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Research article

An integrated approach for early *in vitro* seizure prediction utilizing hiPSC neurons and human ion channel assays

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Abstract

Seizure liability remains a significant cause of attrition throughout drug development. Advances in stem cell biology coupled with an increased understanding of the role of ion channels in seizure offer an opportunity for a new paradigm in screening. We assessed the activity of 15 pro-seizurogenic compounds (7 CNS active therapies, 4 GABA receptor antagonists, and 4 other reported seizurogenic compounds) using automated electrophysiology against a panel of 14 ion channels (Nav1.1, Nav1.2, Nav1.6, Kv7.2/7.3, Kv7.3/7.5, Kv1.1, Kv4.2, KCa4.1, Kv2.1, Kv3.1, KCa1.1, GABA $\alpha_1\beta_2\gamma_2$, nicotinic $\alpha_4\beta_2$, NMDA 1/2A). These were selected based on linkage to seizure in genetic/pharmacological studies. Fourteen compounds demonstrated at least one "hit" against the seizure panel and 11 compounds inhibited 2 or more ion channels. Next, we assessed the impact of the 15 compounds on electrical signaling using human-induced pluripotent stem cell neurons in microelectrode array (MEA). The CNS active therapies (amoxapine, bupropion, chlorpromazine, clozapine, diphenhydramine, paroxetine, quetiapine) all caused characteristic changes to electrical activity in key parameters indicative of seizure such as network burst frequency and duration. The GABA antagonist picrotoxin increased all parameters, but the antibiotics amoxicillin and enoxacin only showed minimal changes. Acetaminophen, included as a negative control, caused no changes in any of the parameters assessed. Overall, pro-seizurogenic compounds showed a distinct fingerprint in the ion channel/MEA panel. These studies highlight the potential utility of an integrated *in vitro* approach for early seizure prediction to provide mechanistic information and to support optimal drug design in early development, saving time and resources.

Keywords: drug development; drug discovery; seizure; ion channels; MEA; hiPSC neurons; NAMs

CNS toxicity account for around 25% of failures across the spectrum of drug discovery and development and is the most frequently cause of safety failure in clinical development, a phase where consequences are higher in terms of resources and patient impact (Cook *et al.*, 2014; Morgan *et al.*, 2018). Of the CNS issues encountered, seizures and tremors are frequent, accounting for 67% and 65% of CNS-related observations in preclinical studies, respectively (Authier *et al.*, 2016). Confirming this, the seizures/convulsions that were reported in patients for drugs approved in Japan were not identified during preclinical studies (Nagayama, 2015). Compounds associated with seizure liability span a wide variety of pharmacological classes, chemical classes, and therapy areas including cardiovascular, gastrointestinal, respiratory, and inflammation as well as drugs intended to treat the CNS itself (Aagaard and Hansen, 2013; Cook *et al.*, 2014; Easter *et al.*, 2009; Walker *et al.*, 2018). CNS adverse drug reactions may be particularly prevalent in compounds that are designed to penetrate the blood-brain barrier to reach their primary target (Aagaard and Hansen, 2013).

Seizures are characterized by periods of excessive neuronal firing and uncontrolled hyperexcitability. Significant effort is expended in preclinical safety assessment to identify and mitigate this potentially serious side effect (Authier *et al.*, 2016; Easter *et al.*, 2009; Redfern *et al.*, 2008). Currently, the detection of seizures is reliant upon observations in preclinical rodent and nonrodent studies intended to support clinical trials (Easter *et al.*, 2009; Nagayama, 2015) or studies conducted late in nonclinical development that have little impact on the design and optimization of pharmaceutical compounds. These could be CNS related signs such as tremors or other abnormal movements, requiring a follow-up electroencephalogram study. Additionally, stand-alone safety pharmacology studies test relatively low doses, so these studies rarely identify adverse drug reactions suggestive of potential seizure liability risk at higher doses. As such, the primary source of data for seizure liability generally comes from acute and repeat-dose toxicity studies (which include groups at or near the maximum tolerated dose) when either overt convulsions or premonitory clinical signs of convulsions are noted.

Some progress has been made in seizure detection using automated video systems that record and analyze animal movements (Yip *et al.*, 2019), and a lot of effort has been invested in developing screening methods that could be used earlier in drug discovery such as the hippocampal brain slice (Easter *et al.*, 2007) or zebrafish larval locomotor assays (Khan *et al.*, 2017; Winter *et al.*, 2008).

However, there are concerns regarding the translation of rodent and zebrafish data to humans. Additionally, the hippocampal slice is of limited throughput. It would be far preferable to have an earlier, higher throughput, human-based model for the prediction of seizure risk that could be used to identify and eliminate liabilities early in discovery, whereas there are still options for the medicinal chemists making potential new drugs.

We previously proposed that advances in stem cell biology coupled with an increased understanding of the role of neuronal ion channels in seizure offered a new paradigm in seizure screening, similar to that used for cardiac arrythmia (Roberts *et al.*, 2021; Rockley *et al.*, 2019). Specifically, attrition due to cardiac adverse drug reactions has benefited from assessment of cardiotoxicity in human-induced pluripotent stem-cell-derived (hiPSC) cardiomyocytes and routine screening against cardiac ion channels, allowing for optimization of medicinal chemistry away from these liabilities (Crumb *et al.*, 2016; Gintant *et al.*, 2016; Park *et al.*, 2018; Pollard *et al.*, 2017). The development of a similar approach for seizure liability screening is especially pertinent in the context of the recent FDA modernization act (FDA, 2022) which allows applicants to use methods other than animal testing to establish drug safety and effectiveness. Our proposal for a human ion channel assay coupled with assessment of electrical activity in stem cells (Roberts *et al.*, 2021) fits these criteria and has been highlighted by the FDA/CDER as a useful novel alternative method (NAM) that should be considered as a component of the overall safety assessment for seizure liability (Avila *et al.*, 2023).

Here, we describe the development and evaluation of an *in vitro* screening method for detecting seizure liability based on 2 novel *in vitro* assays: a panel of 14 seizure-related ion channels that can be screened using automated electrophysiology and a human-derived neuronal stem cell MEA assay that detects potential seizurogenic compounds via their effect on electrical activity. Together, the data provide evidence that an integrated approach to early seizure liability screening can support optimal drug design in early development before animals, resources, and time have been wasted.

Materials and methods

Compounds

The compound set used to validate our approach consisted of 15 seizure-associated compounds (Table 2) and 1 negative control (acetaminophen) purchased from either Sigma-Aldrich (Gillingham, Dorset, UK) or TOCRIS (Bristol, UK). Stock solutions were made in dimethyl sulfoxide (DMSO) and stored at −20°C until use. The compounds were selected from Easter *et al.* (2009) and the compound set described by the HESI Neurotoxicity MEA Subteam (HESI, 2020). Overall, the test set reflects chemicals with varying mechanisms of action that cause seizures clinically or are used experimentally to reduce the seizure threshold.

Ion channel panel

Cell lines (either Chinese hamster ovary [CHO] or human embryonic kidney [HEK]) stably expressing the wild-type human ion channels (Table 1) were generated by standard recombinant techniques (Green and Sambrook, 2012). The voltage-gated sodium channels and potassium channels were created at ApconiX, and the ligand-gated channels were generously provided by Bristol-Myers Squibb for this project. The activity of the validation compounds on our ion channel panel was then assessed using automated patch clamp (Bell and Dallas, 2018).

Cell culture

CHO cells were cultured in F12-HAM medium supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, 1% penicillin-streptomycin, and the appropriate selection antibiotics. HEK cells were cultured in Dulbecco's Modified Eagles Medium supplemented with 10% FBS, 1% L-glutamine, 1% nonessential amino acids, 1% penicillin-streptomycin, and the appropriate selection antibiotics.

Screening of validation compounds

Ion Works Quattro (Molecular Devices LLC, San Jose, California).

Compounds were tested in an 8-point concentration-response curve generated using 3.16 serial dilutions from the top test concentration. Single cell recordings were made in perforated-patch configuration at room temperature (21–23°C). Cells were accessed using 100 µg/ml amphotericin. The access buffer contained (in mM): 145 KCl, 1 $MgCl_2$, 1 EGTA, 20 HEPES and was buffered to pH 7.25 using KOH. The internal solution contained (in mM): 100 K gluconate, 40 KCl, 3.2 $MgCl_2$, 3 EGTA, 5 HEPES and was buffered to pH 7.25 using KOH. The external buffer (Dulbeccos phosphate-buffered saline) contained (in mM): 0.9 $CaCl_2$, 0.5 $MgCl_2$-($6H_2O$), 2.7 KCl, 1.47 KH_2PO_4, 137 NaCl, 8 Na_2HPO4-($7H_2O$) and was buffered to pH 7.4. Cells were clamped at a holding potential of −90 mV before depolarizing steps appropriate for each ion channel. Currents were measured from the step and referenced to the holding current. Compounds were incubated for approximately 3–4 min. The difference in current between the pre- and postcompound measurements was exported and analyzed using GraphPad Prism software. The IC_{50} values were obtained from a 4-parameter logistic fit of normalized concentration-response data.

QPatch and PatchLiner (see Bell and Dallas, 2018).

Stock compounds in DMSO were diluted to their top test concentration in HEPES-buffered saline. Five- or 6-point concentration-response curves were generated using 3.16 serial dilutions from the top test concentration. Single cell ionic currents were measured in whole-cell configuration at room temperature (25°C). Buffer composition and voltage protocols are listed in Table 3. Compounds were incubated for at least 120 s. Concentration-response curves were generated by cumulative addition of compound with concentrations low to high. In all cases, steady-state inhibition was achieved before the next concentration of compound was added. The difference in current between the pre- and postcompound measurements was exported and analyzed using GraphPad Prism software. The IC_{50} values were obtained from a 4-parameter logistic fit of normalized concentration-response data.

hiPSC neuronal coculture assay

Cell plating and culture

iCell Glutaneurons (lot 106060: 80% glutamatergic neurons/20% GABAergic neurons) and iCell astrocytes (lot 105981) were thawed and cultured according to the supplier's instructions (FujiFilm Cellular Dynamics, Madison, Wisconsin). Briefly, each vial was thawed sequentially for 2 min and added to complete BrainPhys medium at room temperature (BrainPhys neuronal medium [Stem Cell Technologies] supplemented with 2% iCell Neural Supplement B, 1% Nervous System Supplement [FujiFilm Cellular Dynamics], 1% N2 [Thermofisher Scientific], 1% penicillin/streptomycin, and 0.1% laminin [Sigma Aldrich]). After a manual cell count with trypan blue, the cells were resuspended

