX7 after 72 hr incubation with control compounds. Tetrodotoxin (25 µM), GABA (100 µM) and picrotoxin (50 µM) show no effect, similar to negative vehicle control DMSO (0.5%). Chlorpromazine (100 µM) is cytotoxic at the top concentration tested. Nocodazole (1 µM) and vincristine (1 µM) demonstrate a complete inhibition of neurite outgrowth. Paclitaxel (25 uM) demonstrates an inhibition and morphological change to neurites.

Conclusions

- The neurotoxin domoic acid was tested in both a microelectrode array assay and a neurite outgrowth assay to assess *in vitro* CNS liabilities. Domoic acid was found to have a significantly different toxicity profile on each platform. This toxin completely eliminates spontaneous spike activity at concentrations as low as 1 µM on the MEA but does not affect cell health or neurite outgrowth when tested up to 25 µM.
- An additional 12 control compounds were tested on each platform for comparison
- In addition to domoic acid; haloperidol, picrotoxin, strychnine, GABA and tetrodotoxin demonstrated CNS liabilities using the MEA assay at significantly lower concentrations when compared to the neurite outgrowth assay
- Alternatively, vincristine, paclitaxel and nocodazole demonstrated a greater liability when tested in the neurite outgrowth assay as compared to the MEA assay
- Chlorpromazine, clozapine, fluoxetine and sertraline had similar results in both assays
- In conclusion, we have demonstrated that testing compounds in both the microelectrode array assay and the neurite outgrowth assay increases the chance of identifying CNS liabilities

References

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