Evaluation of the ToxCast Assay Suite for the Detection of Neuroactivity

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Computational Toxicology I

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Introduction

Background

- The U.S. EPA has developed a tiered testing approach for screening thousands of data-poor substances.[1]
- We hypothesized that high-throughput screening assays in the U.S. EPA's Toxicity Forecaster (ToxCast) suite can qualitatively and quantitatively detect neuroactive substances, demonstrating their potential to serve as a second-tier screen for neuroactivity.

Approach

366 neuroactive substances

with evidence of *in vivo* neuroactivity based on:
- manual curation of published literature
- expert-knowledge
- neurotoxicity data in the U.S. EPA's Toxicity Values database

1,668 ToxCast assay endpoints

383 nervous-system relevant (NSR) | **1,285 other**

202 with gene target that is elevated in the nervous system (according to Human Protein Atlas[2,3])*

181 that use a neuronal cell model or tissue*

* except for ABC or nucleoside transporters or endocrine-related proteins

Excluded hit calls based on:
- 3 or more caution flags
- AC_{50} < minimum concentration tested and model top < 20% above the cutoff
- cell viability assay with a gain-loss model fit

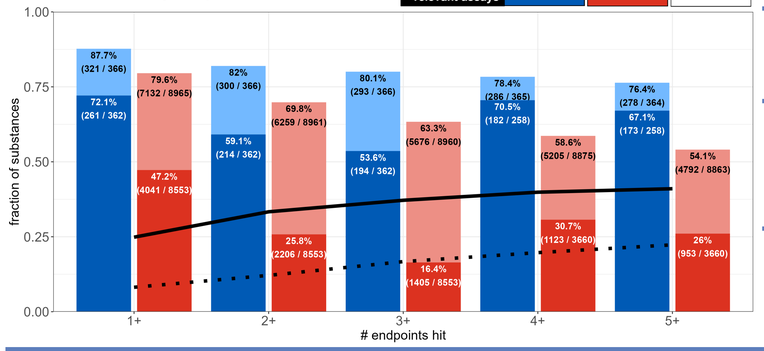
Primary questions

- Are neuroactive substances detected qualitatively in ToxCast?
- Do the undetected neuroactive substances reveal any biological gaps in ToxCast?
- Are endpoints derived from whole-cell neuronal assays more sensitive to neuroactive substances?
- Are neuroactive substances detected at lower concentrations in NSR assays than other assays?

ToxCast detects activity from 88% of neuroactive substances

Fraction of substances detected by substance type and assay subset, for substances tested in >=1 of the 1,668 ToxCast assay endpoints

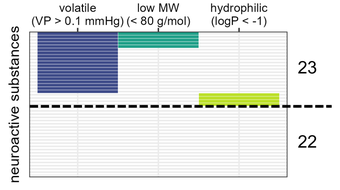
	neuroactive substances	other tested substances	difference % detected
All ToxCast	% detected	% detected	·····
Nervous-system relevant assays	% detected	% detected	——



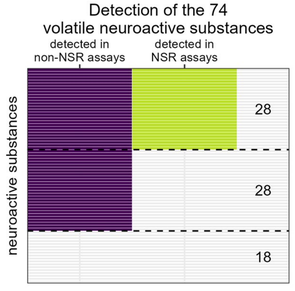
- 88% of neuroactive substances are detected in >=1 ToxCast endpoint.
- 80% of other tested substances are also detected in >= 1 ToxCast endpoint.
- NSR assay endpoints provide comparable sensitivity with higher specificity for neuroactive substances.
- Difference in % neuroactive vs. other substances detected increases with higher hit count threshold.

Neuroactive substances not detected in ToxCast are largely volatile or have not been extensively tested in NSR assays

45 neuroactive substances were tested in >= 3 ToxCast endpoints but not detected. 23 may have physicochemical properties that are not be amenable to *in vitro* screening.



Low detection rates of semi-volatile neuroactive substances (24% in NSR assays, 62% in non-NSR assays) may indicate a shortcoming in ToxCast, particularly in the NSR assays.

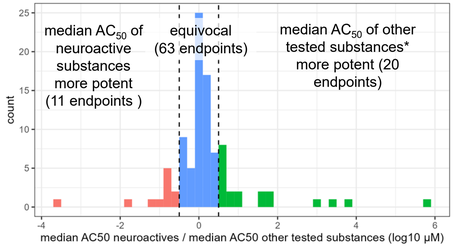


22 remain undetected. Possible explanations for lack of activity may include lack of testing (particularly in NSR assays) or possibly insufficient concentration tested. For 6 substances (*), we identified an alternative salt form with activity, most of which were more extensively tested. Testing more chemicals may be necessary to demonstrate lack of sensitivity or lack of molecular targets in NSR assays.

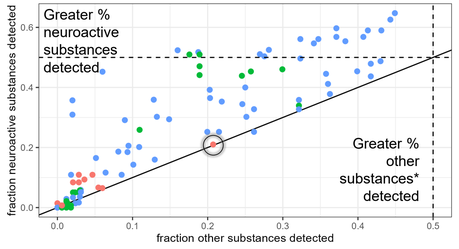
	Chemical	# NSR tested	# non-NSR tested	Other forms tested (# hit / # tested) in any assay
*	Cyclophosphamide	0	3	Cyclophosphamide monohydrate (13/1065)
*	Paraquat	0	4	Paraquat dichloride (30/57)
	Formothion	0	7	-
*	3,6-Diacetylmorphine	3	76	(-)-Heroin hydrochloride (3/79)
*	Ketamine	3	76	Esketamine hydrochloride (6/52) (+)-Ketamine (0/79) Esketamine (0/79)
	Bemegride	3	76	-
	Pentylenetetrazol	3	76	-
*	Sodium barbital	3	232	Barbital (3/235)
	Naloxone	3	232	Naloxone hydrochloride dihydrate (0/57)
	2-N-Dibutylaminoethanol	3	232	-
	4-Methylimidazole	3	232	-
	Amprolium hydrochloride	3	232	-
	Demeton	3	232	-
	Glutethimide	3	232	-
	Hexachlorobenzene	3	232	-
	Mephenoxalone	3	232	-
	Meprobamate	3	232	-
	Metoclopramide	3	232	-
	Pralidoxime chloride	3	232	-
	2-Methylimidazole	17	305	-
*	Phenobarbital	39	232	Phenobarbital sodium (23/1124)
	1,4-Butanediol	51	355	-

