

Detection of seizurogenic compounds in human and rat neurons in vitro: A multi-laboratory, multi-model assessment

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ABSTRACT

Seizure liability remains a key risk for drug development and following chemical exposure. Advances in induced pluripotent stem cell (iPSC) biology and in vitro detection methodologies such as multielectrode array (MEA) offer an opportunity for a new paradigm in compound screening. Thus, a coordinated effort is needed to determine the methods, models, and parameters that are optimal for larger scale nonclinical assessment for seizure liability. Here we present the results of a multi-laboratory MEA assessment of seizurogenic detection in both human iPSC-derived neural cultures and primary rat cortical neurons. We evaluated the impact of 10 pro-convulsant compounds and 3 negative controls on spontaneous electrical activity in both types of neurons utilizing MEA. The work was conducted at 7 different laboratories across 3 continents. Both rat and human models showed similar changes to common metrics such as mean of interspike distance (mean ISI) and median burst rate across facilities and across all compounds, with more consistency in the human model. Regarding differences between compounds, seizurogenic compounds caused the largest changes in MEA profile and the parameter with the most commonality was mean ISI, which was decreased in all tested compounds except picrotoxin. Overall, all laboratories generated MEA data indicative of a seizurogenic phenotype but the human model was more consistent across sites, despite some variation in protocols. Lessons learned from this work include having a clear

Abbreviations: 3Rs, reduction, refinement, replacement; ACH R, Acetylcholine receptor; CDER, FDA Center for Drug Evaluation and Research; CNS, Central nervous system; COU, context of use; COX-2, Cyclooxygenase-2; CV, coefficient of variation; D2R, Dopamine D2 Receptor; DIV, days in vitro; EEG, electroencephalogram; GABA, Gamma Aminobutyric Acid; GABAAR, Gamma Aminobutyric Acid Receptor & Ligand Gated Channel; HiPSC, Human induced pluripotent stem cell; iPSC, induced pluripotent stem cell; ICH, The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use; ISI, median inter-spike-interval MEA, multielectrode array; IQR, normalized interquartile range; IQR burst duration, normalized IQR burst duration; MAD burst spk, normalized median absolute deviation burst spike number; MAO, monoamine oxidase; Mean ISI, mean of interspike distance; Median ISI, median of ISI-distance; NAM, new approach methodology; NAM, new approach methodology; SD, standard deviation; TSS, Total Standard Score.

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aim before choosing a model, understanding and characterizing the model in the actual test site, and carefully considering the most appropriate timepoints to assess seizure liability.

1. Introduction

Central nervous system (CNS) toxicity contributes to 25% of failures throughout drug discovery and development and it remains the leading cause of drug attrition. It is one of the most frequent causes of safety related failure during clinical development, a phase where consequences are higher in terms of resources and patient impact (Cook et al., 2014; Morgan et al., 2018). Amongst CNS related findings, seizures and tremors are particularly common, accounting for 67% and 65% of reported observations in nonclinical studies, respectively (Authier et al., 2016; Orciani et al., 2024). Notably, several approved drugs in Japan with reported seizures/convulsions in patients were not identified as seizurogenic during preclinical studies (Nagayama, 2015). Compounds associated with seizure liability arise across diverse pharmacological classes, including cardiovascular, gastrointestinal, respiratory, and anti-inflammatory agents, as well as drugs specifically targeting the CNS (Aagaard and Hansen, 2013; Easter et al., 2009; Nagayama, 2015; Walker et al., 2018). Adverse events may be particularly prevalent in compounds that are designed to penetrate the blood-brain barrier to reach their primary target (Aagaard and Hansen, 2013).

Beyond pharmaceuticals, seizures also occur after exposure to several classes of environmental chemicals, including organophosphate nerve agents such as sarin (Pessah et al., 2016; Williamson et al., 2019), explosive components such as RDX (Brown et al., 2023; Fang et al., 2023), herbicides such as glufosinate, (Calas et al., 2016; Park et al., 2018) and organochlorine insecticides such as dieldrin (Costa, 2015). Accordingly, understanding potential seizure liability of environmental chemicals is important for occupational safety and human health protection.

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) provides safety guidelines (S7A) aimed at protecting human clinical trial subjects and patients. ICH S7A includes only general guidance for CNS safety pharmacology and does not specify in vitro approaches. Currently, detection of seizures relies on observations made during in vivo nonclinical studies intended to support clinical development (Easter et al., 2009; Nagayama, 2015). However, these late-stage assessments have limited impact on early compound design and optimization. CNS side-effects, such as tremors or other abnormal movements, often require a follow-up electroencephalogram (EEG) assessment. Moreover, at lower exposures, compounds may induce focal seizures that do not progress to an observable convulsion, delaying the detection and prevention of detrimental CNS compound adverse effects. Similarly, chemical regulation has historically also relied heavily on animal studies to identify neurotoxicity hazard (Jett, 2012).

Current ICH S7A revision efforts emphasize the need for more predictive approaches for CNS safety assessment. Consequently, there is a demand for cost effective methods that could detect seizure liability earlier in drug development process or be used for more rapid toxicological hazard characterization. Recent advances in the use of neural cells cultured for use on multielectrode array (MEA) have enabled continuous, non-invasive measurement of spontaneous electrical spikes and groups of spikes (bursts) associated with neuronal action potentials (Nam and Wheeler, 2011). These activity patterns demonstrate synchrony and oscillatory behaviors, which are related to synaptogenesis, neural network development, and reflect the balance of excitatory and inhibitory synapses (Chiappalone et al., 2006; Maeda et al., 1995; Muramoto et al., 1993). Such activity on MEA has been consistently demonstrated using both primary rodent and human iPSC-derived neural cell cultures.

