Evaluation of the ToxCast Assay Suite for the Detection of Neuroactivity

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Introduction

Background

- The U.S. EPA has developed a tiered testing approach for screening thousands of data-poor compounds.[1]
- Tier 2 assays are needed to confirm potentially neuroactive compounds identified in Tier 1 assays.
- We hypothesized that high-throughput screening assays in the U.S. EPA's Toxicity Forecaster (ToxCast) suite can qualitatively and quantitatively detect neuroactive compounds.

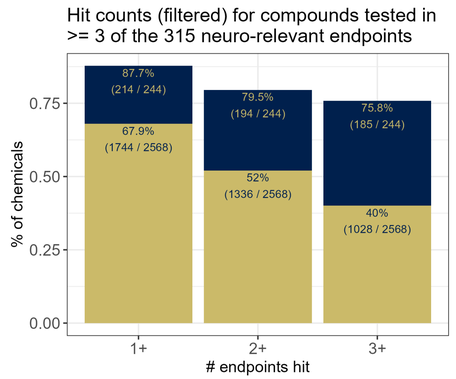
Approach

- We identified 244 compounds with evidence of *in vivo* neuroactivity based on manual curation of published literature, expert-knowledge, and neurotoxicity data in the U.S. EPA's Toxicity Values database.
- Collected ToxCast data from 1,789 assay endpoints.
- Excluded hit calls based on a) 3 or more caution flags, b) AC_{50} < minimum concentration tested and model top < 20% above the cutoff, and c) cell viability assay with a gain-loss model fit.
- Identified 315 neurologically-relevant (NR) assay endpoints based on neuronal cell model or neurologically-relevant target.

Primary questions

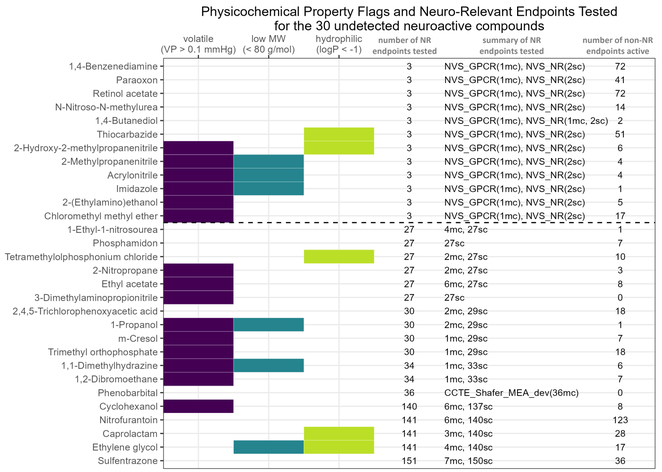
- Are neuroactive compounds detected qualitatively in ToxCast?
- Do the undetected neuroactive compounds reveal any concerning gaps in ToxCast?
- What is a minimal set of NR assay endpoints that can detect all neuroactive compounds?
- Do the NR assays detect activity from neuroactive compounds at lower concentrations than other assays?

ToxCast assays detect 88% of neuroactive compounds



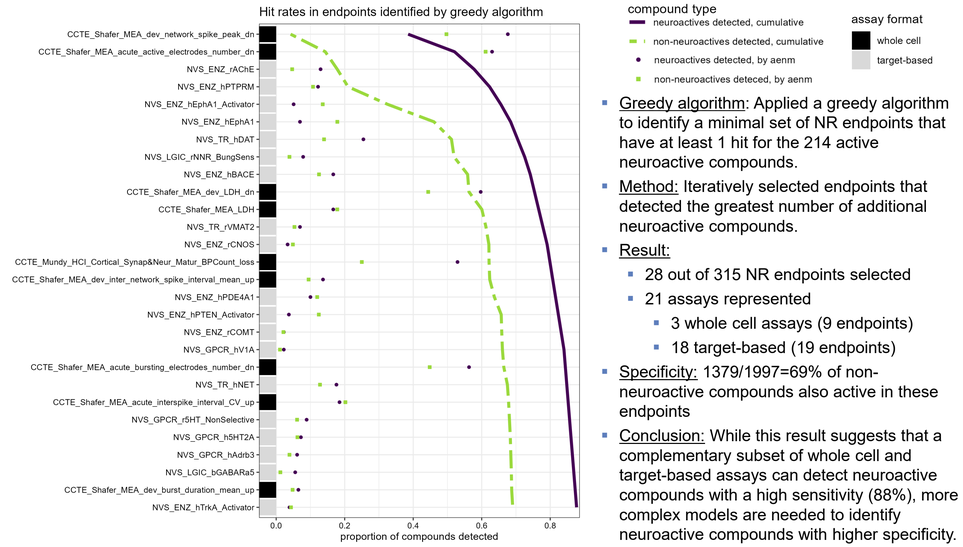
- 214/244=88% of neuroactive compounds tested are active in >= 1 endpoint.
- 68% of non-neuroactive compounds also active in >= 1 endpoint.
- As the hit count threshold increases, the difference between the percentage of neuroactive and non-neuroactive compounds increases.
- All neuroactive compounds with activity are also active in other ToxCast assays.