Table 1. Selected ion channels for *in vitro* seizure screening panel

	Ion Channel	Gene	Associated Epileptic Syndromes	Mechanism(s)	References
Voltage-gated sodium channels	Nav1.1	*SCN1A*	Dravet syndrome, Developmental and epileptic encephalopathy, generalized epilepsy with febrile seizures	Predominantly LOF	Lossin *et al.* (2002), Claes *et al.* (2001), Mantegazza *et al.* (2005)
	Nav1.2	*SCN2A*	Developmental and epileptic encephalopathy, benign familial infantile seizures	GOF/LOF	Howell *et al.* (2015), Wolff *et al.* (2017), Berecki *et al.* (2018), Schwarz *et al.* (2016)
	Nav1.6	*SCN8A*	Developmental and epileptic encephalopathy, benign familial infantile seizures	GOF/LOF	Wagnon *et al.* (2018), Blanchard *et al.* (2015), Veeramah *et al.* (2012)
Potassium channels	Kv1.1	*KCNA1*	Episodic ataxia/myokymia syndrome, partial epilepsy, developmental and epileptic encephalopathy	Predominantly LOF	Dinoi *et al.* (2022), Miceli *et al.* (2022), Scheffer *et al.* (1998), Zuberi *et al.* (1999)
	Kv2.1	*KCNB1*	Developmental and epileptic encephalopathy	Predominantly LOF	Torkamani *et al.* (2014), Calhoun *et al.* (2017), De Kovel *et al.* (2017), Kang *et al.* (2019)
	Kv3.1	*KCNC1*	Progressive myoclonic epilepsy, developmental and epileptic encephalopathy	LOF	Muona *et al.* (2015), Li *et al.* (2021), Cameron *et al.* (2019)
	Kv4.2	*KCND2*	Autism with severe intractable seizures	GOF	Lee *et al.* (2014)
	Kv7.2/7.3	*KCNQ2/3*	Developmental and epileptic encephalopathy, benign familial neonatal convulsions	Predominantly LOF	Singh *et al.* (1998), Biervert *et al.* (1998), Bassi *et al.* (2005), Mulkey *et al.* (2017), Miceli *et al.* (2015)
	Kv7.3/7.5	*KCNQ3/5*	Developmental and epileptic encephalopathy	LOF/GOF	Nappi *et al.* (2022), Lehman *et al.* (2017), Krüger *et al.* (2022)
	KCa1.1	*KCNMA1*	Generalized epilepsy	GOF	Du *et al.* (2005), Li *et al.* (2018)
	KCa4.1	*KCNT1*	Developmental and epileptic encephalopathy, nocturnal frontal lobe epilepsy	GOF	Barcia *et al.* (2012), McTague *et al.* (2018)
Ligand-gated ion channels	GABA α1β2γ2	*GABRA1/B2/G2*	Developmental and epileptic encephalopathy	GOF/LOF	Hernandez and Macdonald (2019)
	Nicotinic α4β2	*CHRNA4/CHRNB2*	Nocturnal frontal lobe epilepsy, Developmental and epileptic encephalopathy	GOF/LOF	Phillips *et al.* (2001), De Fusco *et al.* (2000), Conti *et al.* (2015), Aridon *et al.* (2006)
	NMDA 1/2A	*GRIN1/GRIN2A*	Nocturnal frontal lobe epilepsy	GOF/LOF	Swanger *et al.* (2016)

in dotting media (complete BrainPhys media with 10% laminin) at a density of 15 million cells/ml for iCell Glutaneurons and 6.7 million cells/ml for iCell Astrocytes. For plating, the cells were mixed in a 8:3 ratio so that 11 µl of cell suspension contained 120k iCell Glutaneurons (85%) and 20k iCell Astrocytes (15%). Eleven microliters of droplets (140k cells/droplet) were carefully plated over the recording electrodes of 24-well MEA plates pre-coated with 0.07% polyethyleneimine and left to adhere at 37°C for 1 h. Six hundred microliters of complete BrainPhys medium were added in 2 stages to all wells and 2 ml sterile water was added around the edges of the plate to increase humidity. Fifty percent media changes with complete BrainPhys medium were performed every 24–72 h and the water was topped up as required. The cells were maintained for 21–25 days in culture to form functional networks before testing of compounds.

Compound addition

The day before compound addition the media was completely removed from the MEA plate and 300 µl of the spent media was re-added into the wells with 300 µl of fresh BrainPhys media. This ensured that the volume of media was accurate for compound addition. Compounds were solubilized at 1000× in DMSO and serial dilutions were prepared in BrainPhys media at 10× the desired concentration. After brief incubation at 37°C, these were added directly to the MEA wells to create the final concentrations. The final DMSO exposure was kept at or below 0.1%.

A test range of concentrations for each compound was determined from the literature on human toxic and therapeutic ranges and from *in vivo* data on the seizurogenic threshold in the rat (Table 2 and references therein). The optimal concentration was then selected for further experimentation.

MEA recordings and data analysis

After equilibration of the MEA plate in the axion MEA instrument at 37°C and 5% CO_2 for 15–30 min, a 15-min baseline recording was taken. After 1-h incubation with test compounds 15-min treatment recordings were taken. Data were acquired using Axis Navigator 3.1.2 software in spontaneous neural real-time configuration with the adaptive threshold spike detector set at 6× standard deviation of internal noise level (rms) on each electrode.

Table 2. Compound set used to validate the *in vitro* seizure assays

Clinical Compounds Reported to Be Seizurogenic in Humans						
			Human Blood Plasma Concentration (μM)		Rat Dose	
Name	Mechanism of Action	MEA Conc (μM)	Therapeutic Range (μM)	Toxic Range (μM)	Dose Range (mg/kg)	References
Amoxapine	Tricyclic antidepressant that blocks reuptake of norepinephrine and serotonin, also blocks the dopamine D2 receptor. Many reports of seizures in humans	0.1–10	0.57–1.91	9.56		Kumlien and Lundberg (2010), Litovitz and Troutman (1983)
Bupropion	Atypical antidepressant thst is also used for smoking cessation. Blocks reptake of norepinephrine and dopamine and antagonizes several nicotinic acetylcholine receptors. Many reports of seizures in humans	0.3–30	0.04–0.42	5–8.34		Kumlien and Lundberg (2010), Davidson (1989)
Chlopromazine	Antipsychotic drug that antagonizes the dopamine D2 receptor. Many reports of seizures in humans	0.1–10	0.09–0.31	3.14–6.27		Kumlien and Lundberg (2010), Lertxundi *et al.* (2013)
Clozapine	Atypical antipsychotic that blocks serotonin receptors and dopamine receptors, with the highest affinity for the D4 dopamine receptor. Many reports of seizures in humans	0.1–10	0.3–1.83	1.83–29.06		Kumlien and Lundberg (2010), Lertxundi *et al.* (2013), Wong and Delva (2007)
Diphenhydramine	Antihistamine used to treat allergies and insomnia that acts as an inverse agonist at the H1 receptor. Reported to cause seizures in humans	0.1–10	0.19–3.92	3.92–15.66		Köppel *et al.*, (1987), Jang *et al.* (2010)
Paroxetine	Antidepressant of the selective serotonin reuptake inhibitor class. Reported to cause seizures in humans	0.1–10	0.03–0.3	1.06–1.21		Kumlien and Lundberg (2010), Hill *et al.* (2015)
Quetiapine	Atypical antipsychotic medication that acts as a dopamine, serotonin, and adrenergic antagonist. Also a potent antihistamine with some anticholinergic properties. Many reports of seizures in humans	0.3–30	2.6	4.69		Kumlien and Lundberg (2010), Young *et al.* (2009), Dogu *et al.* (2003)
Strychnine	Glycine receptor antagonist. Known to induce seizures in humans and rats	0.3–30	N/A	0.22–0.3		Rivera and Barrueto (2014)
Amoxicillin	Antibiotic acting as GABA-A receptor antagonist. Reported to cause seizures	1–100	1.37–41.05	no data		Raposo *et al.* (2016)
Enoxacin	Broad-spectrum antibiotic acting as GABA-A receptor antagonist. Reported to cause seizures	0.1–10	3.12–12.49	no data		Wanleenuwat *et al.* (2020)
Compounds Used to Induce Epilepsy/Lower the Seizure Threshold in Rats *In Vivo*						
4-Aminopyridine	Voltage dependent potassium channel blocker with high potency on Kv3.1 channels	1–100			10	Peña and Tapia (2000), Gutman *et al.* (2005), Fedor *et al.* (2020)
Linopirdine	Kv7 channel blocker that enhances the release of acetyclcholine and glutamate	1–100			10–30	Flagmeyer *et al.* (1995)
Pilocarpine	Cholinergic muscarinic receptor agonist	0.3–30			100–400	Cavalheiro *et al.* (1996), Curia *et al.* (2008)
Pentylenetetrazole (PTZ)	GABA-A receptor antagonist	30–3000			30–50 mg/kg used for kindling	Shimada and Yamagata (2018)
Picrotoxin	GABA-A receptor antagonist	0.1–10			3–6	Mackenzie *et al.* (2002)

Table 3. Voltage protocols and buffer composition

Protocol	Holding Potential (mV)	Step (mV, ms)	Pulse Interval (s)	Ligand	Extracellular Buffer	Intracellular Buffer
hNaV1.1	−90	0, 50	0.33		a	c
hNaV1.2	−90	0, 50	0.33		a	c
hNaV1.6	−90	0, 50	0.33		a	c
hKv1.1	−90	+40, 200	10		a	d
hKv2.1	−90	+40, 200	10		a	d
hKv3.1	−90	+40, 200	10		a	D
hKv4.2	−90	+40, 50	10		a	D
hKCa1.1	−90	+40, 200	10		a	D
hKCa4.1	−90	+40, 200	10		a	E
hKv7.2/7.3	−90	+40, 200	10		b	F
hKv7.3/7.5	−90	+40, 200	10		b	F
GABA $\alpha_1\beta_2\gamma_2$	−80			30 μM GABA	a	G
$NMDA_{1A/2A}$	−80			30 μM glutamate, 10 μM glycine	a	D
nAChR$\alpha_4\beta_2$	−80			100 μM acetylcholine	a	D

A, (in mM) 138 NaCl, 4.5 KCl, 1.8 $CaCl_2$, 1.0 $MgCl_2$, 10 HEPES, 10 glucose, buffered to pH 7.4; B, As per buffer A but 0 mM $MgCl_2$; C, (in mM) 140 CsF, 1 EGTA, 10 NaCl, 10 HEPES, buffered to pH 7.3; D, (in mM) 60 KF, 50 KCl, 10 NaCl, 10EGTA, 10 HEPES, buffered to pH 7.3; E, As per buffer D but with 10 mM KCl and 50 mM NaCl; F, (in mM) 120 KF, 20 KCl, 10 HEPES, 10 EGTA, 10 EDTA, buffered to pH 7.3; G, (in mM) 130 KCl, 1 $MgCl_2$, 5 Mg-ATP, 10 HEPES, 5 EGTA, buffered to pH 7.3.

Table 4. MEA parameters included in the analysis

MEA Parameter	Description	Units
Mean firing rate	Total number of spikes divided by the recording time	(Hz)
Burst duration	Average time from the first spike to the last spike in a single electrode burst	(s)
Network burst frequency	Total number of network bursts divided by the recording time	—
Network burst duration	Average time from the first spike to the last spike in a network burst	(s)
Number of spikes per network burst	Average number of spikes in a network burst	—

After recording spontaneous activity, raw files were rerecorded using the AxIS spike detector to create Axis spike files (.spk). These were then loaded on to the Axion Neural Metric tool for analysis of the spikes and generation of raster plots. Only active electrodes (MSR ≥ 5 spikes/min) in active wells (≥8 active electrode) were included in the analysis. Single electrode bursts were detected using ISI threshold with a minimum of 5 spikes and a maximum ISI of 100 ms. Network bursts were identified using the envelope method. Metrics were exported for analysis using the Axion Metric Plotting tool. Here, the baseline and treatment data were compared with identify % change in selected parameters. Five parameters have been included to describe the activity and organization of activity: Mean firing rate, burst duration, network burst frequency, network burst duration, and number of spikes per network burst (Table 4). Similar parameters have been selected in analyses by other groups (Kreir *et al.*, 2018; Tukker *et al.*, 2020a,b).

