Improving the stability and reproducibility of clinical neurotoxicity predictions from a high-throughput compatible neural organoid platform

AxoSim

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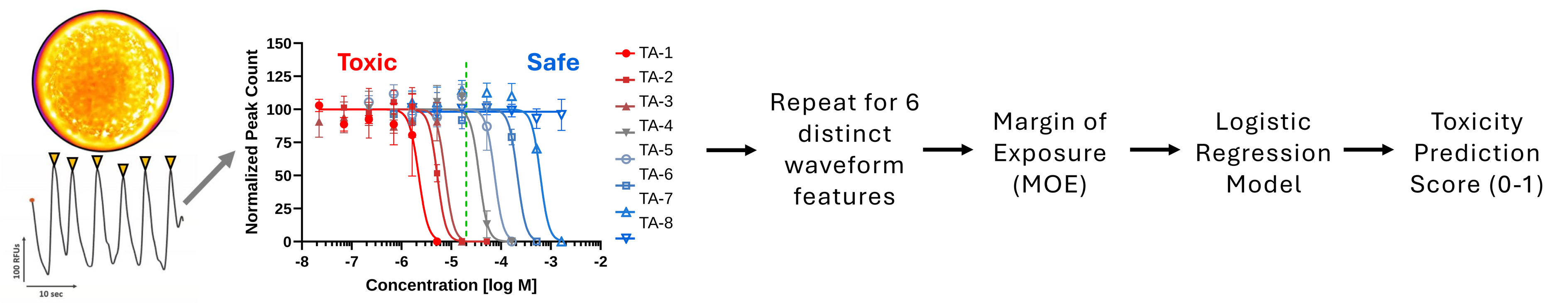
Background and Purpose

The drug development process is fraught with failure due to either safety issues or poor efficacy. When considering safety profile, neurotoxicity is the leading cause of clinical failure [1]. Furthermore, 12% of drugs withdrawn between 1960-1999 were caused by neuro-related adverse events [2]. The use of complex in vitro models (CIVM) derived from human tissue has dramatically expanded in recent years, promising to provide the necessary biological complexity to improve clinical translation and scale to enable adoption early in drug development pipelines. We have developed a cortical brain organoid model that exhibits robust spontaneous "waveform" activity that is compatible with HTS methodology and provides a clinically-relevant endpoint for phenotypic profiling. In 2022, this organoid platform was used to develop a predictive clinical neurotoxicity model that showed remarkable specificity (>90%) and good sensitivity (>50%), making it an ideal pre-screening method prior to standard 2-species animal testing [3]. Here, we tested the stability and reproducibility of these predictions over time and used these replicate experiments to refine and automate neurotoxicity score predictions.

Methods

3D cortical organoids were derived from healthy donor iPSCs, which were differentiated into NPCs, then seeded seeded into ultra-low attachment 384-well plates, wherein they self-organize and co-differentiate into cortical neurons and astrocytes. After 10 weeks of differentiation, once cultures exhibited strong coordinated network activity, acute (0 - 4 hours) neuromodulation screening of 84 known neurotoxic and safe compounds was performed using a calcium flux assay and high-throughput kinetic plate reader (FLIPR).

