Evaluating two analysis methods for the microelectrode array network formation assay

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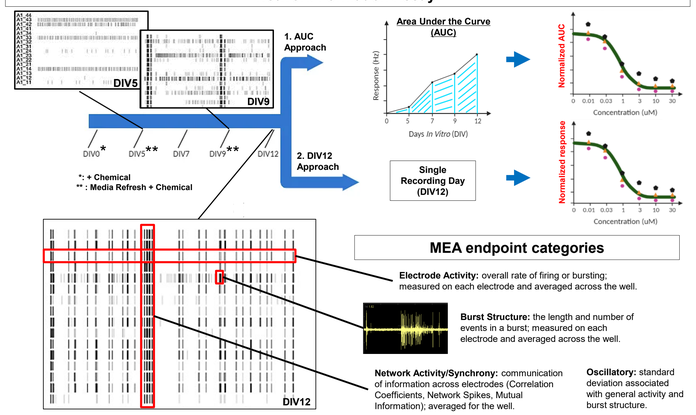
Introduction to the Microelectrode Array (MEA) and Methods

- *In vitro* new approach methods (NAMs) help evaluate developmental neurotoxicity (DNT) potential.
- Network Formation Assay (NFA)[1]: 12-day exposure model using MEAs to measure neuronal network activity changes in primary rat cortical cell cultures, including 17 endpoints measuring features such as bursting and network connectivity.
- An area under the curve (AUC) analysis is performed to compute a single value to represent total activity across four recording days in vitro (DIV) (5, 7, 9, and 12) for each chemical and endpoint. NFA data in the ToxCast database[2] are analyzed using an AUC analysis approach.
- Differences in bioactivity based on analysis of a single recording day versus using the AUC for all days has yet to be evaluated.
- **Hypothesis: The AUC method is a more sensitive readout of bioactivity compared to activity measured on a single recording day (DIV12) given that the AUC metric captures activity changes occuring on DIV5, 7, 9 and 12, while DIV12 activity only captures changes that are cumulative and lasting from the 12-day exposure.**
- Dataset included 396 chemicals screened in the NFA at a concentration range of 0-30μM; data were curve-fit using the ToxCast pipeline software (tcpl R package, tcpl v3.0)[3].

Network Formation Assay[4]



MEA endpoint categories

Electrode Activity: overall rate of firing or bursting; measured on each electrode and averaged across the well.

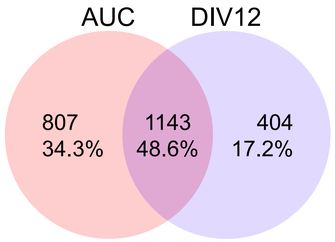
Burst Structure: the length and number of events in a burst; measured on each electrode and averaged across the well.

Network Activity/Synchrony: communication of information across electrodes (Correlation Coefficients, Network Spikes, Mutual Information); averaged for the well.

Oscillatory: standard deviation associated with general activity and burst structure.

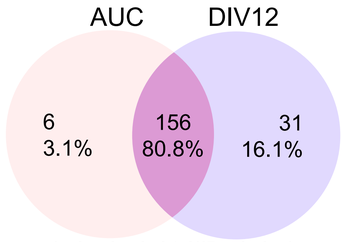
The DIV12 analysis method identified 31 active chemicals that were not identified as active by the AUC approach.

Concordance of activity by curve (chemical x endpoint)



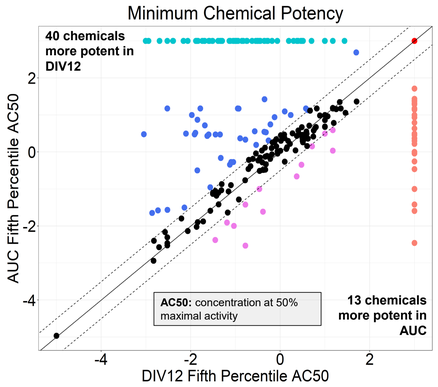
(tcpl hitcall ≥ 0.9)

Concordance of activity by chemical



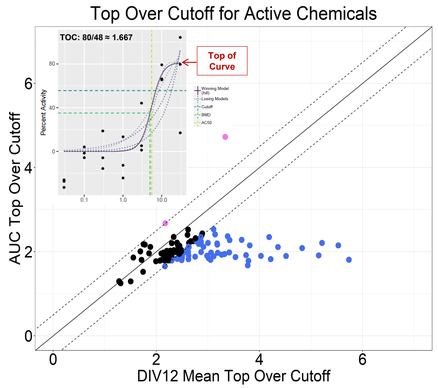
(active chemical: ≥ 3/17 endpoints)

DIV12 endpoints captured more potent minimum effect levels per chemical compared to the AUC.



Chemical Activity Identifier
- Chemicals Inactive in AUC
- Chemicals Inactive in DIV12
- Chemicals Inactive in DIV12 and AUC
- Chemicals More Potent in AUC
- Chemicals More Potent in DIV12

DIV12 endpoints detected larger effect sizes compared to AUC endpoints.



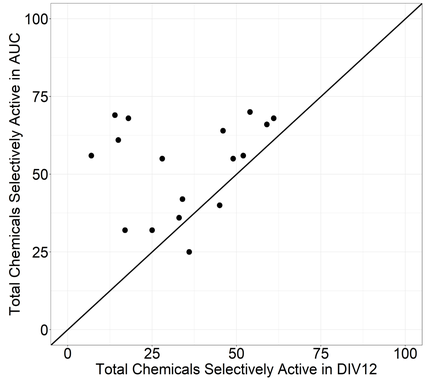
TOC Identifier
- TOC fell within 0.5 log10-μM in DIV12 and AUC
- TOC greater in AUC
- TOC greater in DIV12

AUC endpoints detect more selective activity by endpoint compared to the DIV12 endpoints.

