Benefits of early in vitro screening for seizure liability in problem solving and decision making

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Seizure liability remains a significant cause of attrition throughout drug development both in pre-clinical and clinical studies. This emphasizes the need for improved methodologies to detect seizure liability prior to in vivo toxicology studies, ideally with reduced reliance on animals and better translation to humans. Much like the Comprehensive in vitro Proarrhythmia Assay (CiPA) which is now widely accepted for early assessment of cardiovascular safety, we have developed an approach utilizing hiPSC-neuronal cell microelectrode array (MEA) and ion channel screening for early seizure prediction. These studies provide mechanistic information, and are useful for early de-risking, and support optimal drug design.

EXAMPLES DEMONSTRATING THE UTILITY OF IN VITRO SEIZURE LIABILITY ASSAYS

① Known Seizurogenic compounds identified correctly with high predictivity

② De-risk and prioritize a chemical series at single concentration

③ Good correlations between *in vitro - in vivo* responses with novel compounds

④ Assess human relevance of convulsions observed in nonclinical studies

① *PREDICITIVITY OF MEA ASSAY*

- ✓ 14/16 known seizurogenic compounds were identified correctly (87%)
- ✓ Correlations between *in vitro* responses and clinical exposures
- ✓ E.g. amoxapine – An antidepressant where 8.75% ADRs are convulsions

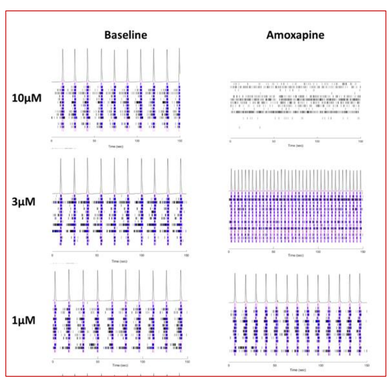
MEA parameters		Amoxapine 0.1µM	0.3µM	1µM	3µM	10µM
Spike	Mean firing rate (Hz)	NC	NC	↑	↑↑	↓↓↓
	Interspike Interval (ISI) Coefficient of variation - Avg	NC	NC	NC	↓	↓↓↓
Bursts	Burst frequency - Avg (Hz)	NC	NC	↑↑	↑↑↑	↓↓↓
	Burst duration - Avg (sec)	NC	NC	↓	↓↓	↓↓↓
	Number of spikes per burst - Avg	NC	NC	↓	↓↓	↓↓↓
	Mean ISI within burst - Avg (sec)	NC	NC	NC	↓↓	UNC
Network Bursts	Network burst frequency	NC	NC	↑↑	↑↑↑	N/A
	Network burst duration - Avg (sec)	NC	NC	↓	↓↓	N/A
	Number of spikes per network burst - Avg	NC	NC	↓	↓↓↓	N/A
	Mean ISI within network burst - Avg (sec)	NC	NC	NC	↑↑	N/A
	Network IBI Coefficient of variation - Avg	NC	NC	NC	↓↓	UNC
Synchrony	Area under normalized cross-correlation	NC	NC	NC	NC	↓↓↓

NC	no change (±10%)
one arrow	0 – 29% change
two arrows	30 – 49% change
three arrows	>50% change

Therapeutic and toxic doses taken from Schulz et al., 2020 – Revisited: Therapeutic and toxic blood concentrations of more than 1100 drugs and other xenobiotics

Therapeutic range 0.57 - 1.91µM

Toxic dose 9.56µM



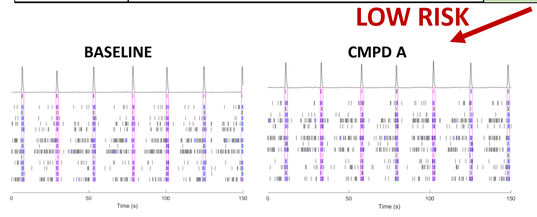
② *DE-RISKING A CHEMICAL SERIES*

- ✓ Testing a chemical series revealed one with low seizure risk (compound A)
- ✓ This compound had distinct structural features.