Neuronal network activity measured on MEA can be modulated by

compounds with a wide variety of modes of action (Cohen et al., 2025; Johnstone et al., 2010; Tukker et al., 2018), including voltage-gated sodium channel modulators, voltage-gated potassium channel modulators, and GABA receptor antagonists, all of which are often included in panels designed for screening antiepileptic and pro-seizurogenic compounds (Bradley et al., 2018; Fan et al., 2019; Grainger et al., 2018; Kreir et al., 2018). Recent work has shown that combining MEA-derived spontaneous activity profiles with human ion channels assay data can successfully predict seizure liability in drug discovery programs (Rockley et al., 2023). This approach was highlighted by the FDA Center for Drug Evaluation and Research (CDER) as a promising new approach methodology (NAM) for safety assessment (Avila et al., 2023). These advances underscore the potential of MEA platforms to improve early detection of seizurogenic risk in vitro. However, broader implementation requires coordinated evaluation of the methods, cell models and analytical parameters needed to standardize MEA based assessments for larger scale non-clinical compound screening for seizure liability. To address this need, we conducted a multi-laboratory, multi-model assessment of seizure detection using both rat primary neurons and human iPSC-derived neural cultures. Our aims were to characterize baseline MEA activity, evaluate compound induced changes across models, and identify reproducible features and parameters across test sites. Strengthening adoption of these approaches could substantially enhance the efficiency of neuronal toxicity screening, reduce late-stage development failures and support the 3Rs (reduction, refinement, replacement) principles by decreasing reliance on animal testing.

2. Materials and methods

2.1. Compounds

The compound test set used in this study consisted of 10 different small molecules, including 3 negative controls (Table 1). All compounds were purchased from Cayman Chemical or Sigma-Aldrich. Compounds were selected based on prior seizure liability studies (Easter et al. (2009) and previous work from the HESI Neurotoxicity MEA Subteam (Roberts et al., 2021). Stock solutions were made in DMSO and stored at -20°C until use. Test concentrations were derived from reported human toxic exposure or from published rodent data on the seizurogenic threshold. Overall, the panel reflects chemicals with various mechanisms of action that can cause seizures clinically or are used experimentally to reduce the seizure threshold.

3. Cell culture

3.1. Human iPSC-derived neurons and astrocytes

Seven different laboratories, including BMS, Tohoku Institute of Technology, FUJIFILM CDI, Cyprotex, EPA (Herr, 2026), FDA, and NeuCyte, utilized commercially available sources of human iPSC-derived human neurons composed of mixed populations of glutamatergic and GABAergic neurons. These cells were typically co-cultured with human iPSC-derived astrocytes or primary human astrocytes (Table 2a). Culture methods differed in commercial source, plating density, and culture medium as noted in Table 2a. Briefly, cells were seeded onto the multi-well MEA plates at a ratio of 5:1 or 6:1 neurons to astrocytes, with most labs using 140,000 cells per well and a range from 95,000 to 740,000 cells per well. All labs used fully supplemented BrainPhys neuronal medium except for two sites that used NeuCyte SynFire culture media. Culture media was refreshed on the indicated schedules as shown in Table 2a, and testing was performed from 13 to 28

days post-plating. All plates were maintained at 37°C with, and 5% CO₂ and 95% air.

3.2. Primary rat cortical neurons

Six laboratories used primary cultures of primary rat cortical cells with three sites (EPA, J&J, Eisai) preparing the cultures in house, and the other three (Cypotex, GSK, NIHS Japan) purchasing frozen cells from QBM Cell Science (Catalog #R-Cx-500). Culture methods were generally standardized, with differences in commercial cell source, plating density, and culture medium composition summarized in Table 2b. Briefly, cells were seeded onto MEA plates at densities ranging from 50,000–150,000 cells per well. Most laboratories used Neurobasal A medium with B-27 supplement for culture and testing. Media changes were performed according to the schedules shown in Table 2b and MEA recordings and compounds testing were carried out between 13 and 28 days post-plating. All laboratories maintained cultures in humidified incubators at 37 °C with 5% CO₂.

4. MEA recordings and data analysis

After a 60-minute equilibration on the instrument, baseline MEA recordings (≥15 min) were acquired to assess culture quality based on uniformity of spikes, networks bursts and synchrony across wells. Baseline activity for each model was compared both within and across different laboratories (see Table 3). Cells were dosed with compounds at 5 different concentrations with a minimum of 3 technical replicates, incubated for 60 min, and then MEA activity was recorded again for ≥ 15 min. Data were acquired and analyzed using Axis Navigator software. Each site reviewed their own data and applied internal quality control criteria to determine which plates and/or wells were suitable for subsequent compound testing. From the qualified wells, individual spike, network burst and network synchrony characteristics were extracted. Neuronal activity was visualized as Raster plots, which depict spiking patterns over time. For comparison across sites, nine MEA analytic parameters were defined (terminology used in figures is shown in brackets):

1. median inter-spike-interval (ISI)
2. mean of ISI-distance (mean ISI)
3. median of ISI-distance (median ISI)
4. normalized interquartile range (IQR) interburst interval (IQR inter-burst interval)

5. median burst duration
6. normalized IQR burst duration (IQR burst duration)
7. normalized median absolute deviation burst spike number (MAD burst spk)
8. median burst rate (bursts/sec)
9. raw firing rate.

All data were reviewed to ensure they met minimum quality control criteria, including DMSO firing rate within acceptable range (defined as 25% of the mean), at least 8 active electrodes per well and robust activity in the positive control wells. Plates that did not meet these criteria were excluded from further analysis.

5. Data standardization

To ensure consistency across sites, a data standardization procedure was performed before parameter analysis. First, for each well, the difference between the baseline value and the post-compound value was calculated for every parameter. A Standard Score was then computed using the following equation:

$$\text{Standard score} = \frac{((\text{Post treatment} - \text{Baseline}) - \text{DMSO}_{\text{Ave}})}{\text{DMSO}_{\text{SD}}}$$

where DMSO_{Ave} means the mean average of all difference values calculated from wells treated with DMSO; and DMSO_{SD} means the standard deviation (SD) of all DMSO treated wells. Finally, the calculated Standard score was standardized to the DMSO treated wells, as the mean average of Standard score for DMSO was set as 0 and the SD was set as 1. The standardization was conducted for each parameter on a well-by-well basis, after which the mean and coefficient of variation (CV) were calculated.