Data were reproducible over time, especially for network bursting which was a more reproducible parameter than mean firing rate. The critical parameters in ensuring data reproducibility were the proportion of glutaneurons (85%) to astrocytes (15%) and the elapsed time of 21–25 days in culture allowed to establish functional networks.

Results

Ion channel panel potency

The potency of the validation compounds against the ion channel panel is presented as IC_{50} values (Figure 1) with a grading system for activity derived from experience with hERG (Leishman *et al.*, 2020; Redfern *et al.*, 2003) as follows: IC_{50}>100 μM=no activity at ion channel, low risk (green); 30–100 μM=moderate activity at ion channel, intermediate risk (orange); <30 μM=strong inhibition of ion channel, high risk (red). Although both inhibition and activation were assessed in the assay, only inhibition was observed. Example concentration response curves for Nav1.1, Kv2.1, and GABA $\alpha_1\beta_2\gamma_2$ are included as part of Supplementary Data.

To facilitate ranking the ion channels for their potential association with seizure, a value of 2, 1, or 0 was assigned for high inhibition hits (red; IC_{50}<30 μM), intermediate inhibition hits (orange; IC_{50} 30–100 μM), and low inhibition hits (green IC_{50}>100 μM), respectively (Figure 1, right hand column). The ion channels ranked from high risk to low risk as follows: Nicotinic $\alpha_4\beta_2$=21, Kv2.1 = 15, Kv4.2 = 15, Kv7.3/7.5 = 14, Kv7.2/7.3 = 12, Kv1.1 = 10, GABA $\alpha_1\beta_2\gamma_2$=9, Nav1.1 = 9, Nav1.2 = 8, Nav1.6 = 7, Kv3.1 = 6, KCa4.1 = 5, NMDA1/2A = 5, KCa1.1 = 0 (Figure 1). The nicotinic $\alpha_4\beta_2$ channel was the most frequent hit and the lowest activity was at KCa4.1, NMDA1/2A, and KCa1.1.

CNS active therapies.

Looking at the CNS active compounds, the ion channels ranked from high to low risk as follows: Chlorpromazine = 21, amoxapine = 20, paroxetine = 19, clozapine = 17, diphenhydramine = 14, quetiapine = 10, and bupropion = 6 (Figure 1). For most CNS therapies some sodium, potassium and ligand-gated channels were inhibited, except bupropion and quetiapine which inhibited potassium channels and the nicotinic $\alpha_4\beta_2$ channel only.

GABA antagonists.

Looking at the GABA antagonists, pentylenetetrazole (PTZ) and picrotoxin inhibited GABA $\alpha_1\beta_2\gamma_2$ only. The only activity for amoxicillin and enoxacin was at nicotinic $\alpha_4\beta_2$, and Kv2.1 for enoxacin (Figure 1).

Other compounds (4-AP, linopirdine, pilocarpine, strychnine).

Looking at the other test compounds, the potassium channel inhibitor 4-AP showed activity at Kv.3.1 and nicotinic $\alpha_4\beta_2$.

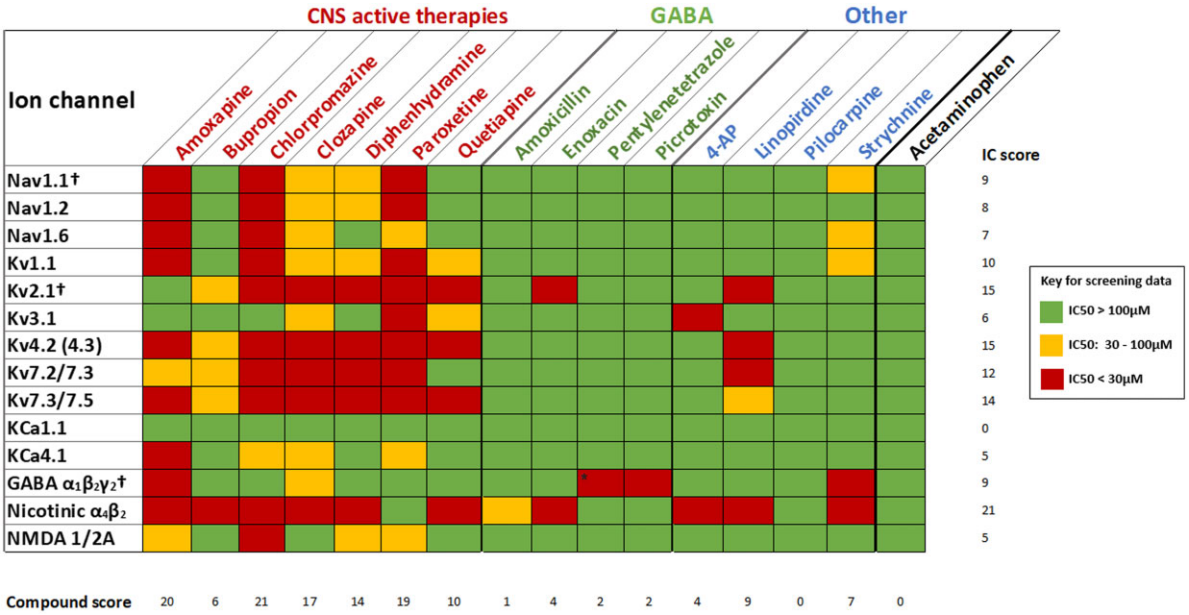

Figure 1. Activity of 15 CNS active therapies, GABA receptor antagonists, and other reported seizurogenic compounds at 14 ion channels implicated in seizure. Key depicts highly active (IC_{50}<30 μM red), moderately active (IC_{50} 30–100 μM orange), and inactive active (IC_{50}>100 μM green) compounds. Right-hand column: cumulative ranking of each ion channel for its potential association with seizure based on a value of 2, 1, or 0 for high, intermediate, and low inhibition hits, respectively. Bottom row: cumulative ranking of each compound for its activity at ion channels based on a value of 2, 1, or 0 for high, intermediate, and low inhibition hits, respectively. *IC_{50} of pentylenetetrazole is >100 μM; however, this is expected to be high and still considered inhibition. †Concentration response curves included as part of Supplementary Data.

Linopirdine inhibited Kv7.2/7.3, Kv2.1, Kv4.2, and nicotinic $\alpha_4\beta_2$ at IC_{50}<30 μM and Kv7.3/7.5 at IC_{50} 30–100 μM. Strychnine moderately inhibited 2 sodium channels (Nav1.1, Nav1.6) and 1 potassium channel (Kv1.1), and strongly inhibited GABA $\alpha_1\beta_2\gamma_2$ and nicotinic $\alpha_4\beta_2$. Pilocarpine was the only seizureogenic compound that did not inhibit any ion channels on our panel.

Acetaminophen was included as a negative control and did not inhibit any ion channels.

hiPSC neuronal coculture assay

Spike Raster plots depicting baseline activity and activity after 1-h incubation with selected concentrations of each compound are shown in Figure 2. The full dose range of concentrations tested is shown in Table 2. The changes in mean firing rate (Hz), burst duration (s), network burst frequency (Hz), network burst duration (s), and the number of spikes per network burst for the compounds is summarized in Figure 3. To facilitate ranking the compounds for their potency in causing changes in these 5 key parameters, each compound was assigned 1, 2, or 3 up or down arrows depending on the fold and direction of change.

CNS active therapies.

The CNS active therapies (amoxapine, bupropion, chlorpromazine, clozapine, diphenhydramine, paroxetine, quetiapine) all caused clear changes to neuronal firing which is visible in the raster plots. This was mostly characterized by decreased burst duration and an increased number of network bursts that are shorter and contain fewer spikes. Raster plots showed some distinct banding patterns of networks bursts with bupropion and diphenhydramine, whereas amoxapine and paroxetine cause sustained network bursting. Changes to mean firing rate mostly decreased across the group, however, amoxapine and paroxetine increased the firing rate.

GABA antagonists.

Picrotoxin increased all parameters, and increased network bursting was evident in the raster plots. PTZ caused minimal changes to the selected parameters, and the antibiotics amoxicillin and enoxacin did not change any parameter >20%.

Other compounds (4-AP, linopirdine, pilocarpine, strychnine).

4-AP did not change the number of network bursts; however, raster plots depicted a distinct pattern of network bursting of one large burst followed by many smaller bursts of activity. Linopirdine increased network bursting, and also increased the mean firing rate and number of spikes per network burst. Pilocarpine was the only compound to decrease the number of network bursts, which were also longer and contained more spikes. This pattern is evident in the raster plots. Strychnine shows a similar profile to the CNS active therapies—an increased number of network bursts that are shorter and contain fewer spikes.

Acetaminophen was included as a negative control and caused no change to any of the parameters assessed.

Discussion

Seizure liability remains a significant cause of attrition throughout drug development. Advances in stem cell biology coupled with an increased understanding of the role of ion channels in seizure offer an opportunity for a new paradigm in screening. Here, we describe an integrated approach for early detection of seizure *in vitro* using an ion channel panel coupled with perturbation of electrical activity in human derived iPSC neuronal cocultures. There is clear evidence for the involvement of ion channels in seizure with around 25% of genes identified in epilepsy