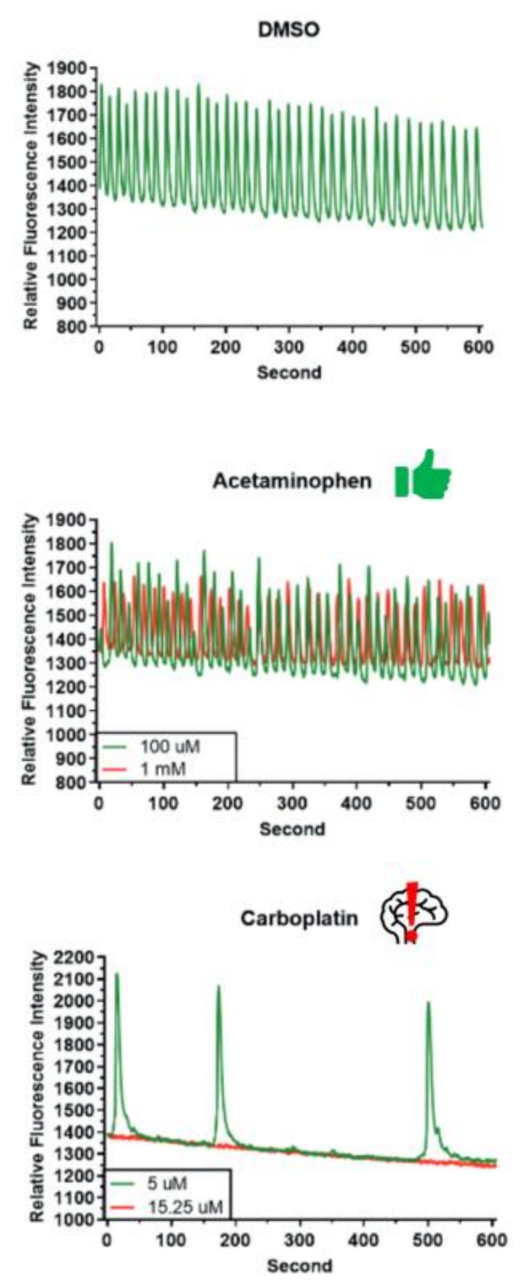


Changes in the number, size, shape, and variability of the spontaneous activity waveforms were quantified using custom written code in Python. A margin of exposure (MOE) value was calculated for each waveform feature as the ratio of total plasma C_{max} (tpC_{max}) to the EC/IC_{50}. MOE values were used to train and test a logistic regression model to predict safe (category 1) or neurotoxic (category 2, 3, 4) compounds.

	Sets	Neurotoxicity severity category[a]		Drugs
Model Training	CNS-targeting set (N = 58)	1. Negative (N = 17)	👍	Tolcapone, pimavanserin, L-DOPS (droxidopa), sertindole, duloxetine HCl, galantamine, zotepine, talipexole, ralfinamide, MK-8931 (verubecestat), levodopa, benzatropine mesylate, bicifadine, tasimelteon, reserpine, lazabemide, moclobemide
		2. Rare (N = 9)	neurotoxic	Varenicline tartrate, mifepristone, maprotiline, rotigotine, ketorolac, pramipexole, chlorprothixene, almotriptan malate, lidocaine
		3. Infrequent (N = 13)		Enoxacin, pergolide, cefazolin, sertraline, safinamide, propranolol HCl, cyclobenzaprine, ropinirole, memantine HCl, riluzole, ropivacaine, atomoxetine HCl, aripiprazole
		4. Frequent (N = 8)		Rivastigmine, chlorpromazine, theophylline, aminophylline, bupropion, donepezil HCl, BIA 10-2474, indomethacin
		5. Anticonvulsant (N = 11)		Topiramate, lamotrigine, divalproex Na, levetiracetam, oxcarbazepine, ziconotide, carbamazepine, valproic acid, zonisamide, vigabatrin, tetrabenazine
Model Testing	Test set (N = 26)	1. Negative (N = 13)	👍	Pilocarpine HCl, atropine, flupirtine, ambroxol, nifedipine, ibuprofen, acetaminophen, aspirin, naproxen, diclofenac, buspirone HCl, fluoxetine HCl, paroxetine
		2. Rare (N = 2)	neurotoxic	Simvastatin, pravastatin
		3. Infrequent (N = 1)		Gabapentin
		4. Frequent (N = 10)		Doxorubicin, cisplatin, paclitaxel, vincristine, carboplatin, ixabepilone, isoniazid, colchicine, aminopyridine (dalfampridine), phenytoin

Modified from Wang et al., ALTEX 2022 (Takeda)

Compounds were classified into 1 of 4 categories based on its clinical adverse event rate: 1: negative (<0.01%), 2: rare (0.01-0.1%), 3: infrequent (0.1%-1%), 4: frequent (>1%). Dosing concentrations were selected to span 0.1x – 100x the in vivo Cmax in 7-point dose response.