5.1. Calculating coefficients of variation (CV)

The coefficient of variation (CV) is a normalized measure of dispersion that expresses the standard deviation as a percentage of the mean. CVs were calculated using baseline DMSO data according to the following equation:

$$\text{CV} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

This provides a measure of relative variability across datasets that differ in scale or units.

Table 1
Compounds and drug targets *Cmax ug/ml.

Compound	Concentrations (μM)	Source	Catalog Number	CAS	Free Cmax (μM)	Class/ Target
Pentylentetrazol (PTZ)	10, 31.6, 100, 316, 1000	Cayman Chemical	18,682	54-95-5	N/A	GABA/GABAAR
Picrotoxin (PTX)	0.1, 0.316, 1, 3.16, 10	Sigma Aldrich	P1675	124-87-8	N/A	GABA/GABAAR
Amoxicillin	1, 3.16, 10, 31.6, 100	Sigma Aldrich	A8523	26787-78-0	0.5-1*	PBP2
Chlorpromazine	0.1, 0.316, 1, 3.16, 10	Cayman Chemical	16,129	69-09-0	0.15	CNS active/D2R
Amoxapine	0.316, 1, 3.16, 10, 31.6	Cayman Chemical	17,347	14028-44-5	0.04-0.6	CNS active/ MAO Inhibitor
Phenytoin	1, 3.16, 10, 31.6, 100	Sigma Aldrich	PHR1139	57-41-0	4	CNS active/ Na Channels
Linopirdine (HCl)	1, 3.16, 10, 31.6, 100	Cayman Chemical	19,547	105431-72-9	3	Other/K Channels
4-Aminopyridine	0.316, 1, 3.16, 10, 31.6	Sigma Aldrich	275,875	504-24-5	0.26-0.8	Other/K Channels
Strychnine	0.316, 1, 3.16, 10, 31.6	Sigma Aldrich	S0532	57-24-9	0.9	Other/Glycine R
Pilocarpine (HCl)	0.316, 1, 3.16, 10, 31.6	Cayman Chemical	14,487	54-71-7	0.55	Other/Muscarinic ACH R
Acetaminophen	1, 3.16, 10, 31.6, 100	Sigma Aldrich	A5000	103-90-2	5-25*	COX-2 inhibitor
Picrotoxin	3, 10	Sigma Aldrich	P1675	124-87-8	N/A	GABAAR
Bicuculline	25	Sigma Aldrich	14,343	40709-69-1		GABAAR

GABA – Gamma Aminobutyric Acid.

GABAAR – Gamma Aminobutyric Acid Receptor & Ligand Gated Channel.

CNS – Central Nervous System.

D2R – Dopamine D2 Receptor.

MAO - monoamine oxidase.

ACH R – Acetylcholine receptor.

COX-2 – Cyclooxygenase-2.

5.2. Calculating total standard score

To identify which analytical parameter(s) were generally applicable for detecting seizurogenic potential across different facilities, a Total Standard Score (TSS) was calculated for each of the nine MEA-derived parameters. For each compound, the TSS for a given parameter was computed as the sum of its Standard Scores across all tested concentrations. Parameters were then assigned to one of three categories based on the direction and magnitude of the TSS: an increase of ≥ 2 , no meaningful change (between -2 and 2), or a decrease of ≤ -2 . The overall consistency of each parameter across facilities was assessed by aggregating these TSS classifications across all participating sites.

5.3. Statistical analysis

One-way ANOVA followed by Dunnett's test was used to calculate significant differences between each compound and baseline. Welch's *t*-test was used to calculate significant differences of the variations in each parameter between human iPSC neuronal cultures and primary rat cortical neurons.

6. Results

6.1. Comparison of human and rat neuronal activity

Both rat (6 laboratories) and human (7 laboratories) neuronal models displayed robust firing, bursting and synchronous network activity across all sites, with similarities in baseline data for DMSO. Minor differences were observed between the two models in baseline activity patterns (Supplementary Figure 1), and some variability was also evident between laboratories using the same model (Table 3). Overall, the human iPSC-derived neural cultures showed lower variability across laboratories, as reflected by smaller CV values and narrower CV ranges compared with the rat neurons for the nine MEA parameters analyzed (Table 3).

6.2. Effect of seizurogenic compounds and negative controls on network activity

A total of 10 compounds and 3 controls were tested in two neuronal cell models, primary rodent cortical and human induced pluripotent stem cell-derived, across 7 different laboratories on 3 continents.

Neuronal responses to compound exposure are illustrated using raster plots, which depict spiking activity over time. Representative raster plots for human iPSC-derived co-cultures (Fig. 1) and primary rat cortical neurons (Fig. 2) show activity from the same well before and after compound addition (with DMSO serving as the vehicle control). Baseline recordings were generally stable and consistent, with spontaneous firing detected across electrodes (black tick marks) and regular synchronized bursts (pink boxes indicating peaks in activity), reflecting intact and connected neural networks. Following compound addition, cultures exhibited activity changes, including notable increases in firing and increased network burst synchrony, indicative of patterns associated with seizure and consistent with activity patterns reported in previous literature (Bradley and Strock, 2019; Ishibashi et al., 2023; Matsuda et al., 2022; Rockley et al., 2019).