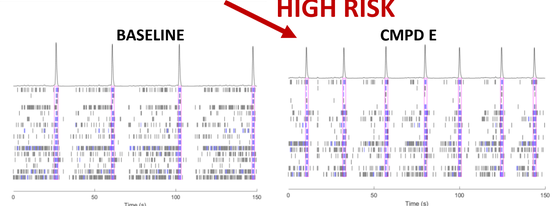
MEA parameters		A	B	C	D	E
Spike	Mean firing rate (Hz)	0	-8	+7	-5	-18
	Interspike Interval (ISI) Coefficient of variation - Avg	-4	+5	+5	+6	+4
Bursts	Burst frequency - Avg (Hz)	+3	-4	+1	+10	-6
	Burst duration - Avg (sec)	-6	-8	-6	-8	-11
	Number of spikes per burst - Avg	-12	-14	-12	-19	-19
	Mean ISI within burst - Avg (sec)	+3	0	-1	-6	-22
Network Bursts	Network burst frequency	+9	+34	+51	+46	+62
	Network burst duration - Avg (sec)	-2	-11	-8	-12	-15
	Number of spikes per network burst - Avg	-9	-27	-20	-29	-43
	Mean ISI within network burst - Avg (sec)	+8	-42	+16	+23	+211
Synchrony	Area under normalized cross-correlation	+9	-10	-4	-2	-13
Cell attachment	Resistance	0	-11	-2	0	-14

no change (±10%)
0 – 29% change
30 – 49% change
>50% change

LOW RISK



HIGH RISK



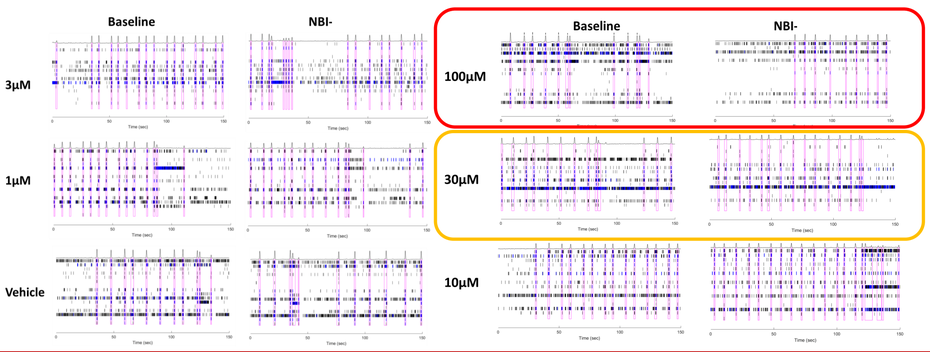
③ *IN VITRO – IN VIVO TRANSLATION*

MEA parameters		NBI compound B 1µM	3µM	10µM	30µM	100µM
Spike	Weighted mean firing rate (Hz)	NC	NC	↓	↓	↓↓
	Interspike Interval (ISI) Coefficient of variation - Avg	NC	NC	NC	NC	NC
Bursts	Burst frequency - Avg (Hz) *	↑	↑	↑	↑	↑
	Burst duration - Avg (sec)	NC	↓	↓	↓	↓↓
	Number of spikes per burst - Avg	NC	↓	↓	↓	↓
	Mean ISI within burst - Avg (sec)	NC	NC	↓	↓	↓
Network Bursts	Network burst frequency *	NC	NC	NC	↑	↑
	Network burst duration - Avg (sec)	NC	↓	↓	↓↓	↓↓↓
	Number of spikes per network burst - Avg	NC	↓	↓	↓	↓↓↓
	Mean ISI within network burst - Avg (sec)	NC	NC	NC	NC	↑
	Network IBI Coefficient of variation - Avg	↓	NC	NC	NC	↓
Synchrony	Area under normalized cross-correlation	NC	NC	NC	↑	↑