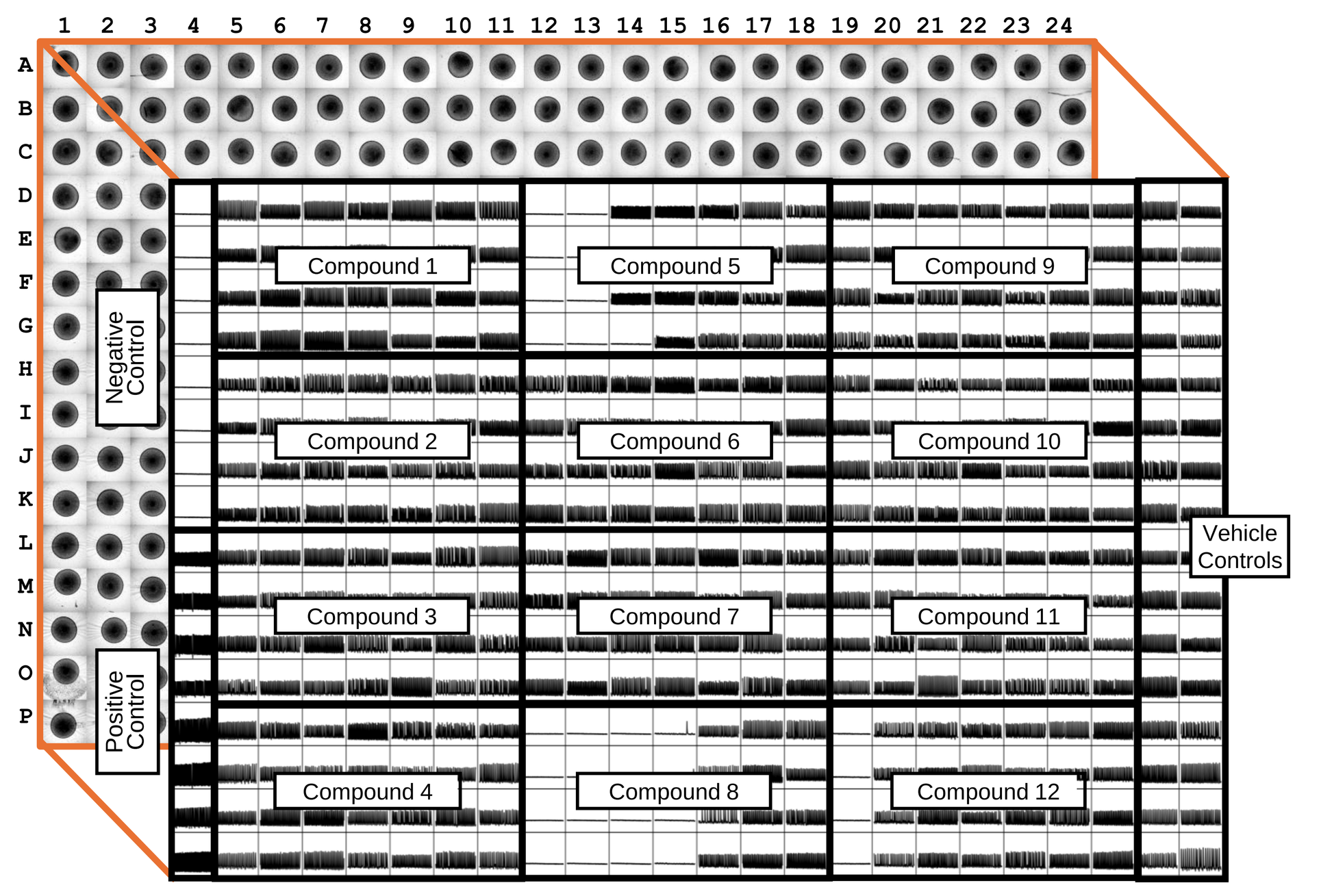


Normalize activity data to vehicle control wells

Safe compounds show little-to-no functional changes

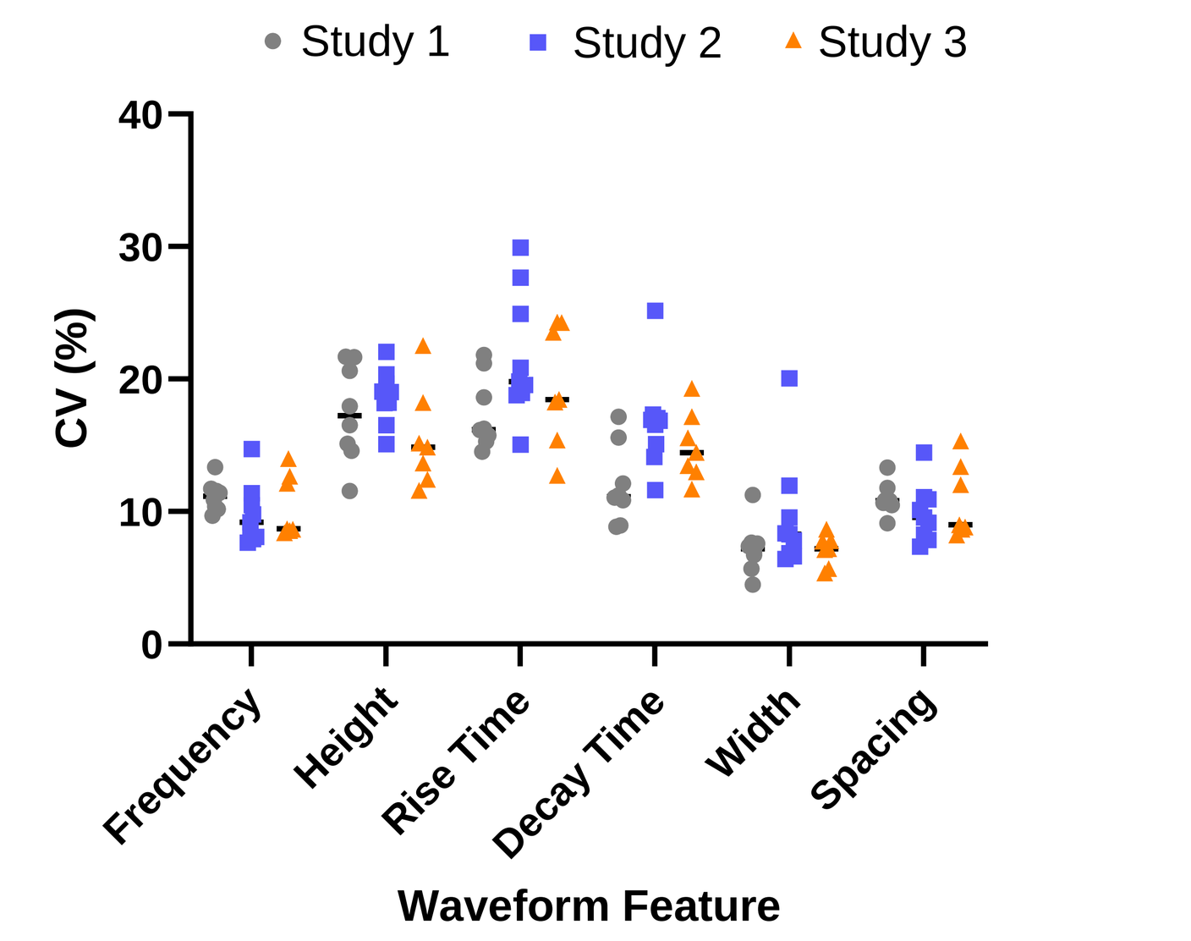
Toxic compounds show strong modulation and functional deterioration

Consistency of Cortical Organoid Function