6.3. Effect of seizurogenic compounds and negative controls on analytical parameters

To identify which analytical parameters were most effective for detecting seizurogenic potential, heat maps were generated to illustrate changes in nine MEA-derived parameters upon administration of compounds known to induce seizures in vivo and negative controls for human iPSC-derived neural cultures (Fig. 3A) and for primary rat cortical neurons (Fig. 3B). In the human iPSC neurons model, several parameters showed consistent changes across facilities and across most compounds. However, some variation in the pattern and magnitude of parameter responses was observed between laboratories. In both the human and rat models, seizurogenic compounds generally produced the largest changes in some parameter values, particularly at high concentrations, although the specific parameters affected varied by compound.

Table 2a
Human iPSC-derived neurons culture conditions.

Testing Site	BMS	Fujifilm CDI	Cyprotex	EPA (Herr, 2026)	FDA	Tohoku Inst.	NeuCyte
Cell Vendor	FUJIFILM Cellular Dynamics*	FUJIFILM Cellular Dynamics*	FUJIFILM Cellular Dynamics*	FUJIFILM Cellular Dynamics*	FUJIFILM Cellular Dynamics*	NeuCyte	NeuCyte
Cell Density	80,000 iCell GlutaNeurons 15,000 iCell Astrocytes	120,000 iCell GlutaNeurons 20,000 iCell Astrocytes	120,000 iCell GlutaNeurons 20,000 iCell Astrocytes	120,000 iCell GlutaNeurons 20,000 iCell Astrocytes	120,000 iCell GlutaNeurons 20,000 iCell Astrocytes	38,000 SynFire Glutamatergic Induced Neurons 16,000 GABAergic Induced Neurons 19,000 primary human astrocytes 73,000	140,000 SynFire® Glutamatergic Induced Neurons 60,000 GABAergic Induced Neurons 70,000 primary human astrocytes 270,000
Total Cells/well	95,000	140,000	140,000	140,000	140,000		
Coating	PEI/laminin	PEI/laminin	PEI/laminin	PEI/laminin	PEI/laminin	PEI/laminin	PEI/laminin
Substrate							
Culture Media	BrainPhys + iCell Neural Supplement B + iCell Nervous System Supplement					NeuCyte SynFire basal media and supplements media Yes	NeuCyte SynFire® basal media and supplements media Yes
Antibiotics	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Media Change Schedule	M-W-F	Every 2 days	Every 2 days	Every 2–3 days	Every 2 days	Every 2–3 days	Every 2–3 days
Maturation Period	19–24 days	21–28 days	15 days	14 days	14 days	28–30 days	21–22 days
Assay Platform	Axon Maestro	Axon Maestro	Axon Maestro	Axon Maestro	Axon Maestro	Axon Maestro	Axon Maestro
Plate Type	Classic MEA 48-well Plate	Classic MEA 48-well Plate	Classic MEA 48-well Plate	Classic MEA 48-well Plate	Classic MEA 48-well Plate	Classic MEA 24-well Plate	Classic MEA 48-well Plate
Dosing Scheme	1 Concentration per well	1 Concentration per well	1 Concentration per well	1 Concentration per well	1 Concentration per well	1 Concentration per well	1 Concentration per well

* Derived from the same lot of iCell GlutaNeurons provided by FUJIFILM CDI.

Table 2b

Rat cortical neurons culture conditions.

Testing Site	EPA (Herr, 2026)	GSK	Cyprotex	NIHS Japan	J&J	Eisai
Cell Vendor	In house, prepared fresh from PND 0 Long Evans, Charles River	QBM Cat# R-CX-500, Sprague Dawley, E18-19	QBM Cat# R-CX-500, Sprague Dawley, E18-19	QBM Cat# R-CX-500, Sprague Dawley, E18-19	In house, prepared fresh E-18, Wistar	In house, prepared fresh, E18, Wistar
Cell Density (cells/well)	150,000	80,000	75,000	120,000	65,000	50,000
Culture Media	Neurobasal A + B27 at 2%	Neurobasal B-27	Neurobasal B-27	Neurobasal-A + B27	Neurobasal B-27	Neurobasal B-27 + Neuron Culture Medium (Wako, 148-09671) was added at 20% during the first week
Antibiotics	Yes	Yes	No	No	Yes	No
Test/Assay Media	NBA+B27	NBA+B27	NBA+B27	NBA+B27	NBA+B27	Neurobasal + B27
Media Change Schedule	DIV 5, 9, 12	M-W-F	M-W-F	M-W-F	M-W-F	M-F
Maturation Period	13-15 days	14-17 days	16 days	19-21 days	22-28 days	19 days
Assay Platform	Axon Maestro	Axon Maestro	Axon Maestro	Axon Maestro	Axon Maestro	Axon Maestro
Plate Type	Classic MEA 48-well plate	Classic MEA 48-well plate	Classic MEA 48-well plate	Classic MEA 48-well plate	Classic MEA 48-well plate	Classic MEA 48-well plate
Dosing Scheme	1 Concentration per well	1 Concentration per well	1 Concentration per well	1 Concentration per well	1 Concentration per well	1 Concentration per well

Table 3

Average coefficients of variation (CV) of cortical neuron culture.

Parameter*	Human neurons Avg CV (Range)	Rat neurons Avg CV (Range)
median inter-spike-interval (ISI)	50.5 (21-114)	108.8 (46.5-182)
mean of ISI-distance (mean ISI)	5.7 (1.2-14.3)	30.8 (1.0-69.9)
median of ISI-distance (median ISI)	5.8 (2.2-14.1)	35.4 (1.1-75.5)
normalized interquartile range (IQR) interburst interval	38.6 (16.8-71.9)	31.7 (9.8-71.6)
median burst duration	26.4 (3.3-48.2)	29.4 (10.8-47.7)
normalized IQR burst duration (IQR burst duration)	28.0 (6.8-52.7)	20.4 (5.9-40.3)
normalized median absolute deviation burst spike number (MAD burst spk #)	19.5 (11.6-53.1)	28.8 (5.4-57.9)
median burst rate (bursts/sec)	29.7 (13.5-56.8)	16.1 (7.4-23.7)
raw firing rate.	39.0 (15.8-64.3)	36.1 (19.0-51.3)

* Selected parameters for DMSO (baseline data) across all laboratories. A higher CV indicates greater relative variability, while a lower CV indicates a lower spread around the mean.