Most whole-cell NSR endpoints appear qualitatively but not quantitatively more sensitive toward neuroactive substances than other tested substances

Distribution of median AC_{50} ratios of neuroactive:other substances tested for whole-cell neuronal assay endpoints



Hit rate comparison by substance type for whole-cell neuronal assay endpoints

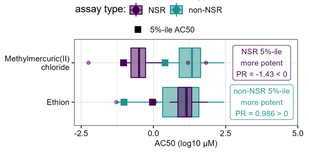


- We hypothesized that the 94 endpoints derived from microelectrode array recordings of neuronal networks, high-content imaging of developing neurons, and zebrafish brain morphogenesis are more sensitive toward neuroactive substances.
- Quantitatively, only 11/94 whole-cell neuronal endpoints have a median concentration that caused a 50% of maximal response (AC_{50}) of neuroactives >0.5 $\log_{10}$ µM more potent than the median AC_{50} of other substances.
- Qualitatively, 84/94 whole-cell neuronal endpoints detect a greater percentage of neuroactive than other substances tested.
- 10/11 endpoints with more potent median AC_{50} of neuroactive vs. other tested substances have low hit rates, indicating that potency shifts may be driven by a few specific chemicals rather than indicative of general trends.
- An endpoint measuring a decrease in the robustness of coordinated activity in a neuronal network after an acute exposure (◯) has a more substantial hit rate for both substance types as well as a relatively potent median AC_{50} of neuroactive substances.

* Other tested substances may include salts or stereoisomers of neuroactive substances, which may cause an apparent lack of specificity towards neuroactive substances.

Potency in NSR versus other assays varies by substance

Example distribution of AC_{50}s by assay type

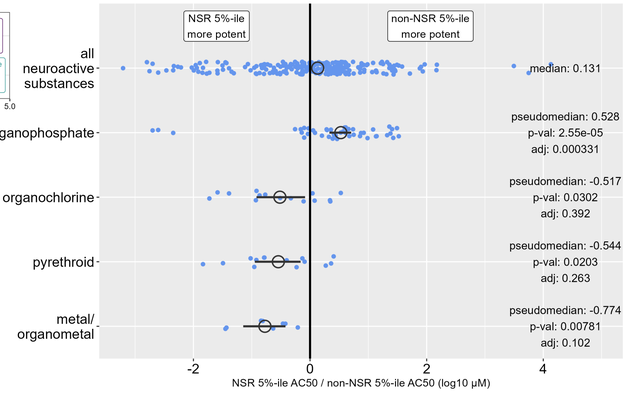


- Calculation of Potency Ratio (PR): The lower 5th percentile of concentrations that caused a 50% of maximal response (AC_{50}) in NSR assays was compared to the 5th percentile AC_{50} in non-NSR assays.

$$PR = \log_{10}\left(\frac{5\%ile\ AC_{50}\ NSR\ assays}{5\%ile\ AC_{50}\ non\ NSR\ assays}\right)$$

- PR < 0 indicated greater potency in NSR assays for 103/238 = 43.3% of neuroactive substances with sufficient data.

Distribution of 5%-ile AC_{50} ratios of NSR:non-NSR assay endpoints with Wilcoxon signed rank test 95% confidence intervals



- Wilcoxon signed rank test: Tested for shift in distribution of PRs towards greater potency in NSR or non-NSR assays by chemical class.
- PR's for organophosphates shifted ~0.5 $\log_{10}$ µM towards greater potency in non-NSR assays.
- Several organochlorines, pyrethroids, and metals have PR < 0, though trend is not statistically significant after Bonferroni adjustment by chemical class.

Concluding remarks

Summary

- 88% of neuroactives substances are active in >=1 ToxCast assay endpoint; 72% are active in >=1 NSR assay endpoint.
- Lack of detection in the remaining 12% of neuroactive substances may be due problematic physicochemical properties for some. The number and lack of testing of the remaining undetected neuroactive substances seems insufficient to reveal a gap in the biological space for the detection of neuroactivity.
- Most whole-cell neuronal assay endpoints detect a greater percentage of neuroactive than other substances tested.
- Trends for metals, pyrethroids, and organochlorines suggest that NSR assays may detect activity at lower concentrations than other assays.

Conclusions

- Overall, the majority of neuroactive substances evaluated here were detected by ToxCast assays.
- NSR assays will likely play a role in detecting neuroactive substances at sufficiently sensitive concentrations.

Future directions

- Use *in vivo* to *in vitro* extrapolation to compare concentrations at which neurological effects are seen *in vivo* to the concentrations at which activity is observed *in vitro*.
- Assess the activity of neuroactive substances with a known mechanism of action in appropriate target-based assays.

1. Russell S Thomas et al., "The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency," *Toxicological Sciences* 169, no. 2 (June 2019): 317–332. https://doi.org/10.1093/toxsci/kfz058.

2. Human Protein Atlas proteinatlas.org

3. Mathias Uhlén et al., "Tissue-based map of the human proteome," *Science* 347, no. 6220 (January 2015). https://doi.org/10.1126/science.1260419.

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