- Minimal changes at lower doses
- Changes to firing rate, bursts/network bursts at 100µM

No CNS signs *in vivo* at free total plasma concentrations up to 38µM (300 mg/kg, PO)

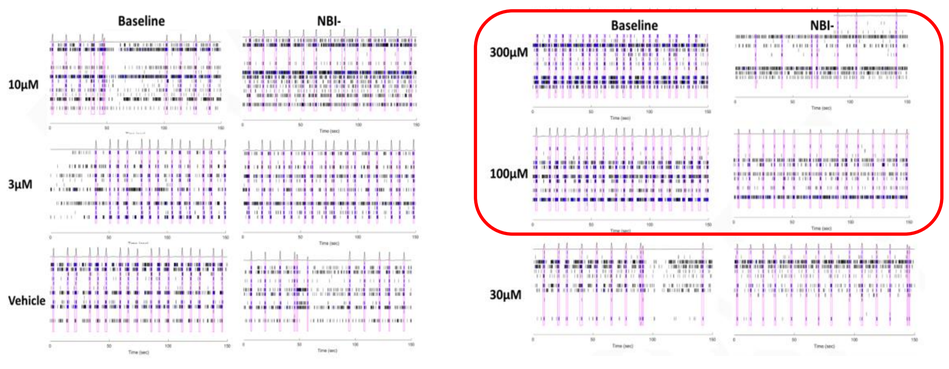
Convulsions *in vivo* at total plasma concentration of 148 – 297µM (600 – 1000 mg/kg, PO)



MEA parameters		NBI compound A 3µM	10µM	30µM	100µM	300µM
Spike	Weighted mean firing rate (Hz)*	↑	↑	↑	↓	↓
	Interspike Interval (ISI) Coefficient of variation - Avg	NC	↓	↓	↓	↓
Bursts	Burst frequency - Avg (Hz) *	NC	↑	↑	NC	↓
	Burst duration - Avg (sec)	NC	NC	↓	↓	↓
	Number of spikes per burst - Avg	NC	NC	↓	↓	↓
	Mean ISI within burst - Avg (sec)	↓	NC	NC	↓	↓
Network Bursts	Network burst frequency *	↑	↑	↑	↑	↓
	Network burst duration - Avg (sec)	NC	NC	NC	↓	↓
	Number of spikes per network burst - Avg	NC	NC	↓	↓↓	↓↓
	Mean ISI within network burst - Avg (sec)	NC	NC	NC	↑	↑↑
	Network IBI Coefficient of variation - Avg	UNC	NC	↓↓	↓↓↓	↓↓↓
Synchrony	Area under normalized cross-correlation	NC	NC	NC	NC	↓

- Minimal changes at lower doses
- Changes to network bursts at higher doses

No CNS signs *in vivo* at plasma concentrations up to 0.35 µM (free) and 2.54µM (total) (1000mg/kg, PO)



④ HUMAN RELEVANCE OF CONVULSIONS IN DIFFERENT SPECIES

- ✓ Nonclinical testing of a compound caused convulsions only in dogs
- ✓ On the MEA assay only the dog-specific metabolite caused a seizurogenic phenotype
- ✓ Only the dog metabolite hit an ion channel on the panel providing mechanistic insight into the possible cause of seizure

Parent drug

MEA parameters		1µM	3µM	10µM	30µM	100µM
Spike	Weighted mean firing rate (Hz)	NC	NC	↑	NC	NC
	Interspike Interval (ISI) Coefficient of variation - Avg	↑	NC	NC	NC	↑
Bursts	Burst frequency - Avg (Hz)	NC	NC	NC	NC	NC
	Burst duration - Avg (sec)	NC	NC	↑	↑	↑
	Number of spikes per burst - Avg	NC	NC	↑↑	↑	↑
	Mean ISI within burst - Avg (sec)	NC	NC	↓	↓	NC
Network Bursts	Network burst frequency	NC	NC	NC	NC	↓
	Network burst duration - Avg (sec)	NC	NC	NC	NC	NC
	Number of spikes per network burst - Avg	NC	NC	NC	↑	NC
	Mean ISI within network burst - Avg (sec)	NC	NC	NC	NC	NC
	Network IBI Coefficient of variation - Avg	↓	↑	↑	↓	UNC
Synchrony	Area under normalized cross-correlation	NC	NC	NC	NC	NC