For human iPSC-derived neuronal cultures (Figs. 3A), 4-AP showed significant differences in ISI and mean ISI in many facilities; however, the direction of change varied among facilities. Amoxapine exhibited consistent changes across facilities in all parameters except median ISI, with a significant decrease observed. Chlorpromazine showed significant differences in many facilities across all parameters except median ISI and IQR burst duration; however, the direction of change varied among facilities. Enoxacin, phenytoin, and strychnine consistently showed a significant decrease in ISI and mean ISI. Linopirdine showed significant decreases in ISI, mean ISI, and MAD burst spike number in many facilities. PTZ and picrotoxin exhibited relatively weak responses, and no consistent changes were observed across facilities. Pilocarpine showed variability in response magnitude among facilities. As expected, the negative control acetaminophen (APAP) produced minimal changes across any of the parameters in either model.

For primary rat cortical neurons (Fig. 3B), amoxapine and chlorpromazine produced the most reproducible effects across laboratories,

again with the strongest responses at the highest concentrations. Overall, however, parameter responses in the rat model displayed greater variability across facilities compared with the human iPSC-derived neural cultures, reflecting more diverse response patterns and reduced cross-site consistency (Fig. 3A).

As shown in Fig. 4, among the seven facilities that generated MEA data from human iPSC-derived neural cultures, the parameter with the highest degree of cross-site commonality was mean ISI, which decreased in all tested compounds except picrotoxin. This indicates that mean ISI is a generally robust parameter for detecting seizurogenic potential. Three additional parameters (median ISI, MAD burst spike number, and median burst rate) also showed consistent trends across facilities. Notably, MAD burst spike number increased in response to both PTZ and picrotoxin, compounds that act as GABA_A receptor antagonists, whereas median burst rate increased in response to 4-AP and linopirdine, both K⁺ channel blockers.

In the primary rat cortical neuron model, many compounds also produced measurable changes in MEA parameters; however, the direction, magnitude, and presence of these changes varied substantially across laboratories (Fig. 4). As a result, cross-site reproducibility was lower than in the human model. Only three compounds (Amoxapine, Chlorpromazine, and Picrotoxin) showed consistent TSS classification changes across sites, reflecting the higher inter-laboratory variability observed in rat cultures.

7. Discussion

Here, we present the results of a multicenter, multi-model assessment evaluating the ability of MEA platform to detect seizure liability. We characterized neuronal network phenotypes across wells, plates, and sites for each cell-platform combination, quantifying spiking, bursting and synchrony, and the variability parameters describing these characteristics. Importantly, we evaluated both human and rat neurons as these remain the most commonly used systems. Overall, all participating centers generated MEA data indicative of seizurogenic phenotype in both rat and human neurons although notable differences were observed between parameters and between species.

MEA data can be visualized in raster plots, which provide an immediate qualitative impression of neuronal firing patterns and increased synchrony associated with a seizurogenic phenotype. However, the complexity of the data makes it difficult to interpret differences in seizure intensity between compounds or concentrations and cannot be