1 Study = 7 plates = 84 compounds

Representative plate-view of waveform traces shows consistency between replicate organoids.



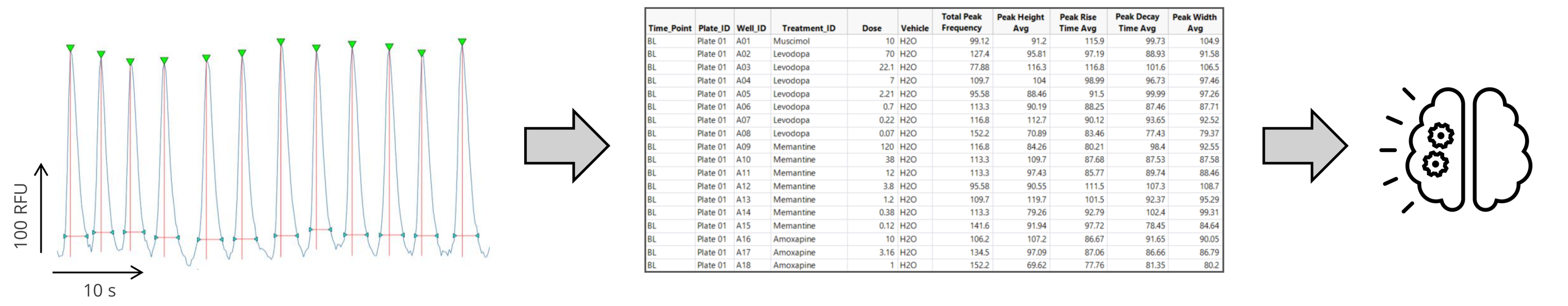
Cortical organoids exhibited consistent spontaneous functional activity at 10 weeks of differentiation across multiple studies.