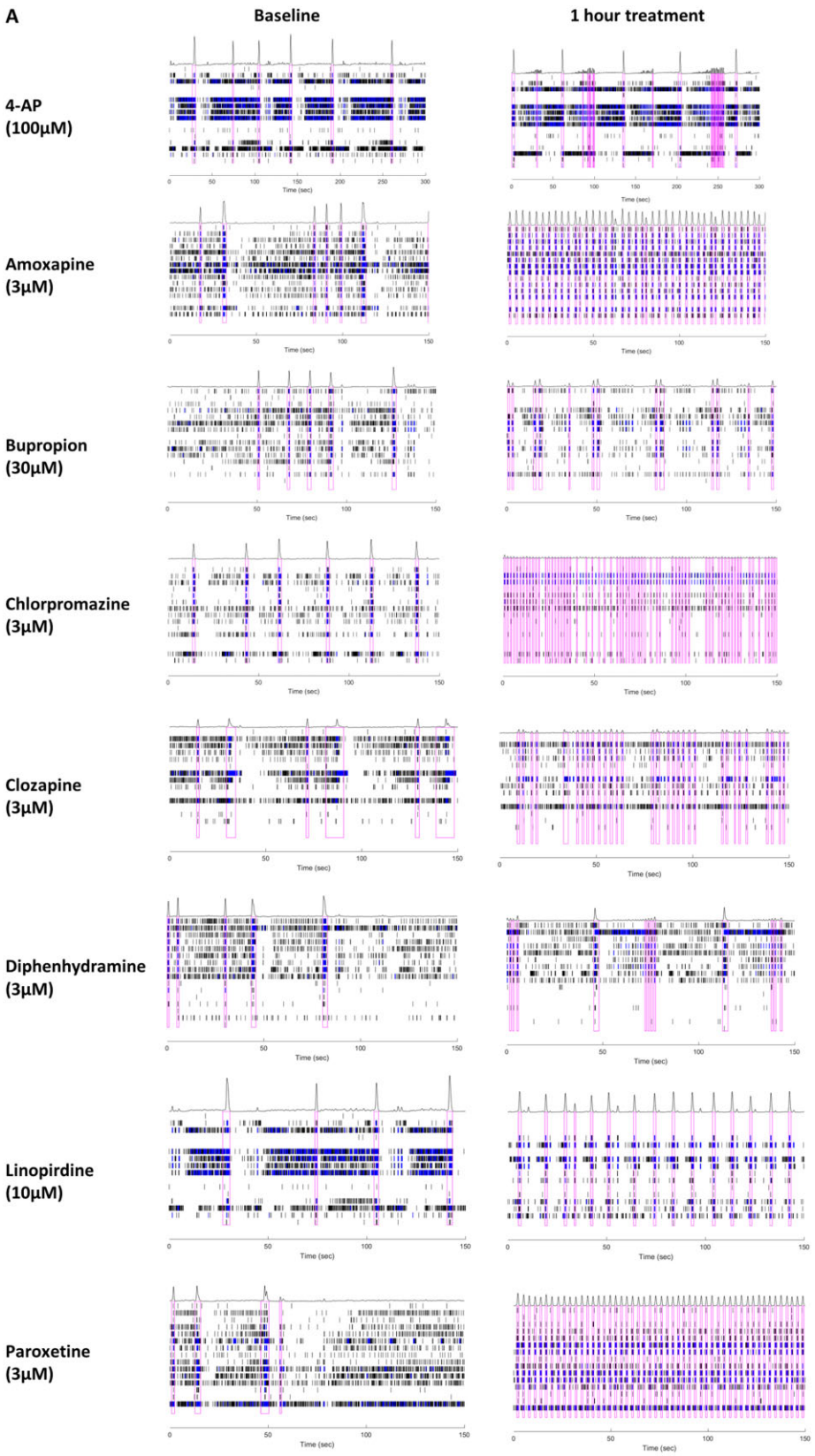

Figure 2. Raster plots depicting baseline activity and activity after 1-h incubation for 7 CNS active therapies, 4 GABA receptor antagonists, and 4 other reported seizurogenic compounds plus acetaminophen as a control (see Table 2). Only one concentration is shown for each compound; the full range of concentrations tested is shown in Table 2. Extracted key MEA parameters are shown in Figure 3.

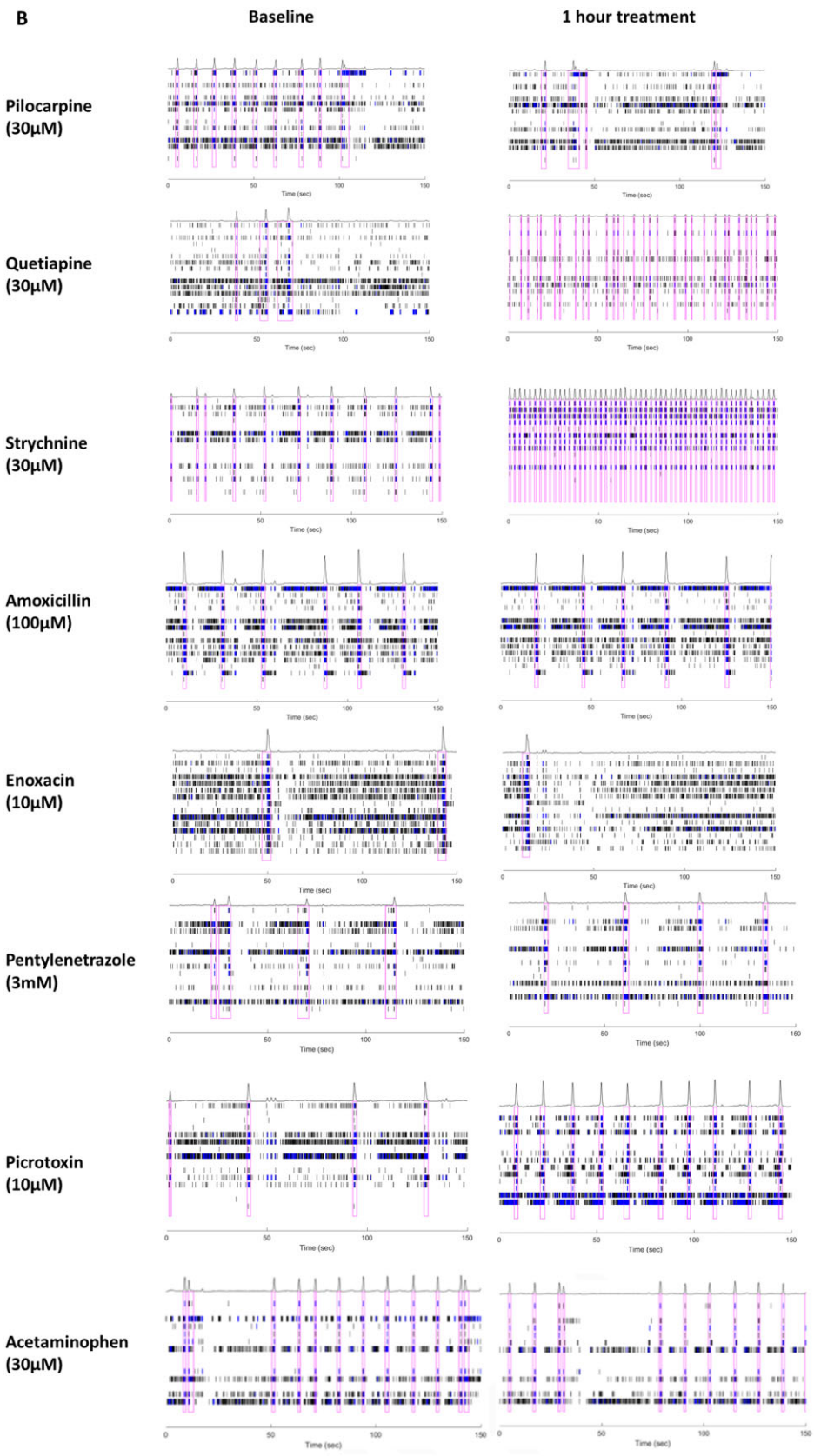

Figure 2. (Continued).

	MEA parameters					
	Mean firing rate	Burst duration	Network burst frequency	Network burst duration	Number of spikes per network burst	Tick score
Amoxapine (3μM)	↑↑	NC	↑↑↑	↓↓	↓	8
Bupropion (30μM)	↓	↓	↑↑↑	↓↓	↓↓	9
Chlorpromazine (3μM)	↓↓	↓↓	↑↑↑	↓↓	↓↓	11
Clozapine (3μM)	↓	↓	↑↑↑	↓↓	↓↓	9
Diphenhydramine (3μM)	↓	↓	↑↑↑	↓	↓↓	8
Paroxetine (3μM)	↑	↓	↑↑↑	↓	↓	7
Quetiapine (30μM)	↓	↓↓	↑↑↑	↓↓	↓↓	10
Amoxicillin (100μM)	NC	NC	NC	NC	NC	0
Enoxacin (10μM)	NC	NC	NC	NC	NC	0
Pentylenetetazole (1mM)	↓	NC	NC	↓	NC	2
Picrotoxin (10μM)	↑	↑↑	↑	↑	↑↑	7
4-AP (100μM)	NC	↓	NC	↓	↓	3
Linopirdine (10μM)	↑↑	NC	↑↑↑	↓	↑↑	8
Pilocarpine (30uM)	↓	NC	↓↓	↑↑	↑↑	7
Strychnine (30μM)	↑	NC	↑↑↑	↓	↓	6
Acetaminophen (30μM)	NC	NC	NC	NC	NC	0

Figure 3. Changes in 5 key MEA parameters in hiPSC neuronal cocultures for 7 CNS active therapies, 4 GABA receptor antagonists, and 4 other reported seizurogenic compounds plus acetaminophen as a negative control. Each compound was assigned 1 (20%–49% change), 2 (50%–100% change), or 3 (>100% change) up (blue) or down (green) arrows depending on the fold and direction of change. NC depicts no notable change. Right hand column: cumulative ranking for each compound where 1 arrow (either up or down) equals a score of 1.

encoding ion channels (Oyrer *et al.*, 2018). A comprehensive list of ion channels involved in seizure equates to over 100 targets (Kullmann and Waxman, 2010; Lerche *et al.*, 2013; Oyrer *et al.*, 2018) but weight of evidence from genetic and pharmacological studies (Easter *et al.*, 2007) suggested an initial panel comprising Nav1.1, Nav1.2, Nav1.6, Kv7.2/7.3, Kv7.3/7.5, Kv1.1, Kv4.2, KCa4.1, Kv2.1, Kv3.1, KCa1.1, GABA $\alpha_1\beta_2\gamma_2$, nicotinic $\alpha_4\beta_2$, NMDA 1/2A (Table 1) as a starting point for testing (Roberts *et al.*, 2021; Rockley *et al.*, 2019).

In our study, the CNS active therapies generally inhibited the most ion channels outside of their accepted mechanisms of action (see Table 2) whereas acetaminophen, the negative control, did not change any parameters. In terms of potency for ion channel inhibition the compounds ranked as follows: Chlorpromazine> amoxapine>paroxetine>clozapine>diphenhydramine>quetiapine >bupropion. Bupropion, clozapine, amoxapine, and quetiapine are within the 10 drugs most frequently associated with convulsive ADRs (Kumlien and Lundberg, 2010). These drugs are known to be promiscuous (Bianchi, 2010). For example, in isolated rat neurons clozapine inhibits the GABA-induced Cl^- current (Yokota *et al.*, 2002) and chlorpromazine inhibits K-transient and K-delayed rectifier currents (Ogata and Tatebayashi, 1993; Wooltorton and Mathie, 1993). In human recombinant cell lines clozapine and quetiapine inhibit the hERG potassium channel (Crumb *et al.*, 2006) and paroxetine, clozapine, and chlorpromazine inhibit Nav1.2 (Lenkey *et al.*, 2010). These reports are concordant with our findings which link inhibition of 14 seizure-related ion channels with the clinical observation of convulsions. This supports the notion that CNS-penetrant compounds may benefit from early ion channel screening to identify and design out potential liabilities as illustrated in an example of convulsions due to GABA-A (Delaunois *et al.*, 2020). The nicotinic $\alpha_4\beta_2$ ion channel was most frequently implicated in seizure across all the compounds assessed, followed by the potassium channels Kv2.1, Kv4.2, Kv7.3/7.5, Kv7.2/7.3, and Kv1.1 making these key components for screening.

