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A Multiplexed Assay for Determination of Neurotoxicant Effects on Spontaneous Network Activity and Cell Viability from Multi-well Microelectrode Arrays

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Introduction

❖ *In vitro* methods are needed to rapidly and efficiently screen chemicals for hazard characterization and rank them for additional testing.

❖When using cell-based screens, chemical effects on endpoints of interest vs cell health (e.g. viability) need to be distinguished

❖ Assessment of spontaneous activity in neuronal cultures on microelectrode arrays (MEAs) has been proven as a sensitive, rapid method to screen and prioritize drugs and chemicals for neurotoxicity.

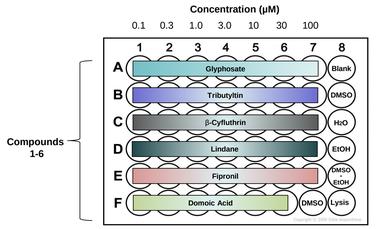
❖However, determination of cell viability is often done in "sister" cultures, sometimes at different densities. While providing information of cell health, this protocol is not optimal.
❖This is also inefficient because it requires additional culture materials and dosing solutions.

❖**Goals**: 1) To develop a multiplexed assay that allows determination of compound effects on spontaneous network activity and cell health. 2) To compare the results from a "Screening Mode" design (concurrent replicates) to a traditional design (replicates over several experiments).

Methods

Compound Selection: Six compounds were selected as test compounds for these experiments (Table 1).

Compound	Effect on Network Activity	Effect on Viability	Reference
Glyphosate	No Effect	No Effect	1, 2
Tributyltin	Inhibits	50% reduction	2
β-Cyfluthrin	Inhibits	No Effect	1, 2
Fipronil	Inhibits	35% reduction	1
Lindane	Increases	50% reduction	1
Domoic Acid	Inhibits	No Effect	1, 2



Compare

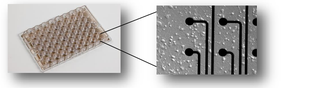
Traditional Design: Test each compound once over several different cultures and dates

Screening Mode Design: Test each compound in three replicate multi-well MEA plates from the same culture on the same date

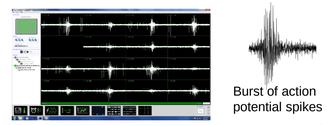
Culture: Primary cortical neurons from Long-Evans rats (PND 0-1) were plated at 150K cells/array on PEI coated 48 well MEA plates and maintained at 37° C in 500 µL of media per well.
MEA system: Comprised of the Maestro 768-channel amplifier, Middle-man data acquisition interface, personal computer with Axion Integrated Studio (AxIS) software, and 48-well plates (Axion M768-KAP-48). Each well contains 16 individual nano-textured gold microelectrodes (~40-50 µm diameter, 350 µm center-to-center spacing) with 4 integrated ground electrodes.
Experimental Recording: Baseline spontaneous neuronal activity was recorded for 1 hr between DIV 12-14. Following baseline recording, test compounds were added to individual well at the concentrations indicated on the plate map (above).
Data Analysis: Mean firing rate (MFR) determined in the presence of compound was expressed as a percentage of its pre-treatment value (% Control) to determine the percent increase or inhibition of MFR. These data were averaged across experiments to produce the concentration-response curves illustrated. CellTiter Blue data are expressed as mean % Control across all replicates. LDH data are expressed as the mean % of total of LDH released (% Total) across all replicates.

Experimental Design

Determine Effects on Spontaneous Network Activity



1. Primary cortical neurons are cultured in 48 well MEA plates and allowed to mature for 14 Days

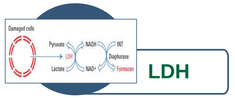


Burst of action potential spikes

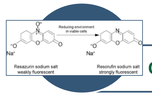
2. Plates are placed in the Axion Maestro MEA amplifier

3. Determine firing rate in each well for 60 min prior to and after treatment with compounds

Determine Effects on Cell Health



LDH



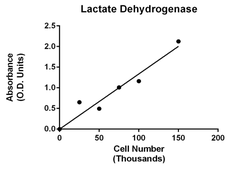
CellTiter Blue™

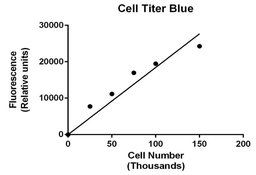
4. Transfer 50 µL of media from mw MEA to 96 well assay plate.