Building an Automated Analysis Pipeline

Three automations were used to stabilize predictions and remove user-bias from waveform analysis.

1 Remove user-bias from peak detection

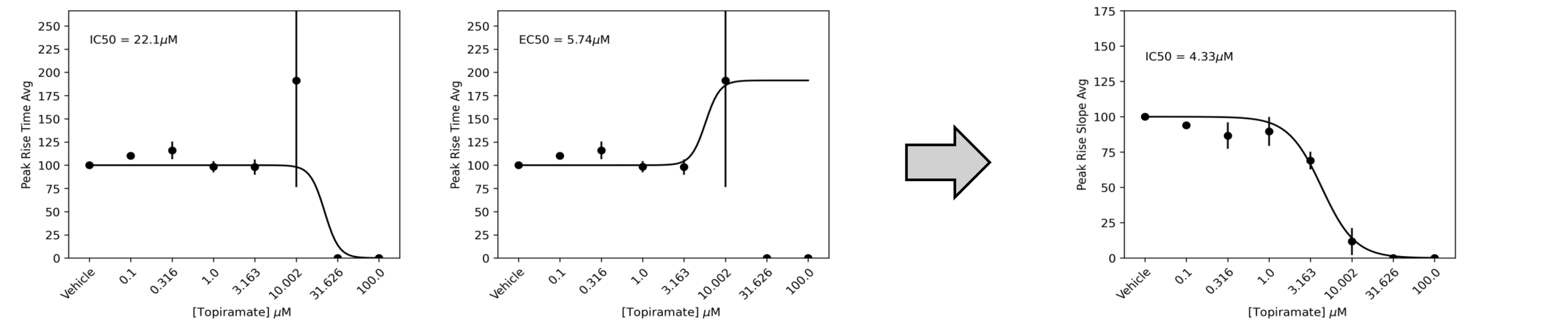
AnalytiX™ software detects and quantifies six robust features describing the number, size, and shape of organoid spontaneous activity bursts, which are then utilized in the clinical neurotoxicity prediction model.



2 Adjust features describing peak shape

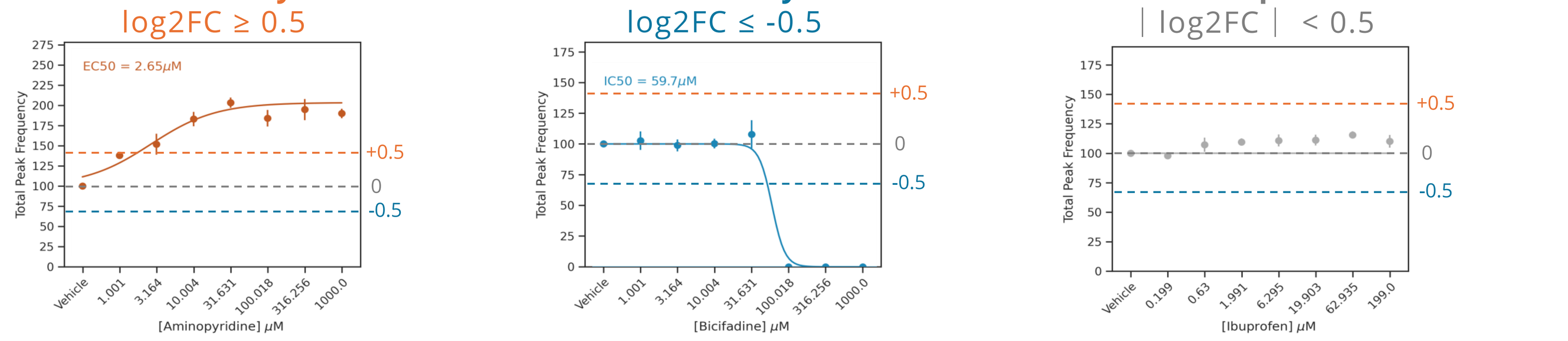
TIME features - Difficult to assess potency in rise and decay time features that often display biphasic responses.