A range of convulsants have been shown to change the firing of neuronal networks using MEAs and complex cultures of primary rodent neuronal cells, human-derived iPSC-derived neuronal cultures, and hippocampal slices (Accardi *et al.*, 2018; Bradley and Strock, 2019; Bradley *et al.*, 2018; Fan *et al.*, 2019; Frega *et al.*, 2012; Koerling *et al.*, 2019; Kreir *et al.*, 2018). The Health and Environmental Science Institute (HESI) Neurotoxicity MEA Subteam (HESI, 2020) has developed and conducted pilot studies using MEAs for predicting the seizure liability of drugs using both primary rodent and hiPSC-derived neurons, although human-derived cells are expected to provide a better translation to humans than rodent cells (Grainger *et al.*, 2018; Ishii *et al.*, 2017; Matsuda *et al.*, 2018; Odawara *et al.*, 2018; Ojima and Miyamoto, 2018; Tukker *et al.*, 2020b).

In our studies, the CNS active therapies all caused clear changes to neuronal firing similar to seizure prediction pattern 2 proposed by Bradley *et al.* (2018) whereas acetaminophen, the negative control, did not change any parameters. Our findings for amoxapine and chlorpromazine are in concordance with similar studies with respect to network burst frequency, but with differences in mean spike rate for chlorpromazine (Tukker *et al.*, 2020b) pointing towards network bursting as a more reproducible parameter. The increased network bursting seen with amoxapine in hiPSC-neuronal cultures has not been observed with rodent primary cortical cultures in other studies (Kreir *et al.*, 2018; Tukker *et al.*, 2020b) whereas seizures were demonstrated in zebrafish (Winter *et al.*, 2008), highlighting interspecies differences in amoxapine sensitivity. The same may be true of bupropion because seizures were not reported in rodent hippocampal slice

assays (Easter *et al.*, 2009). However, we detected clear changes in our hiPSC-MEA assay.

The GABA antagonists PTZ and picrotoxin are both used *in vivo* to reduce seizure threshold and have been widely used for validation studies of preclinical models (Bradley *et al.*, 2018; Easter *et al.*, 2007; Fan *et al.*, 2019; Kreir *et al.*, 2018; Tukker *et al.*, 2020b; Winter *et al.*, 2008) They both inhibited the GABA $\alpha1\beta_2\gamma_2$ ion channel only, and picrotoxin increased all MEA parameters assessed. Our MEA findings with picrotoxin agree with other studies of hiPSC in the literature (Tukker *et al.*, 2020a), and align with seizure prediction pattern 1 proposed by Bradley *et al.* (2018). PTZ minimally affected the electrical activity in our hiPSC-neuronal assay, contrasting with previous reports and possibly due to differences in concentration ranges tested (Odawara *et al.*, 2018; Tukker *et al.*, 2020b).

Amoxicillin and enoxacin are broad spectrum antibiotics that are reported to inhibit GABA-A and cause seizures in humans (Raposo *et al.*, 2016; Wanleenuwat *et al.*, 2020). Both compounds did not inhibit the GABA $\alpha1\beta_2\gamma_2$ ion channel and caused no significant changes to neuronal firing in the MEA assay at the clinically relevant concentrations used. In other studies, minimal changes to neuronal activity were reported with these compounds in hiPSC-neuronal cocultures (Tukker *et al.*, 2020b). Seizurogenic responses have been observed with enoxacin in zebrafish, rat hippocampal slice, and rat primary cortical cells, so perhaps the lack of activity in human iPSC-neuronal cells in this study reflects a species-related difference (Easter *et al.*, 2009; Tukker *et al.*, 2020b; Winter *et al.*, 2008).

4-AP is widely used in the validation of preclinical models for seizure detection including zebrafish, rodent hippocampal slice, and nonhuman primate slice with positive results (Accardi *et al.*, 2018; Bradley *et al.*, 2018; Easter *et al.*, 2007; Fan *et al.*, 2019; Tukker *et al.*, 2020b; Winter *et al.*, 2008). 4-AP is a highly potent inhibitor of Kv3.1 channels (IC_{50}=29 μM) and a less potent inhibitor of other Kv channels (Gutman *et al.*, 2005). 4-AP at concentrations of up to 100 μM inhibited Kv3.1 with an IC_{50} of 5 μM. No other Kv channels were identified as hits in this study, which agrees with IC_{50} values in the literature of 290 μM, 18 mM, and 5 mM for Kv1.1, Kv2.1, and Kv4.2, respectively (Gutman *et al.*, 2005). Kv3.1 is expressed primarily on inhibitory GABAergic neurons (Protein Atlas, 2023) where it plays an important role in the rapid repolarization of fast-firing cortical neurons (Rudy and McBain, 2001) In our MEA assay, 4-AP did not change the number of network bursts but the raster plots showed a distinct pattern of one large network burst followed by many smaller bursts of activity, as anticipated pattern from hippocampal slice assays (Codadu *et al.*, 2019). This illustrates the importance of studying the pattern of raster plots as well as the quantitative changes to MEA parameters.

One of our primary aims was to look for correlations between ion channel activity and electrical perturbation by MEA. Table 5 shows an agreement between potency at ion channels and strength of perturbation in MEA with a few exceptions such as enoxacin and pilocarpine. To develop the alignment further, data from rat hippocampal brain slices (Easter *et al.*, 2007) were added to Table 5, showing good agreement with 1 or 2 exceptions. In rodent hippocampal slice models, seizurogenicity was not detected with bupropion or pilocarpine (Easter *et al.*, 2007). In this study pilocarpine was the only compound that did not inhibit any ion channels on our panel and decreased network bursting in the MEA assay. This suggests that the seizurogenic mechanism is independent of ion channel perturbation and is likely related to activity on the M1 muscarinic receptor (Cruickshank *et al.*, 1994). A similar study using hiPSC-neuronal cells also reported reduced activity with 30 μM pilocarpine. Interestingly, in rodent primary cortical cultures 3 and 10 μM pilocarpine have the opposite effect and strongly increase network bursting (Kreir *et al.*, 2018; Tukker *et al.*, 2020b). Finally, acetaminophen was included as a negative control and caused no change to any of the parameters assessed. A limitation of this study is the inclusion of only one negative control. Going forward it would be useful to assess the effects of more nonseizurogenic compounds to get a true measure of the assays negative predictive power. Similarly, inclusion of seizureogenic compounds with different mechanisms of action would also be worthwhile.

Overall, our data indicate the potential of an ion channel panel coupled with hiPSC-neuronal MEA assays for predictive early *in vitro* assessment of seizure liability providing the opportunity to assess seizure in human-based *in vitro* assays, reducing reliance on animal studies. Since CNS active compounds were most likely to show activity in these assays, it is particularly important to screen these potential drugs early in discovery. These data support the idea originally outlined in Roberts *et al.* (2021), recently cited by the FDA/CDER (Avila *et al.*, 2023), that the panel constitutes a useful human-based NAM that can play a key part in the overall safety assessment for seizure liability ranging from lead generation to later stages of development (Figure 4). The 14 ion channels were selected based on their strong genetic links to seizure (Table 1). As perturbation of non-ion channel targets such as some GPCRs and transporters enzymes could also cause seizure (Easter *et al.*, 2009), it is important to perform a broad range of safety assessments to fully appraise the potential for seizure liability. Together, the data provide evidence for an integrated approach to early seizure liability screening to support

	Ion Channel Panel	MEA hiPSC Assay	Hippocampal Slice
Amoxapine	20	+	
Bupropion	6	+	[a]
Chlorpromazine	21	+	[a]
Clozapine	17	+	
Diphenhydramine	14	+	
Paroxetine	19	+	
Quetiapine	10	+	
Tiagabine	1		
Enoxacin	4	+	+ [a]
Pentylenetetrazole	2	+	+ [a]
Picrotoxin	2	+	+ [a,b]
Amoxicillin	4	+	+ [a]
Lidocaine	9	+	
Pilocarpine	0	+	[a]
Strychnine	7	+	+ [a,b]
Acetaminophen	0		

Table 5. Alignment of human ion channel activity with hiPSC MEA perturbations and data from the rat hippocampal slice assay

Score for ion channel panel calculated using a value of 2, 1, or 0 for high, intermediate, and low inhibition hits, respectively. A positive MEA hit was determined by demonstrating distinctive changes in firing on raster plots and/or changes in 3 or more parameters. Red, green and blue text depicts CNS active, GABA receptors antagonists and other compounds, respectively. Acetaminophen is included as a negative control.

[a] Rat hippocampal slice work was performed at AstraZeneca as described by Easter *et al.* (2009).

[b] Rat hippocampal slice work was performed by Bristol-Myers Squibb as described by Fan *et al.* (2019).

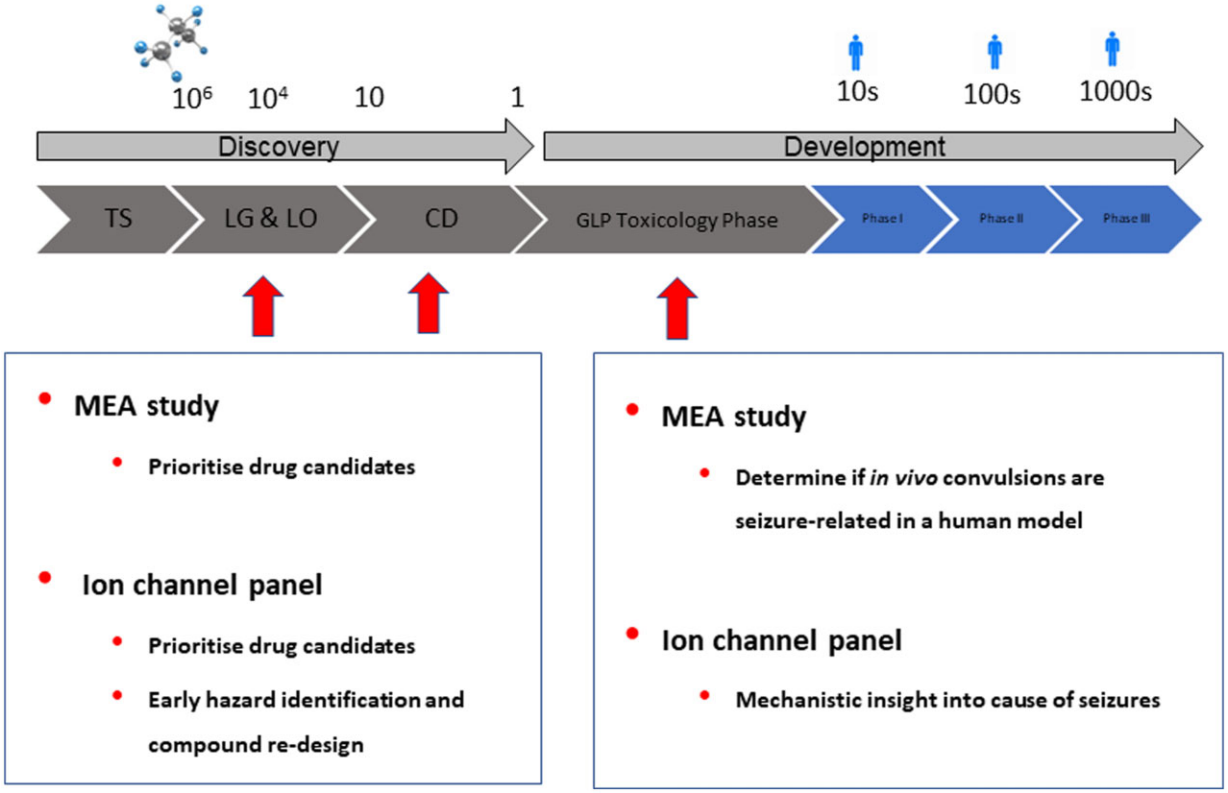

Figure 4. Placement of MEA studies and ion channel screening for seizure liability assessment within drug discovery and development. The timing of the assessment is likely to be project specific. For example, in lead generation the ion channel panel may be useful in early hazard identification so potential liabilities can be designed out, and the MEA may prove useful in ranking drug candidates for selection. In the later stages of development, the MEA assay could provide insight into whether convulsions observed in GLP toxicology studies are seizure related, and the ion channel panel could provide mechanistic information into the cause of seizure.

optimal drug design in early development, saving time and resources.