Neuroactive compounds not detected by neuro-relevant assays are largely volatile or insufficiently tested



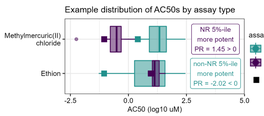
- **Possible explanations for lack of activity**:
  - **Lack of testing:** 12/30 compounds only tested in 3 endpoints
  - **Physicochemical properties:** some compounds may not be amenable to *in vitro* screening
  - **Dose:** possibly insufficient concentration tested, especially for compounds with low molecular weight
- All except Phenobarbital and 3-Dimethylaminoproprionitrile are active in other ToxCast assays
- These 30 compounds highlight known issues regarding physicochemical properties, but do not suggest major gaps in the biological space for detection of neuroactivity.

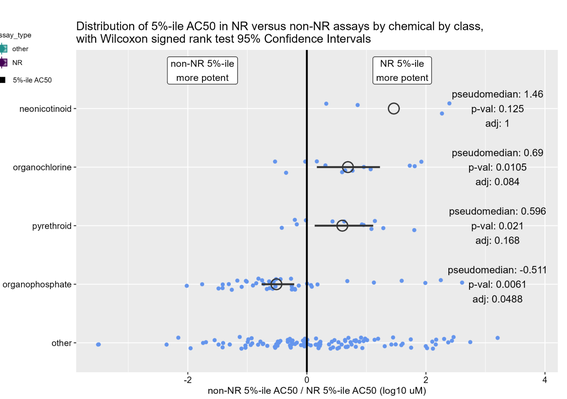
28 assay endpoints are sufficient to detect all active neuroactive compounds



- Greedy algorithm: Applied a greedy algorithm to identify a minimal set of NR endpoints that have at least 1 hit for the 214 active neuroactive compounds.
- Method: Iteratively selected endpoints that detected the greatest number of additional neuroactive compounds.
- Result:
  - 28 out of 315 NR endpoints selected
  - 21 assays represented
    - 3 whole cell assays (9 endpoints)
    - 18 target-based (19 endpoints)
- Specificity: 1379/1997=69% of non-neuroactive compounds also active in these endpoints
- Conclusion: While this result suggests that a complementary subset of whole cell and target-based assays can detect neuroactive compounds with a high sensitivity (88%), more complex models are needed to identify neuroactive compounds with higher specificity.

Potency in neuro-relevant versus other assays varies by compound





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

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

- PR > 0 indicated greater potency in NR assays for 94/185 = 51% of neuroactive compounds with sufficient data.
- Wilcoxon signed rank test: Tested for shift in distribution of PRs towards greater potency in NR or non-NR assays by chemical class.
- PR's for organophosphates shifted ~0.5 log-units towards greater potency in non-NR assays
- Several neonicotinoids, organochlorines, and pyrethroids have PR > 0, though trend is not statistically significantly by chemical class.

Summary and Future Directions

Summary

- 88% of neuroactive compounds are active in at least 1 NR assay.
- Lack of detection in the remaining 12% of neuroactive compounds seems to be due to lack of testing and/or problematic physicochemical properties.
- A set of 28 endpoints from 3 whole cell and 18 target-based assays are sufficient to capture activity from 88% of neuroactive compounds, as well as 69% of non-neuroactive compounds.
- Trends for neonicotinoids, organochlorines and pyrethroids suggest that NR assays may detect activity at lower concentrations than other assays.

Conclusions

- Overall, ToxCast assays detect the vast majority of neuroactive compounds.
- Both whole cell and target-based NR assays could serve as a second tier screen to support identification of putative mechanism or targets and inform potency for neuro-relevant targets, processes, and cells.

Future Directions

- Explore models that utilize all NR assay data to identify neuroactive compounds with higher specificity.
- Assess the activity of neuroactive chemicals with a known mechanism of action in appropriate target-based and whole cell neuronal assays.
- Use *in vivo* to *in vitro* extrapolation to compare concentrations in at which neurological effects are seen *in vivo* to the concentrations at which activity is observed *in vitro*.

[1] Thomas, R. S., Bahadori, T., Buckley, T. J., Cowden, J., Deisenroth, C., Dionisio, K. L., Frithsen, J. B., Grulke, C. M., Gwinn, M. R., Harrill, J. A., Higuchi, M., Houck, K. A., Hughes, M. F., Hunter, E. S., Isaacs, K. K., Judson, R. S., Knudsen, T. B., Lambert, J. C., Linnenbrink, M., Martin, T. M., … Williams, A. J. (2019). The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. *Toxicological sciences : an official journal of the Society of Toxicology*, *169*(2), 317–332. https://doi.org/10.1093/toxsci/kfz058

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