SLOPE features - Monotonic responses are amenable to standard IC_{50} curve fitting.



3 Automate detection of excitatory vs inhibitory compound responses

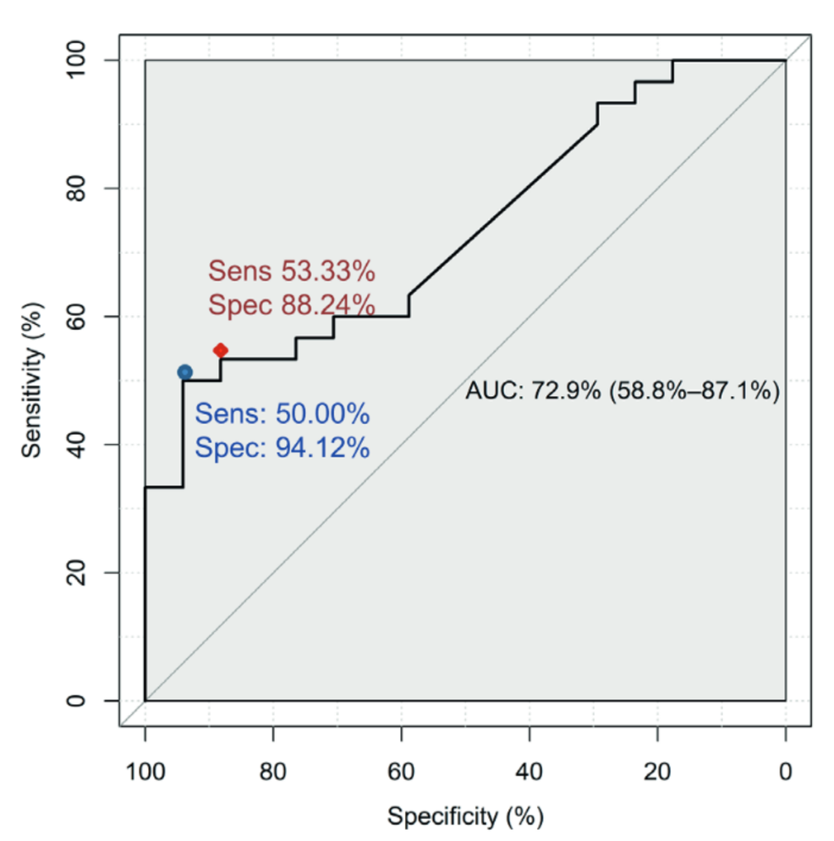
Quantitative criteria were selected to automate response-type assignments to each waveform feature.



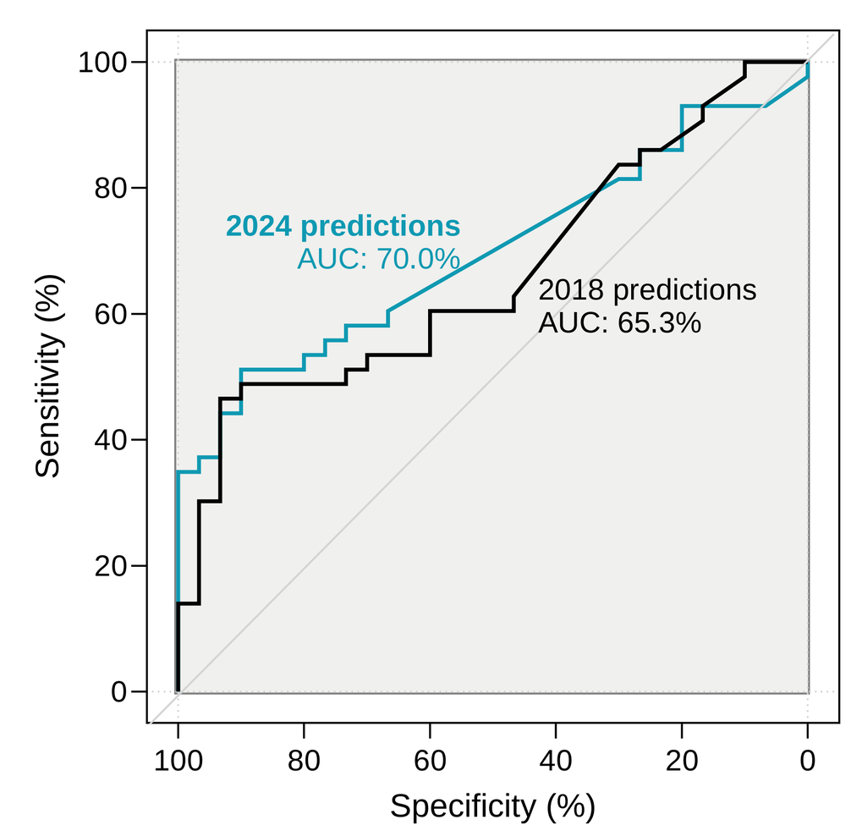
Reproducible Clinical Neurotoxicity Predictions