Supplementary data

Supplementary data are available at *Toxicological Sciences* online.

Declaration of conflicting interests

R.R., K.R., H.J., K.J., and M.M. are all employees of ApconiX, an integrated ion channel research and toxicology consultancy that provides expertise to academia, industry, NGOs, and government on all aspects of nonclinical program design and delivery. M.D. and P.L. are employees of BMS, a multinational pharmaceutical company.

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References

Aagaard, L., and Hansen, E. H. (2013). Adverse drug reactions reported by consumers for nervous system medications in Europe 2007 to 2011. *BMC Pharmacol. Toxicol.* **14**, 30.

Accardi, M. V., Huang, H., and Authier, S. (2018). Seizure liability assessments using the hippocampal tissue slice: Comparison of non-clinical species. *J. Pharmacol. Toxicol. Methods* **93**, 59–68.

Aridon, P., Marini, C., Resta, C. D., Brilli, E., De Fusco, M., Politi, F., Parrini, E., Manfredi, I., Pisano, T., Pruna, D., *et al.* (2006). Increased sensitivity of the neuronal nicotinic receptor α2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. *Am. J. Hum. Genet.* **79**, 342–350.

Authier, S., Arezzo, J., Delatte, M. S., Kallman, M. J., Markgraf, C., Paquette, D., Pugsley, M. K., Ratcliffe, S., Redfern, W. S., Stevens, J., *et al.* (2016). Safety pharmacology investigations on the nervous system: An industry survey. *J. Pharmacol. Toxicol. Methods* **81**, 37–46.

Avila, A. M., Bebenek, I., Mendrick, D. L., Peretz, J., Yao, J., and Brown, P. C. (2023). Gaps and challenges in nonclinical assessments of pharmaceuticals: An FDA/CDER perspective on considerations for development of new approach methodologies. *Regul. Toxicol. Pharmacol.* **139**, 105345.

Barcia, G., Fleming, M. R., Deligniere, A., Gazula, V. R., Brown, M. R., Langouet, M., Chen, H., Kronengold, J., Abhyankar, A., Cilio, R., et al (2012). De novo gain-of-function KCNT1 channel mutations cause malignant migrating partial seizures of infancy. *Nat. Genet.* **44**, 1255–1259.

Bassi, M. T., Balottin, U., Panzeri, C., Piccinelli, P., Castaldo, P., Barrese, V., Soldovieri, M. V., Miceli, F., Colombo, M., Bresolin, N., *et al.* (2005). Functional analysis of novel KCNQ2 and KCNQ3 gene variants found in a large pedigree with benign familial neonatal convulsions (BFNC). *Neurogenetics* **6**, 185–193.

Bell, D. C., and Dallas, M. L. (2018). Using automated patch clamp electrophysiology platforms in pain-related ion channel

research: Insights from industry and academia. *Br. J. Pharmacol.* **175**, 2312–2321.

Berecki, G., Howell, K. B., Deerasooriya, Y. H., Cilio, M. R., Oliva, M. K., Kaplan, D., Scheffer, I. E., Berkovic, S. F., and Petrou, S. (2018). Dynamic action potential clamp predicts functional separation in mild familial and severe de novo forms of SCN2A epilepsy. *Proc. Natl. Acad. Sci. U.S.A.* **115**, E5516–E5525.

Bianchi, M. T. (2010). Promiscuous modulation of ion channels by anti-psychotic and anti-dementia medications. *Med. Hypotheses* **74**, 297–300.

Biervert, C., Schroeder, B. C., Kubisch, C., Berkovic, S. F., Propping, P., Jentsch, T. J., and Steinlein, O. K. (1998). A potassium channel mutation in neonatal human epilepsy. *Science* **279**, 403–406.

Blanchard, M. G., Willemsen, M. H., Walker, J. B., Dib-Hajj, S. D., Waxman, S. G., Jongmans, M., Kleefstra, C. J., van de Warrenburg, T., Praamstra, B. P., Nicolai, P., *et al.* (2015). De novo gain-of-function and loss-of-function mutations of SCN8A in patients with intellectual disabilities and epilepsy. *J. Med. Genet.* **52**, 330–337.

Bradley, J. A., and Strock, C. J. (2019). Screening for neurotoxicity with microelectrode array. *Curr. Protoc. Toxicol.* **79**, e67.

Bradley, J. A., Luithardt, H. H., Metea, M. R., and Strock, C. J. (2018). In vitro screening for seizure liability using microelectrode array technology. *Toxicol. Sci.* **163**, 240–253.

Calhoun, J. D., Vanoye, C. G., Kok, F., George, A. L., and Kearney, J. A. (2017). Characterization of a KCNB1 variant associated with autism, intellectual disability, and epilepsy. *Neurol. Genet.* **3**, e198.

Cameron, J. M., Maljevic, S., Nair, U., Aung, Y. H., Cogné, B., Bézieau, S., Blair, E., Isidor, B., Zweier, C., Reis, A., *et al.* (2019). Encephalopathies with KCNC1 variants: Genotype-phenotype-functional correlations. *Ann. Clin. Transl. Neurol.* **6**, 1263–1272.

Cavalheiro, E. A., Santos, N. F., and Priel, M. R. (1996). The pilocarpine model of epilepsy in mice. *Epilepsia* **37**, 1015–1019.

Claes, L., Del-Favero, J., Ceulemans, B., Lagae, L., Van Broeckhoven, C., and De Jonghe, P. (2001). De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. *Am. J. Hum. Genet.* **68**, 1327–1332.

Codadu, N. K., Graham, R. T., Burman, R. J., Jackson-Taylor, R. T., Raimondo, J. V., Trevelyan, A. J., and Parrish, R. R. (2019). Divergent paths to seizure-like events. *Physiol. Rep.* **7**, e14226.

Conti, V., Aracri, P., Chiti, L., Brusco, S., Mari, F., Marini, C., Albanese, M., Marchi, A., Liguori, C., Placidi, F., *et al.* (2015). Nocturnal frontal lobe epilepsy with paroxysmal arousals due to CHRNA2 loss of function. *Neurology* **84**, 1520–1528.

Cook, D., Brown, D., Alexander, R., March, R., Morgan, P., Satterthwaite, G., and Pangalos, M. N. (2014). Lessons learned from the fate of AstraZeneca's drug pipeline: A five-dimensional framework. *Nat. Rev. Drug Discov.* **13**, 419–431.

Cruickshank, J. W., Brudzynski, S. M., and McLachlan, R. S. (1994). Involvement of M1 muscarinic receptors in the initiation of cholinergically induced epileptic seizures in the rat brain. *Brain Res.* **643**, 125–129.

Crumb, W. J., Ekins, S., Sarazan, R. D., Wikel, J. H., Wrighton, S. A., Carlson, C., and Beasley, C. M. (2006). Effects of antipsychotic drugs on I(to), I (Na), I (sus), I (K1), and hERG: QT prolongation, structure activity relationship, and network analysis. *Pharm. Res.* **23**, 1133–1143.

Crumb, W. J., Vicente, J., Johannesen, L., and Strauss, D. G. (2016). An evaluation of 30 clinical drugs against the comprehensive in vitro proarrhythmia assay (CiPA) proposed ion channel panel. *J. Pharmacol. Toxicol. Methods* **81**, 251–262.

Curia, G., Longo, D., Biagini, G., Jones, R. S. G., and Avoli, M. (2008). The pilocarpine model of temporal lobe epilepsy. *J. Neurosci. Methods* **172**, 143–157.

Davidson, J. (1989). Seizures and bupropion: A review. *J. Clin. Psychiatry* **50**, 256–261.

De Kovel, C. G. F., Syrbe, S., Brilstra, E. H., Verbeek, N., Kerr, B., Dubbs, H., Bayat, A., Desai, S., Naidu, S., Srivastava, S., *et al.* (2017). Neurodevelopmental disorders caused by de novo variants in KCNB1 genotypes and phenotypes. *JAMA Neurol.* **74**, 1228–1236.

Delaunois, A., Accardi, M. V., André, V., Authier, S., Bouzom, F., Hebeisen, S., Lamberty, Y., Laredj, F., Leclercq, K., Letombe, A. G., *et al.* (2020). Unraveling the mechanism and the risk behind seizure liability of lead compounds in a neuroscience project. *J. Pharmacol. Toxicol. Methods* **104**, 106874.

Dinoi, G., Morin, M., Conte, E., Mor Shaked, H., Coppola, M. A., D'Adamo, M. C., Elpeleg, O., Liantonio, A., Hartmann, I., De Luca, A., *et al.* (2022). Clinical and functional study of a de novo variant in the PVP motif of Kv1.1 channel associated with epilepsy, developmental delay and ataxia. *Int. J. Mol. Sci.* **23**, 8079.

Dogu, O., Sevim, S., and Kaleagasi, H. S. (2003). Seizures associated with quetiapine treatment. *Ann. Pharmacother.* **37**, 1224–1227.

Du, W., Bautista, J. F., Yang, H., Diez-Sampedro, A., You, S. A., Wang, L., Kotagal, P., Lüders, H. O., Shi, J., Cui, J., *et al.* (2005). Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat. Genet.* **37**, 733–738.

Easter, A., Bell, M. E., Damewood, J. R., Redfern, W. S., Valentin, J.-P., Winter, M. J., Fonck, C., and Bialecki, R. A. (2009). Approaches to seizure risk assessment in preclinical drug discovery. *Drug Discov. Today* **14**, 876–884.

Easter, A., Sharp, T. H., Valentin, J. P., Pollard, C. E. (2007). Pharmacological validation of a semi-automated in vitro hippocampal brain slice assay for assessment of seizure liability. *J. Pharmacol. Toxicol. Methods* **56**, 223–233.

Fan, J., Thalody, G., Kwagh, J., Burnett, E., Shi, H., Lewen, G., Chen, S. J., and Levesque, P. (2019). Assessing seizure liability using multi-electrode arrays (MEA). *Toxicol. In Vitro* **55**, 93–100.

FDA. (2022). Available at: https://www.congress.gov/bill/117th-congress/senate-bill/5002. Accessed May 15, 2023.

Fedor, F. Z., Paraczky, C., Ravasz, L., Tóth, K., Borhegyi, Z., Somogyvári, Z., Juhász, G., and Fekete, Z. (2020). Electrophysiological and behavioral properties of 4-aminopyridine-induced epileptic activity in mice. *Biol. Futur.* **71**, 427–434.