- Inactive up to 100µM
- No hits on ion channel panel

Human and rodent metabolite

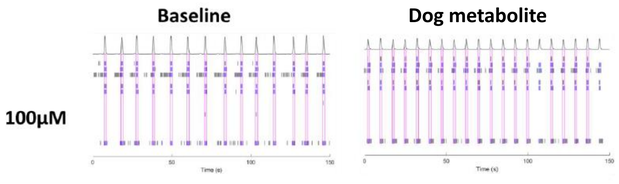
MEA parameters		1µM	3µM	10µM	30µM	100µM
Spike	Weighted mean firing rate (Hz)	NC	NC	NC	NC	↓
	Interspike Interval (ISI) Coefficient of variation - Avg	NC	NC	NC	NC	NC
Bursts	Burst frequency - Avg (Hz)	NC	NC	↓	↓	↓
	Burst duration - Avg (sec)	NC	NC	NC	NC	NC
	Number of spikes per burst - Avg	NC	NC	NC	NC	NC
	Mean ISI within burst - Avg (sec)	NC	NC	NC	NC	↑
Network Bursts	Network burst frequency	NC	NC	↓	NC	↓
	Network burst duration - Avg (sec)	NC	NC	NC	NC	NC
	Number of spikes per network burst - Avg	NC	NC	NC	NC	NC
	Mean ISI within network burst - Avg (sec)	NC	NC	NC	↑	↑
	Network IBI Coefficient of variation - Avg	UNC	UNC	UNC	UNC	UNC
Synchrony	Area under normalized cross-correlation	NC	NC	NC	NC	↓

- Inactive up to 100µM
- No hits on ion channel panel

Dog metabolite

MEA parameters		1µM	3µM	10µM	30µM	100µM
Spike	Weighted mean firing rate (Hz)	NC	NC	NC	↑	NC*
	Interspike Interval (ISI) Coefficient of variation - Avg	NC	NC	NC	NC	↓
Bursts	Burst frequency - Avg (Hz)	NC	NC	↑	↑	↑↑
	Burst duration - Avg (sec)	NC	NC	NC	NC	↓
	Number of spikes per burst - Avg	NC	NC	NC	NC	↓↓
	Mean ISI within burst - Avg (sec)	NC	NC	NC	NC	NC
Network Bursts	Network burst frequency ↑	NC	NC	↑	↑	↑↑
	Network burst duration - Avg (sec)	NC	NC	NC	↓	↓
	Number of spikes per network burst - Avg	NC	NC	NC	↓	↓↓
	Mean ISI within network burst - Avg (sec)	NC	NC	NC	NC	↑↑↑
	Network IBI Coefficient of variation - Avg	UNC	UNC	UNC	UNC	UNC
Synchrony	Area under normalized cross-correlation	NC	↑	NC	↓	↓↓

- Clear seizurogenic phenotype at high doses
- Hit on ion channel panel



DISCUSSION AND CONCLUSIONS

- Currently, the majority of seizure detection for de-risking is done in animal models, with limited success and questionable translation to humans.
- Our approach has been validated using known seizurogenic compounds and shows good translation to clinical exposures (Rockley et al., 2023).
- These assays are useful for early de-risking and problem solving later in drug development.
- Collectively, these studies demonstrate the utility of this approach for early seizure prediction to provide mechanistic information, early de-risking, and support optimal drug design using human in vitro models.