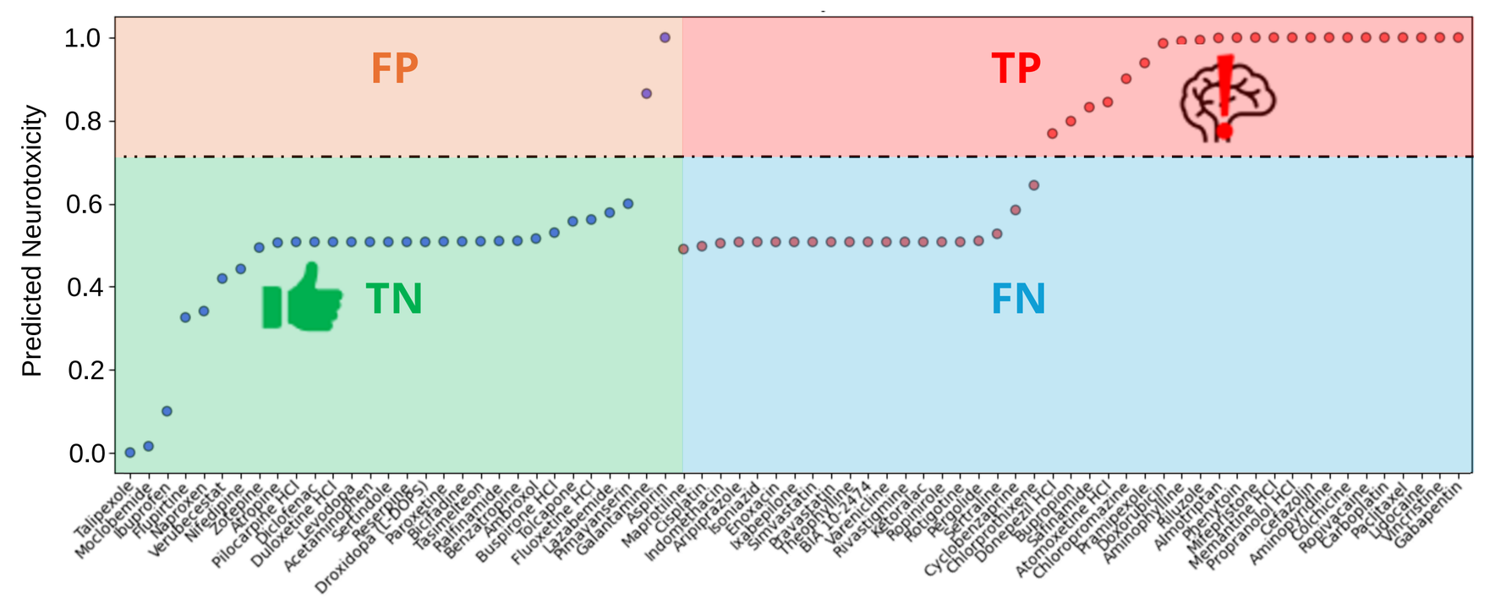
Clinical neurotoxicity predictions, originally published by Takeda [3] were replicated years later in a different lab using a new cell bank. The automated analysis pipeline did not alter model performance on the original dataset (left) and revealed highly-reproducible model performance when trained on the 2024 dataset (right)