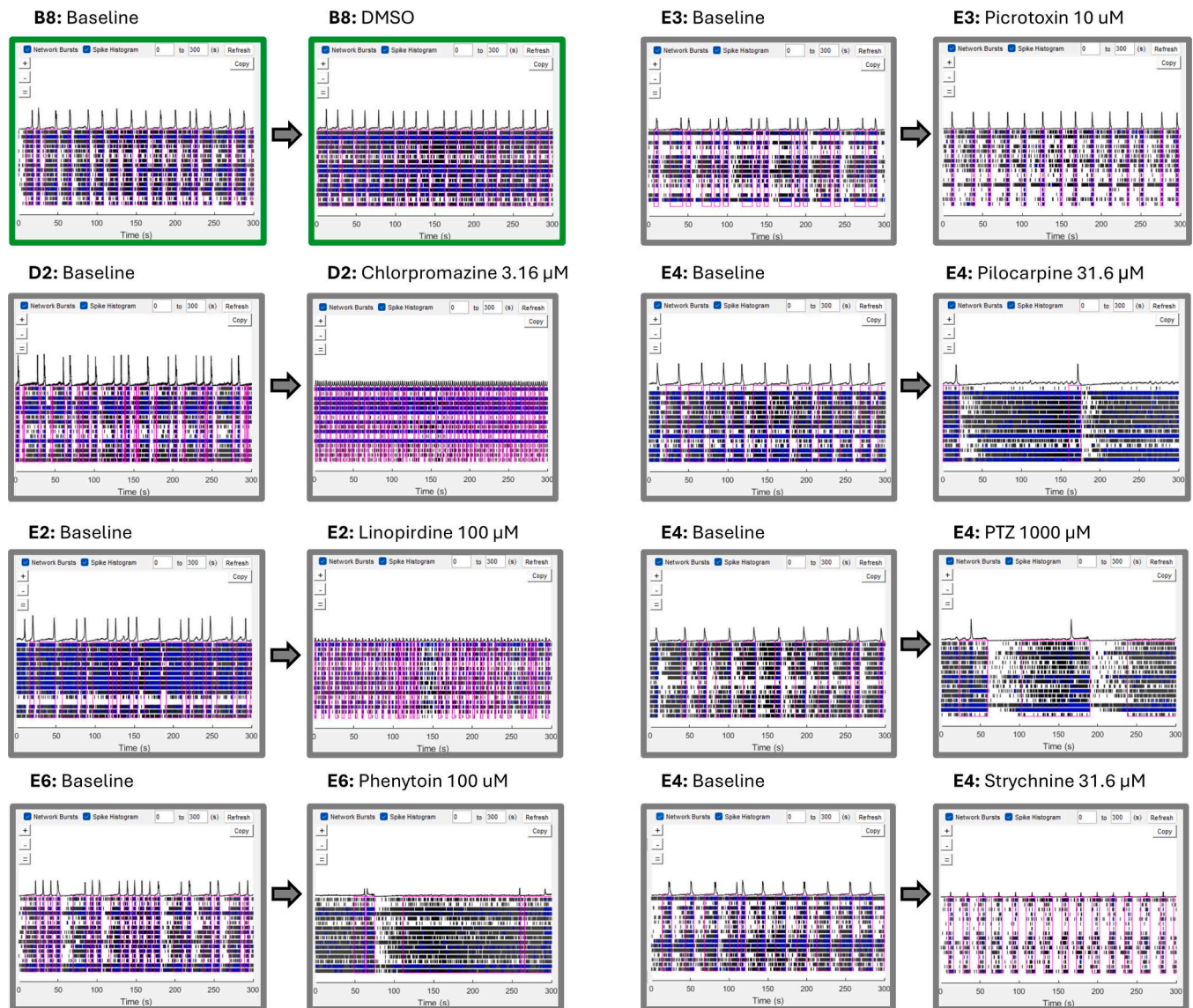


Fig. 1. Example raster plots from human iPSC-derived neurons and astrocytes co-cultured on MEA, where the x-axis is the time in seconds and the y-axis rows shows individual neuron firing events (spikes or action potentials) across 16 different electrodes per well. For each pair of raster plots, the left-hand graph is the baseline activity (pre-dose) and the right-hand side shows activity following compound addition (post-dose.).

compared by visual inspection alone. One goal of this project was to try to identify reproducible MEA features and parameters of potentially seizurogenic compounds in rat and human cultures. Although we found significant variability due to methodological differences, we found that MAD burst spike number, mean ISI and median burst rate were commonly altered. Our results also indicated that, not surprisingly, seizurogenic compounds with different modes of action altered different parameters. To assess parameters that change consistently across sites, we calculated a Total Standard Score (TSS), defined as the sum of Standard Scores across all concentrations for each compound. For human iPSC neural cultures, several parameters showed a high degree of cross-site consistency. In particular, MAD burst spike number increased in response to PTZ and Picrotoxin, both GABA_A receptor antagonists, while median burst rate increased following exposure to 4-AP and linopirdine, both K⁺ channel blockers. These findings suggest that MAD burst spike number and median burst rate reflect the underlying mechanisms of action of these compounds on neuronal networks.

Compared to the human iPSC-derived neural cultures, the majority of compounds tested in primary rat cortical neurons produced measurable changes, but these changes were less consistent across facilities.

Only three compounds (Amoxapine, Chlorpromazine, and Picrotoxin) caused changes in TSS class highlighting lower cross-laboratory reproducibility in the rat model. Since the Standard Scores used to index parameter changes were calculated based on the standard deviation (SD) of DMSO obtained by each facility, higher variability in DMSO baseline activity among rat cultures may have masked treatment-related effects. Additional methodological differences may also have contributed to this variability. Rat cultures showed greater divergence in plating density, media formulations, and especially days in vitro (DIV) at the time of testing, which ranged from 13 to 21 DIV. Since neuronal network maturation typically occurs during the first 0–15 DIV (Chiappalone et al., 2006; Cotterill et al., 2016), differences in culture age could meaningfully influence sensitivity to seizurogenic compounds. Plating density is another important factor, as prior studies have demonstrated that network activity and bursting behavior scale with neuronal density (Biffi et al., 2013; Wagenaar et al., 2006). These culture differences can be seen in Table 2b and further it is noted that two sites used freshly dissociated cells vs cryopreserved cultures. Variability in these parameters likely contributed to the broader range of responses observed in the primary rat cortical neuron model.



Fig. 2. Example raster plots for rat primary cells grown on MEAs, where the x-axis is the time in seconds and the y-axis rows shows individual neuron firing events (spikes or action potentials). For each pair of plots, the left-hand raster plot is the baseline activity and the right-hand plot shows activity following compound addition.

Overall, for the human iPSC-derived neuronal model showed greater cross-site consistency, despite some variation in protocols. In contrast, although several parameters in the primary rat cortical neuron model exhibited consistent changes, the rat data were generally more variable across sites. Across both species, time in culture emerged as a key factor influencing the detectability of compound-induced effects. It is important to note that this study did not aim to establish a fully standardized culture protocol across laboratories, as such uniformity would not reflect typical real-world use of these models. Despite this, the human iPSC-derived neural cultures demonstrated consistent MEA profiles and seizurogenic responses even when different differentiation methods were used. Based on these observations, we recommend adhering closely to the cell and plate manufacturers' guidelines regarding surface coatings, cell density, media, media change schedule and maturation period. Deviations from these parameters may reduce signal strength and decrease the consistency of seizure-related readouts. Most importantly, it is important to understand and characterize the model in the actual test site, and carefully consider the optimal time window for assessing seizure liability, as these factors clearly influence experimental outcomes.

Considering the entire dataset, both human and rat models were effective for detecting a seizurogenic phenotype and in principle could be used interchangeably. However, the two cell models differ in several

ways including biological origin, maturation timelines and functional properties. Rodent cortical neurons typically mature more rapidly with an averaged at 16 days whereas human iPSC neural cultures typically require a longer culture time averaged at 19 days. Human iPSC neural cultures also exhibit a more gradual development of spontaneous network activity and display complex, human-specific dynamic features as they mature. In contrast, primary rat cortical neurons show an earlier onset of stable network activity, reflecting inherent species-specific differences compared with hiPSC-derived models. These distinctions are important when considering translational relevance: although human iPSC-derived neural cultures require longer differentiation and may introduce additional sources of variability, they provide greater physiological relevance for modeling human neural function and drug responses.