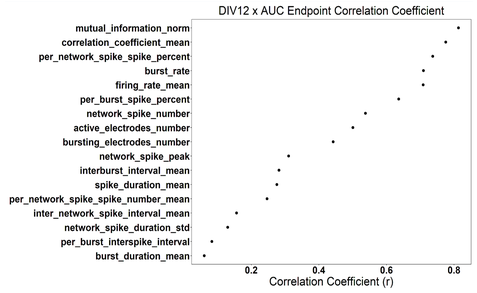
Number of Selectively Active Chemicals Per Endpoint

	Endpoint Name	DIV12	AUC
Network Activity	Active Electrodes Number	46	64
	Mean Internetwork Spike Interval	36	25
	Network Spike Number	28	55
	Network Spike Spike Percent	52	56
Electrode Activity	Bursting Electrodes Number	54	70
	Mean Firing Rate	34	42
	Per Burst Spike Percent	49	55
	Burst Rate	45	40
	Mean Interburst Interval	33	36
Network Synchrony	Mean Correlation Coefficient	59	66
	Mean Network Spike Spike Number	15	61
	Network Spike Peak	14	69
	Mean Spike Duration	18	68
	Normalized Mutual Information	61	68
Burst structure	Per Burst Interspike Interval	25	32
	Mean Burst Duration	17	32
Oscillatory	Network Spike Duration SD	7	56

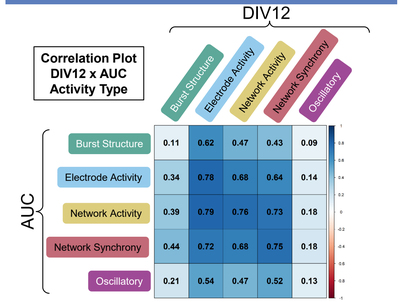
Selective activity was defined as a chemical that demonstrated an AC50 value below the threshold of cytotoxicity for a particular endpoint.



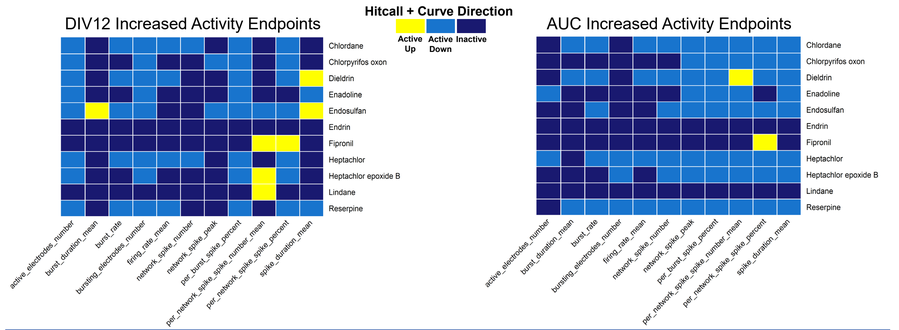
The mutual information endpoint demonstrated the highest correlation between AUC and DIV12 analyses based on selective hit call across 396 chemicals.



Endpoints measuring changes in Electrode Activity were most correlated between DIV12 and AUC.



Neither the DIV12 nor AUC endpoints detected informative changes in the increased activity direction for a subset of chemicals known to increase activity in the MEA acute exposure assay.



Summary and Future Directions

Conclusions:

- The AUC analysis had a higher hit rate of active curves overall compared to the DIV12 analysis.
- The DIV12 endpoints detected more active chemicals (≥3/17 selective hits per chemical) compared to the AUC analysis approach.
- Potency and efficacy estimates suggest that the DIV12 endpoints may capture more sensitive minimum effect levels and larger effect sizes compared to AUC for a subset of chemicals.
- The DIV12 endpoints captured a few more active endpoints in the increased activity direction compared to the AUC, although both methods detected minimal changes in increased activity. This might suggest that changes in increased activity may be transient and/or low efficacy in the NFA and may not be detectable using either approach. Additional testing of chemicals known to increase neural activity may improve the interpretation of this result.

Future Directions: These results indicate that the inclusion of DIV12 endpoints may be additionally informative for detecting bioactivity changes in the NFA. Future studies are needed to explore how the DIV12 endpoints, in addition to endpoints measured on earlier days *in vitro* (i.e. DIV5, 7, 9), may inform distinct bioactivity profiles, with the ultimate goal of building a predictive model of DNT potential using a suite of informative NFA endpoints.

References:

1. Brown, J. P., Hall, D., Frank, C. L., Wallace, K., Mundy, W. R., & Shafer, T. J. (2016). Evaluation of a microelectrode array-based assay for neural network ontogeny using training set chemicals. *Toxicological Sciences*,1-14. doi: 10.1093/toxsci/kfw147
2. Feshuk, M., Kolaczkowski, L., Dunham, K., Davidson-Fritz, S. E., Carstens, K. E., Brown, J.,Judson, R.S., & Paul Friedman, K. (2023). The ToxCast pipeline: updates to curve-fitting approaches and database structure. *Frontiers in Toxicology*. doi: 10.3389/ftox.2023.1275980
3. https://cran.r-project.org/web/packages/tcpl/index.html
4. Image was adapted from Shafer et al., Image was created using *BioRender*.