Follow Promega CytoTox® 96 Non-Radioactive Assay Kit Instructions.

5. Remove remaining 450 µL from all wells of mw MEA.
a) Add 200 µL of fresh media containing CellTiter Blue™ reagent (Promega; 1:6 dilution).
b) Incubate at 37 ºC for 1 h.
c) Transfer 150 µL of media with reagent to an opaque 96 well assay plate.
d) Measure fluorescence at 560Ex/590Em.

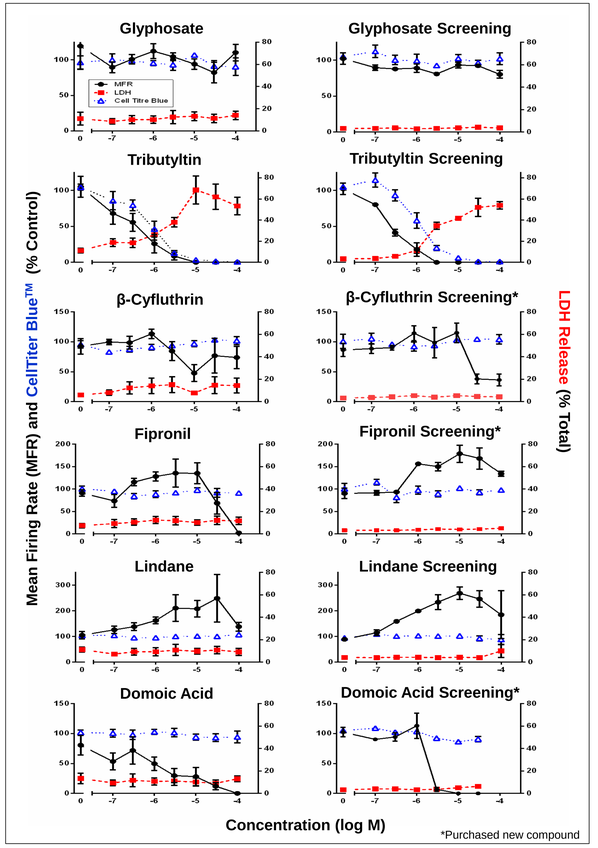
Both lactate dehydrogenase (LDH) and CellTiter Blue™ assays provide linear results up to 150K cells





Results

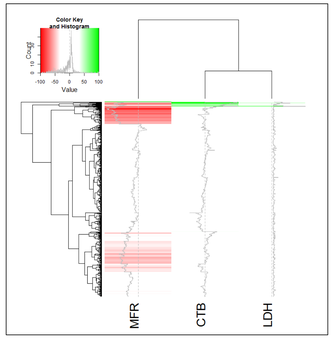
Effects on MFR, LDH and CellTiter Blue™ in the same well: The "Screening Mode" provides results equivalent to testing in multiple cultures at different times



*Purchased new compound

Application to ToxCast Screening

Using the Multiplexed Assay in the "Screening Mode" allows rapid and efficient screening of ToxCast Compounds



- Over 700 ToxCast Phase I and II Compounds Screened
  - Single concentration @ 40 µM
- Screening and Analysis are still ongoing
- Most are not overtly cytotoxic
- ~125 alter MFR beyond a conservative hit threshold

Conclusions

❖Combining measurements of MFR, LDH and CellTiter Blue™ from the same well provides a simple, rapid and economical method to determine compound effects on neural network activity and cell health.

❖Caveat: These methods cannot discriminate between neuronal and glial cytotoxicity

❖Results with these chemicals are similar to previous determinations. However, direct comparisons are confounded by differences in cell density and methodology for viability determination.

❖This multiplexed approach can also be applied to more prolonged or developmental exposure paradigms (see poster Brown et al.; Poster Board No. 446) or to other cell types (e.g. cardiomyocytes).

❖Testing compounds in a "Screening Mode" paradigm provides similar results as multiple experiments and is more efficient and economical.

❖These approaches can be useful when cells and/or compounds are limited or expensive (e.g. patient-derived iPSC neurons).

References

[1]McConnell ER, McClain MA, Ross J, Lefew WR, Shafer TJ. Evaluation of multi-well microelectrode arrays for neurotoxicity screening using a chemical training set. Neurotoxicology. 2012. 33, 1048-57.

[2]Valdivia P, Martin M, LeFew WR, Ross J, Houck KA, Shafer TJ. Multi-well microelectrode array recordings detect neuroactivity of ToxCast compounds. Neurotoxicology. 2014. 204-17.

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