Flagmeyer, I., Gebert, I., and Van Der Staay, F. J. (1995). General pharmacology of the putative cognition enhancer linopirdine. *Arzneimittelforschung* **45**, 456–459.

Frega, M., Pasquale, V., Tedesco, M., Marcoli, M., Contestabile, A., Nanni, M., Bonzano, L., Maura, G., and Chiappalone, M. (2012). Cortical cultures coupled to micro-electrode arrays: A novel approach to perform in vitro excitotoxicity testing. *Neurotoxicol. Teratol.* **34**, 116–127.

Fusco, M. D., Becchetti, A., Patrignani, A., Annesi, G., Gambardella, A., Quattrone, A., Ballabio, A., Wanke, E., and Casari, G. (2000). The nicotinic receptor β2 subunit is mutant in nocturnal frontal lobe epilepsy. *Nat. Genet.* **26**, 275–276.

Gintant, G., Sager, P. T., and Stockbridge, N. (2016). Evolution of strategies to improve preclinical cardiac safety testing. *Nat. Rev. Drug Discov.* **15**, 457–471.

Grainger, A. I., King, M. C., Nagel, D. A., Parri, H. R., Coleman, M. D., and Hill, E. J. (2018). In vitro models for seizure-liability testing using induced pluripotent stem cells. *Front. Neurosci.* **12**, 590.

Green, M. R., and Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual*, 4th ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Gutman, G. A., Chandy, K. G., Grissmer, S., Lazdunski, M., Mckinnon, D., Pardo, L. A., Robertson, G. A., Rudy, B., Sanguinetti, M. C., Stu, W., *et al.* (2005). International union of pharmacology. *Pharmacol. Rev*. **57**, 473–508.

Hernandez, C. C., and Macdonald, R. L. (2019). A structural look at GABA a receptor mutations linked to epilepsy syndromes. *Brain Res*. **1714**, 234–247.

HESI. (2020). Available at: https://hesiglobal.org/committee-ontrans-lational-biomarkers-of-neurotoxicity/. Accessed October 2020.

Hill, T., Coupland, C., Morriss, R., Arthur, A., Moore, M., and Hippisley-Cox, J. (2015). Antidepressant use and risk of epilepsy and seizures in people aged 20 to 64 years: Cohort study using a primary care database. *BMC Psychiatry* **15**, 315.

Howell, K. B., McMahon, J. M., Carvill, G. L., Tambunan, D., Mackay, M. T., Rodriguez-Casero, V., Webster, R., Clark, D., Freeman, J. L., Calvert, S., *et al.* (2015). SCN2A encephalopathy: A major cause of epilepsy of infancy with migrating focal seizures. *Neurology* **85**, 958–966.

Ishii, M. N., Yamamoto, K., Shoji, M., Asami, A., and Kawamata, Y. (2017). Human induced pluripotent stem cell (hiPSC)-derived neurons respond to convulsant drugs when co-cultured with hiPSC-derived astrocytes. *Toxicology* **389**, 130–138.

Jang, D. H., Manini, A. F., Trueger, N. S., Duque, D., Nestor, N. B., Nelson, L. S., and Hoffman, R. S. (2010). Status epilepticus and wide-complex tachycardia secondary to diphenhydramine overdose NIH public access. *Clin. Toxicol. (Phila)* **48**, 945–948.

Kang, S. K., Vanoye, C. G., Misra, S. N., Echevarria, D. M., Calhoun, J. D., O'Connor, J. B., Fabre, K. L., McKnight, D., Demmer, L., Goldenberg, P., *et al.* (2019). High throughput characterization of KCNB1 variants associated with developmental and epileptic encephalopathy. *Ann. Neurol.* **86**, 899–912.

Khan, K. M., Collier, A. D., Meshalkina, D. A., Kysil, E. V., Khatsko, S. L., Kolesnikova, T., Morzherin, Y. Y., Warnick, J. E., Kalueff, A. V., and Echevarria, D. J. (2017). Zebrafish models in neuropsychopharmacology and CNS drug discovery. *Br. J. Pharmacol.* **174**, 1925–1944.

Koerling, A.-L., Fuchsberger, T., Paulsen, O., and Hay, Y. A. (2019). Partial restoration of physiological up-state activity by GABA pathway modulation in an acute brain slice model of epilepsy. *Neuropharmacology* **148**, 394–405.

Köppel, C., Ibe, K., and Tenczer, J. (1987). Clinical symptomatology of diphenhydramine overdose: An evaluation of 136 cases in 1982 to 1985. *J. Toxicol. Clin. Toxicol*. **25**, 53–70.

Kreir, M., Van Deuren, B., Versweyveld, S., De Bondt, A., Van den Wyngaert, I., Van der Linde, H., Lu, H. R., Teuns, G., and Gallacher, D. J. (2018). Do in vitro assays in rat primary neurons predict drug-induced seizure liability in humans? *Toxicol. Appl. Pharmacol*. **346**, 45–57.

Krüger, J., Schubert, J., Kegele, J., Labalme, A., Mao, A., Heighway, J., Seebohm, G., Yan, P., Koko, A., Aslan-Kara, K., *et al.* (2022). Loss-of-function variants in the KCNQ5 gene are associated with genetic generalized epilepsies. *EBioMedicine*. **84**, 104244.

Kullmann, D. M., and Waxman, S. G. (2010). Neurological channelopathies: New insights into disease mechanisms and ion channel function. *J. Physiol*. **588**, 1823–1827.

Kumlien, E., and Lundberg, P. O. (2010). Seizure risk associated with neuroactive drugs: Data from the WHO adverse drug reactions database. *Seizure* **19**, 69–73.

Lee, H., Lin, M., chin, A., Kornblum, H. I., Papazian, D. M., and Nelson, S. F. (2014). Exome sequencing identifies de novo gain of function missense mutation in KCND2 in identical twins with autism and seizures that slows potassium channel inactivation. *Hum. Mol. Genet*. **23**, 3481–3489.

Lehman, A., Thouta, S., Mancini, G. M. S., Naidu, S., van Slegtenhorst, M., McWalter, K., Person, R., Mwenifumbo, J., Salvarinova, R., Adam, S., *et al.*; EPGEN Study. (2017). Loss-of-function and gain-of-function mutations in KCNQ5 cause intellectual disability or epileptic encephalopathy. *Am. J. Hum. Genet.* **101**, 65–74.

Leishman, D. J., Abernathy, M. M., and Wang, E. B. (2020). Revisiting the hERG safety margin after 20 years of routine hERG screening. *J. Pharmacol. Toxicol. Methods* **105**, 106900.

Lenkey, N., Karoly, R., Lukacs, P., Vizi, E. S., Sunesen, M., Fodor, L., and Mike, A. (2010). Classification of drugs based on properties of sodium channel inhibition: A comparative automated patch-clamp study. *PLoS One* **5**, e15568.

Lerche, H., Shah, M., Beck, H., Noebels, J., Johnston, D., and Vincent, A. (2013). Ion channels in genetic and acquired forms of epilepsy. *J. Physiol*. **591**, 753–764.

Lertxundi, U., Hernandez, R., Medrano, J., Domingo-Echaburu, S., García, M., and Aguirre, C. (2013). Antipsychotics and seizures: Higher risk with atypicals? *Seizure* **22**, 141–143.

Li, X., Poschmann, S., Chen, Q., Fazeli, W., Oundjian, N. J., Snoeijen-Schouwenaars, F. M., Fricke, O., Kamsteeg, E. J., Willemsen, M., and Wang, Q. K. (2018). De novo BK channel variant causes epilepsy by affecting voltage gating but not Ca^{2+} sensitivity. *Eur. J. Hum. Genet*. **26**, 220–229.

Li, X., Zheng, Y., Li, S., Nair, U., Sun, C., Zhao, C., Lu, J., Zhang, V. W., Maljevic, S., Petrou, S., *et al.* (2021). Kv3.1 channelopathy: A novel loss-of-function variant and the mechanistic basis of its clinical phenotypes. *Ann. Transl. Med*. **9**, 1397–1397.

Litovitz, T. L., and Troutman, W. G. (1983). Amoxapine overdose. Seizures and fatalities. *JAMA* **250**, 1069–1071.

Lossin, C., Wang, D. W., Rhodes, T. H., Vanoye, C. G., and George, A. L. (2002). Molecular basis of an inherited epilepsy. *Neuron* **34**, 877–884.

Mackenzie, L., Medvedev, A., Hiscock, J. J., Pope, K. J., and Willoughby, J. O. (2002). Picrotoxin-induced generalised convulsive seizure in rat: Changes in regional distribution and frequency of the power of electroencephalogram rhythms. *Clin. Neurophysiol*. **113**, 586–596.

Mantegazza, M., Gambardella, A., Rusconi, R., Schiavon, E., Annesi, F., Cassulini, R. R., Labate, A., Carrideo, S., Chifari, R., Canevini, M. P., *et al.* (2005). Identification of an Nav1.1 sodium channel (SCN1A) loss-of-function mutation associated with familial simple febrile seizures. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 18177–18182.

Matsuda, N., Odawara, A., Katoh, H., Okuyama, N., Yokoi, R., and Suzuki, I. (2018). Detection of synchronized burst firing in cultured human induced pluripotent stem cell-derived neurons using a 4-step method. *Biochem. Biophys. Res. Commun*. **497**, 612–618.

McTague, A., Nair, U., Malhotra, S., Meyer, E., Trump, N., Gazina, E. V., Papandreou, A., Ngoh, A., Ackermann, S., Ambegaonkar, G., et al (2018). Clinical and molecular characterization of KCNT1-related severe early-onset epilepsy. *Neurology* **90**, E55–E66.

Miceli, F., Guerrini, R., Nappi, M., Soldovieri, M. V., Cellini, E., Gurnett, C. A., Parmeggiani, L., Mei, D., and Taglialatela, M. (2022). Distinct epilepsy phenotypes and response to drugs in KCNA1 gain- and loss-of function variants. *Epilepsia* **63**, e7–e14.

Miceli, F., Soldovieri, M. V., Ambrosino, P., De Maria, M., Migliore, M., Migliore, R., and Taglialatela, M. (2015). Early-onset epileptic encephalopathy caused by gain-of-function mutations in the

voltage sensor of Kv7.2 and Kv7.3 potassium channel subunits. *J. Neurosci.* **35**, 3782–3793.

Morgan, P., Brown, D. G., Lennard, S., Anderton, M. J., Barrett, J. C., Eriksson, U., Fidock, M., Hamrén, B., Johnson, A., March, R. E., et al (2018). Impact of a five-dimensional framework on R&D productivity at AstraZeneca. *Nat. Rev. Drug Discov.* **17**, 167–181.

Mulkey, S. B., Ben-Zeev, B., Nicolai, J., Carroll, J. L., Grønborg, S., Jiang, Y. H., Joshi, N., Kelly, M., Koolen, D. A., Mikati, M. A., *et al.* (2017). Neonatal non-epileptic myoclonus is a prominent clinical feature of KCNQ2 gain-of-function variants R201C and R201H. *Epilepsia* **58**, 436–445.