MEA combined with an ion channel panel (Rockley et al., 2023) was highlighted by the FDA CDER as a potential new approach methodology (NAM) (Avila et al., 2023) with a proposed context of use (COU) 'to predict human C_{max} levels of small molecule drugs associated with risk of seizures.' In parallel, ongoing efforts to revise ICH S7A, where neurotoxicity is currently assessed primarily through animal behavioral studies, present an important opportunity to incorporate MEA methods into regulatory safety assessment frameworks (Valentin and Leishman, 2025). In addition, although MEA based approaches are part of a

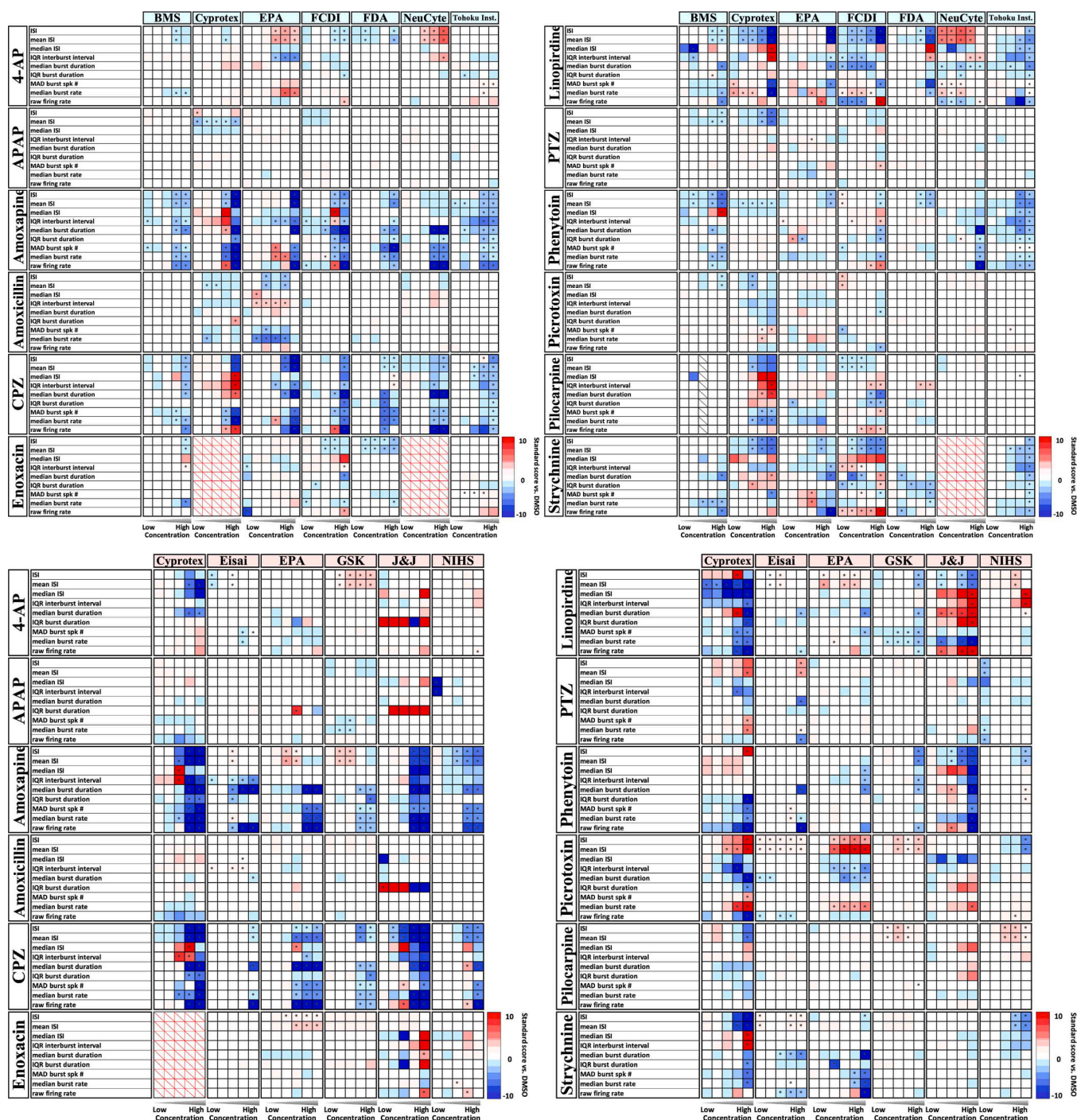


Fig. 3. A. Heat maps of analytical parameters with different seizurogenic compounds and negative control in human iPSC-derived neurons for each facility. Data was calculated to Standard score and standardized to DMSO. Due to the burst disappearance, the Standard scores could not be calculated for some wells (i.e., pilocarpine at dose 3 for BMS) and the data was removed. Enoxacin is not tested at Cyprotex and Neucyte sites, while strychnine is not tested at Neucyte site, which is shown in red columns. **3B.** Heat maps of analytical parameters with different seizurogenic compounds and negative control in rat cortical neurons for each facility. Data was calculated to Standard score and standardized to DMSO. Enoxacin is not tested at Cyprotex site, which is shown in red columns.

battery of in vitro assays for developmental neurotoxicity screening (OECD, 2023), NAMs for acute neurotoxicity (Tal et al., 2024) that propose to include MEA-based approaches are currently under development. Collectively, these emerging potential COUs offer an excellent opportunity for improved seizure prediction and neurotoxicant detection. However, they will require progress in in vitro to in vivo extrapolation. To support this, the concentrations tested in this study were selected to cover the range of exposures reported in rats in vivo and in human clinical studies. Nevertheless, additional work is needed to

predict the human C_{max} levels associated with seizure risk for new compounds, as outlined in the FDA COU.