Wang *et al.*, ALTEX 2022



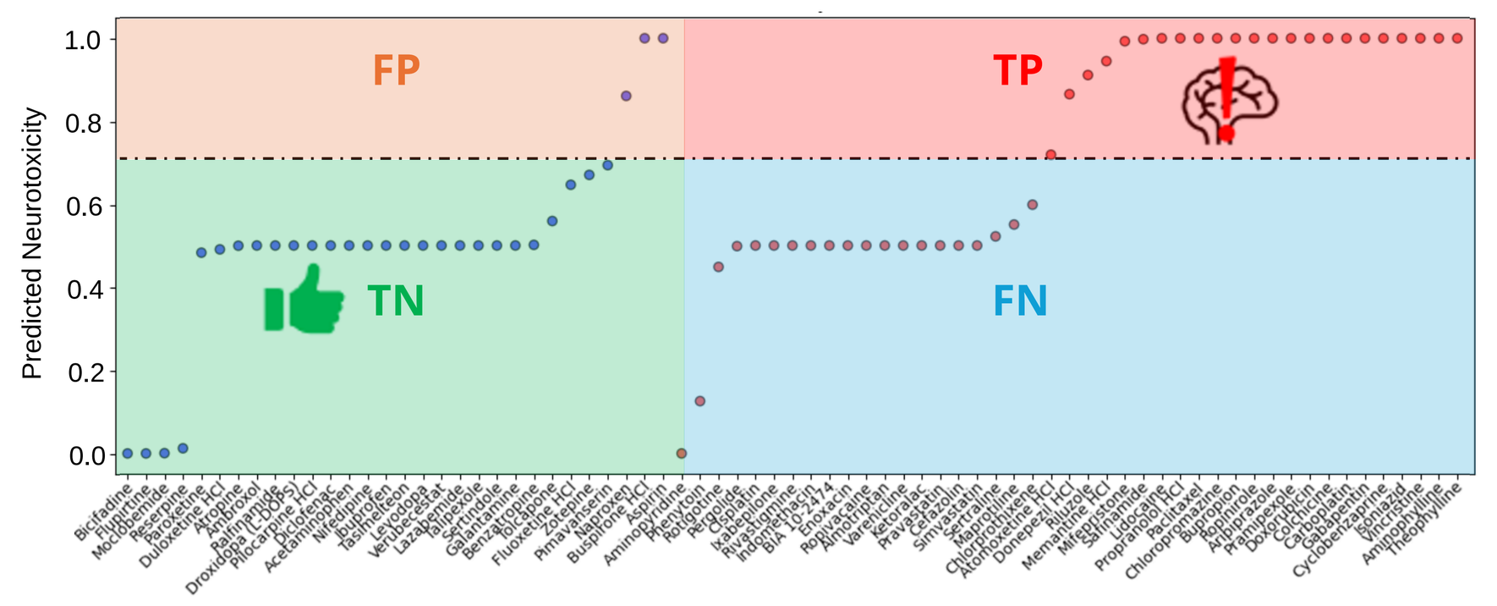
Current work





Sensitivity = 23/43 (**53.5%**)
...of toxic drugs were identified as toxic

Specificity = 28/30 (**93.3%**)
...of safe drugs were identified as safe



Sensitivity = 23/43 (**53.5%**)
...of toxic drugs were identified as toxic

Specificity = 27/30 (**90.0%**)
...of safe drugs were identified as safe

Conclusions & Future Directions

- Functional measurements from human iPSC-derived cortical brain organoids predict clinical neurotoxicity with high specificity (≥90%) and good sensitivity (>50%).
- The stability of neurotoxicity predictions is driven by the reproducibility of the organoid model and was further enhanced through improved peak detection, waveform feature engineering, and automated potency calculations.
- High specificity was maintained across independent experiments conducted at different sites over multiple years with various cell banks, demonstrating model robustness.
- This CIVM approach can enhance preclinical drug screening by identifying neurotoxicity risks without prematurely eliminating viable drug candidates.
- Implementation in drug development pipelines may reduce costly clinical failures by improving the quality of drug candidates before human trials.

References

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[2] Valentin JP, Bialecki R, Ewart L, Hammond T, Leishmann D, Lindgren S, Martinez V, Pollard C, Redfern W, Wallis R. A framework to assess the translation of safety pharmacology data to humans. J. of Pharm. and Tox. Methods. 2009 Sep 1;60(2):152-8.

[3] Wang Q, Cohen JD, Yukawa T, Estrella H, Leonard C, Nunes J, Choi C, Mishra N, Lewis L, Baker KS, Kuga K. Assessment of a 3D neural spheroid model to detect pharmaceutical-induced neurotoxicity. ALTEX. 2022 Oct 18;39(4):560-82.