Muona, M., Berkovic, S. F., Dibbens, L. M., Oliver, K. L., Maljevic, S., Bayly, M. A., Joensuu, T., Canafoglia, L., Franceschetti, S., Michelucci, R., *et al.* (2015). A recurrent de novo mutation in KCNC1 causes progressive myoclonus epilepsy. *Nat. Genet.* **47**, 39–46.

Nagayama, T. (2015). Adverse drug reactions for medicine newly approved in Japan from 1999 to 2013: Syncope/loss of consciousness and seizures/convulsions. *Regul. Toxicol. Pharmacol.* **72**, 572–577.

Nappi, M., Barrese, V., Carotenuto, L., Lesca, G., Labalme, A., Ville, D., Smol, T., Rama, M., Dieux-Coeslier, A., Rivier-Ringenbach, C., et al (2022). Gain of function due to increased opening probability by two KCNQ5 pore variants causing developmental and epileptic encephalopathy. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2116887119.

Odawara, A., Matsuda, N., Ishibashi, Y., Yokoi, R., and Suzuki, I. (2018). Toxicological evaluation of convulsant and anticonvulsant drugs in human induced pluripotent stem cell-derived cortical neuronal networks using an MEA system. *Sci. Rep.* **8**, 10416.

Ogata, N., and Tatebayashi, H. (1993). Differential inhibition of a transient K+ current by chlorpromazine and 4-aminopyridine in neurones of the rat dorsal root ganglia. *Br. J. Pharmacol.* **109**, 1239–1246.

Ojima, A., and Miyamoto, N. (2018). [Method for MEA data analysis of drug-treated rat primary neurons and human iPSC-derived neurons to evaluate the risk of drug-induced seizures]. *Yakugaku Zasshi* **138**, 823–828.

Oyrer, J., Maljevic, S., Scheffer, I. E., Berkovic, S. F., Petrou, S., and Reid, C. A. (2018). Ion channels in genetic epilepsy: From genes and mechanisms to disease-targeted therapies. *Pharmacol. Rev.* **70**, 142–173.

Park, E., Gintant, G. A., Bi, D., Kozeli, D., Pettit, S. D., Pierson, J. B., Skinner, M., Willard, J., Wisialowski, T., Koerner, J., *et al.* (2018). Can non-clinical repolarization assays predict the results of clinical thorough QT studies? Results from a research consortium. *Br. J. Pharmacol.* **175**, 606–617.

Peña, F., and Tapia, R. (2000). Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus in vivo: Role of glutamate- and GABA-mediated neurotransmission and of ion channels. *Neuroscience* **101**, 547–561.

Phillips, H. A., Favre, I., Kirkpatrick, M., Zuberi, S. M., Goudie, D., Heron, S. E., Scheffer, I. E., Sutherland, G. R., Berkovic, S. F., Bertrand, D., *et al.* (2001). CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. *Am. J. Hum. Genet.* **68**, 225–231.

Pollard, C. E., Skinner, M., Lazic, S. E., Prior, H. M., Conlon, K. M., Valentin, J.-P., and Dota, C. (2017). An analysis of the relationship between preclinical and clinical QT interval-related data. *Toxicol. Sci.* **159**, 94–101.

Protein Atlas. (2023). Available at: https://www.proteinatlas.org/ENSG00000129159-KCNC1. Accessed May 2023.

Raposo, J., Teotónio, R., ão Bento, C., and Sales, F. (2016). Clinical commentary amoxicillin, a potential epileptogenic drug. *Epileptic Disord.* **18**, 454–457.

Redfern, W. S., Carlsson, L., Davis, A. S., Lynch, W. G., MacKenzie, I., Palethorpe, S., Siegl, P. K. S., Strang, I., Sullivan, A. T., Wallis, R., et al (2003). Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: Evidence for a provisional safety margin in drug developmentq. *Cardiovasc. Res.* **58**, 32–45.

Redfern, W. S., Waldron, G., Winter, M. J., Butler, P., Holbrook, M., Wallis, R., and Valentin, J.-P. (2008). Zebrafish assays as early safety pharmacology screens: Paradigm shift or red herring? *J. Pharmacol. Toxicol. Methods* **58**, 110–117.

Rivera, H. L., and Barrueto, F. (2014). Strychnine. In *Encyclopedia of Toxicology* (P. Wexler, Ed.), 3rd ed., pp. 407–408. Academic Press, Cambridge. Available at: https://doi.org/10.1016/B978-0-12-386454-3.00788-0. Accessed April 7, 2023.

Roberts, R., Authier, S., Mellon, R. D., Morton, M., Suzuki, I., Tjalkens, R. B., Valentin, J. P., and Pierson, J. B. (2021). Can we panelize seizure? *Toxicol. Sci.* **179**, 3–13.

Rockley, K. L., Roberts, R. A., and Morton, M. J. (2019). Innovative models for in vitro detection of seizure. *Toxicol. Res. (Camb)* **8**, 784–788.

Rudy, B., and McBain, C. J. (2001). Kv3 channels: Voltage-gated K+ channels designed for high-frequency repetitive firing. *Trends Neurosci.* **24**, 517–526.

Scheffer, H., Brunt, E. R. P., Mol, G. J. J., Van der Vlies, P., Stulp, R. P., Verlind, E., Mantel, G., Averyanov, Y. N., Hofstra, R. M. W., and Buys, C. H. C. M. (1998). Three novel KCNA1 mutations in episodic ataxia type I families. *Hum. Genet.* **102**, 464–466.

Schwarz, N., Hahn, A., Bast, T., Müller, S., Löffler, H., Maljevic, S., Gaily, E., Prehl, I., Biskup, S., Joensuu, T., *et al.* (2016). Mutations in the sodium channel gene SCN2A cause neonatal epilepsy with late-onset episodic ataxia. *J. Neurol.* **263**, 334–343.

Shimada, T., and Yamagata, K. (2018). Pentylenetetrazole-induced kindling mouse model. *J. Vis. Exp.* (**136**), 56573.

Singh, N. A., Charlier, C., Stauffer, D., DuPont, B. R., Leach, R. J., Melis, R., Ronen, G. M., Bjerre, I., Quattlebaum, T., Murphy, J. V., *et al.* (1998). A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat. Genet.* **18**, 25–29.

Swanger, S. A., Chen, W., Wells, G., Burger, P. B., Tankovic, A., Bhattacharya, S., Strong, K. L., Hu, C., Kusumoto, H., Zhang, J., et al (2016). Mechanistic insight into NMDA receptor dysregulation by rare variants in the GluN2A and GluN2B agonist binding domains. *Am. J. Hum. Genet.* **99**, 1261–1280.

Torkamani, A., Bersell, K., Jorge, B. S., Bjork, R. L., Friedman, J. R., Bloss, C. S., Cohen, J., Gupta, S., Naidu, S., Vanoye, C. G., *et al.* (2014). De novo KCNB1 mutations in epileptic encephalopathy. *Ann. Neurol.* **76**, 529–540.

Tukker, A. M., van Kleef, R. G. D. M., Wijnolts, F. M. J., de Groot, A., and Westerink, R. H. S. (2020a). Towards animal-free neurotoxicity screening: Applicability of hiPSC-derived neuronal models for in vitro seizure liability assessment. *Altex* **37**, 121–135.

Tukker, A. M., Wijnolts, F. M. J., De Groot, A., Westerink, R. H. S., and Westerink, R. H. S. (2020b). Applicability of hiPSC-derived neuronal co-cultures and rodent primary cortical cultures for in vitro seizure liability assessment. *Toxicol. Sci.* **178**, 71–87.

Veeramah, K. R., O'Brien, J. E., Meisler, M. H., Cheng, X., Dib-Hajj, S. D., Waxman, S. G., Talwar, D., Girirajan, S., Eichler, E. E., Restifo, L. L., *et al.* (2012). De novo pathogenic SCN8A mutation identified by whole-genome sequencing of a family quartet affected by infantile epileptic encephalopathy and SUDEP. *Am. J. Hum. Genet.* **90**, 502–510.

Wagnon, J. L., Mencacci, N. E., Barker, B. S., Wengert, E. R., Bhatia, K. P., Balint, B., Carecchio, M., Wood, N. W., Patel, M. K., and Meisler, M. H. (2018). Partial loss-of-function of sodium channel SCN8A in familial isolated myoclonus. *Hum. Mutat.* **39**, 965–969.

Walker, A. L., Imam, S. Z., and Roberts, R. A. (2018). Drug discovery and development: Biomarkers of neurotoxicity and neurodegeneration. *Exp. Biol. Med. (Maywood)* **243**, 1037–1045.

Wanleenuwat, P., Suntharampillai, N., and Iwanowski, P. (2020). Antibiotic-induced epileptic seizures: Mechanisms of action and clinical considerations. *Seizure* **81**, 167–174.

Winter, M. J., Redfern, W. S., Hayfield, A. J., Owen, S. F., Valentin, J. P., and Hutchinson, T. H. (2008). Validation of a larval zebrafish locomotor assay for assessing the seizure liability of early-stage development drugs. *J. Pharmacol. Toxicol. Methods* **57**, 176–187.

Wolff, M., Johannesen, K. M., Hedrich, U., Masnada, B. S., Rubboli, S., Gardella, G., Lesca, E., Ville, G., Milh, D., Villard, M., *et al.* (2017). Genetic and phenotypic heterogeneity suggest therapeutic implications in SCN2A-related disorders. *Brain* **140**, 1316–1336.

Wong, J., and Delva, N. (2007). Clozapine-induced seizures: Recognition and treatment. *Can. J. Psychiatry* **52**, 457–463.

Wooltorton, J. R., and Mathie, A. (1993). Block of potassium currents in rat isolated sympathetic neurones by tricyclic antidepressants and structurally related compounds. *Br. J. Pharmacol.* **110**, 1126–1132.

Yip, P. K., Chapman, G. E., Sillito, R. R., Ip, T. H. R., Akhigbe, G., Becker, S. C., Price, A. W., Michael-Titus, A. T., Armstrong, J. D., and Tremoleda, J. L. (2019). Studies on long term behavioural changes in group-housed rat models of brain and spinal cord injury using an automated home cage recording system. *J. Neurosci. Methods* **321**, 49–63.

Yokota, K., Tatebayashi, H., Matsuo, T., Shoge, T., Motomura, H., Matsuno, T., Fukuda, A., and Tashiro, N. (2002). The effects of neuroleptics on the GABA-induced Cl− current in rat dorsal root ganglion neurons: Differences between some neuroleptics. *Br. J. Pharmacol.* **135**, 1547–1555.

Young, A. C., Kleinschmidt, K. C., and Wax, P. M. (2009). Late-onset seizures associated with quetiapine poisoning. *J. Med. Toxicol.* **5**, 24–26.

Zuberi, S. M., Eunson, L. H., Spauschus, A., De Silva, R., Tolmie, J., Wood, N. W., McWilliam, R. C., Stephenson, J. P. B., Kullmann, D. M., and Hanna, M. G. (1999). A novel mutation in the human voltage-gated potassium channel gene (Kv1.1) associates with episodic ataxia type 1 and sometimes with partial epilepsy. *Brain* **122 (Pt 5)**, 817–825.