Moving forward, the results, although based on a limited set of compounds, support continued development of MEA approaches for seizure detection and acute neurotoxicity hazard characterization. Key next steps include testing larger panels of drugs and chemicals, further standardizing protocols within and across laboratories, and expanding assessment to mechanisms of action that may elicit adverse neurological outcomes other than seizure (e.g., sedation). Analysis approaches that

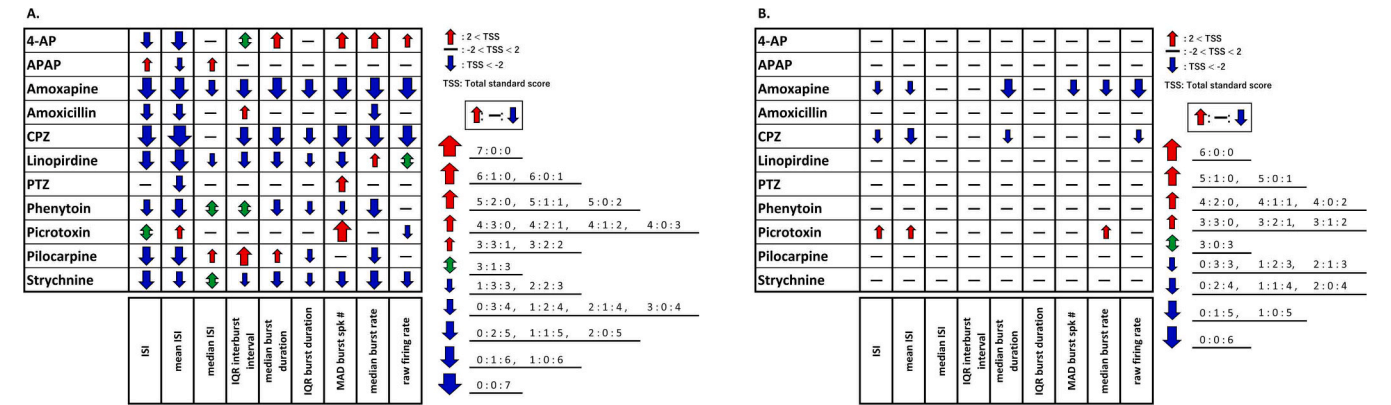


Fig. 4. The common trends for 9 analytic parameters in **A.** human iPSC-derived cortical neurons among all 7 facilities and **B.** rat cortical neurons among all 6 facilities. A Total. Standard score (TSS) was calculated for each parameter, the sum of the Standard scores calculated at each concentration of the compound. A red upward arrow indicates an increase, while a blue downward arrow indicates a decrease in the parameter, with the size of the arrow representing the degree of commonality between the facilities. A green double-headed arrow indicates no notable common change between the facilities. A detailed description of the arrow size and the distribution of the TSS classes in all 6 or 7 facilities is listed at the right panel.

can clearly separate seizure responses from other neuroactivity responses based on analysis of the MEA data should be a high priority moving forward. Recent data from more than 300 compounds demonstrate that this is feasible (Kosnik et al., 2020), but achieving this will require coordinated efforts across the field. One of the key lessons learned from this work is to have a clear aim before choosing a model. For example, if the aim of the work is to evaluate seizures seen in rat studies to avoid them in back up programs, the rat neural cultures would be the most appropriate. Alternatively, if the aim is to predict and avoid human seizures, then the human model would be the first choice. When it comes to drug development, the ultimate aim is protection of human clinical trial subjects and patients, where human-relevant models are generally consider most appropriate.

In summary, we report a unique international, multi-laboratory study evaluating the utility of MEA for in vitro prediction of seizure liability. To better reflect real world data generation practices, study protocols were similar but were not standardized between laboratories. Despite these differences, the data demonstrate that the human model and platform is robust across different sites. Greater variability was observed in the primary rat cortical neuron model, indicating that while this system remains useful, particularly with strong internal positive and negative controls, it may require tighter control of culture conditions in a multi-site evaluation.

CRediT authorship contribution statement

Takashi Yoshinaga: Methodology, Investigation, Data curation. **Tromondae K. Feaster:** Methodology, Investigation, Data curation. **Christopher J. Strock:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Katherine Czyst:** Methodology, Investigation, Data curation. **Yuto Ishibashi:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Ikuro Suzuki:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Xiaobo Han:** Visualization, Investigation, Formal analysis, Data curation. **Mohamed Kreir:** Writing – review & editing, Methodology, Investigation, Data curation. **Yasunari Kanda:** Writing – review & editing, Methodology, Investigation, Data curation. **Norimasa Miyamoto:** Methodology, Investigation, Data curation. **Ruth Roberts:** Writing – review & editing, Writing – original draft, Visualization, Data curation, Conceptualization. **Paul Levesque:** Methodology, Investigation, Data curation. **Scott Schachte:** Writing – review & editing, Methodology. **Timothy J Shafer:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Coby Carlson:** Writing – review & editing,

Writing – original draft, Methodology, Investigation, Data curation. **Pierson Jennifer:** Writing – review & editing, Writing – original draft, Project administration, Data curation, Conceptualization. **Ksenia Bli-nova:** Methodology, Investigation, Data curation. **Matthew Cato:** Writing – review & editing, Methodology, Investigation, Data curation.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Yasunari Kanda reports financial support was provided by Government of Japan Ministry of Health Labor and Welfare. Yasunari Kanda reports financial support was provided by Japan Agency for Medical Research and Development. Ruth Roberts reports a relationship with Apconix that includes: employment. Coby Carlson, Katherine Czyst, Scott Schachte reports a relationship with Fujifilm Cellular Dynamics that includes: employment. Matthew Cato reports a relationship with GlaxoSmithKline LLC that includes: employment. Mohamed Krier reports a relationship with Johnson and Johnson that includes: employment. Paul Levesque reports a relationship with Bristol-Myers Squibb Company that includes: employment. Takashi Yoshinaga reports a relationship with Eisai Co Ltd that includes: employment. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.neuro.2026.103519](https://doi.org/10.1016/j.neuro.2026.103519).

Data availability

Data available in [supplemental materials](#).

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