

Utilising *In Vitro* Assays to Assess Drug-Induced Central Nervous System Liabilities

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CONFIDENTIAL



INTRODUCTION

- Central nervous system (CNS) liabilities, such as seizure and sedation, remain a significant cause of attrition during drug discovery and development. Typically, they are not identified until preclinical toxicology studies, where there are considerable issues with translation of animal data to humans.
- GABA (γ -aminobutyric acid) neurotransmitter signalling in the brain is a critical component of the sleep/wake cycle; increased GABA signalling through the GABA_A receptor results in sedation. Perturbation of GABA_A signalling by drug-induced inhibition and potentiation commonly cause seizure and sedation, respectively.
- We previously developed two novel *in vitro* CNS safety assays: 1) an ion channel panel screened using automated electrophysiology, and 2) a human induced pluripotent stem cell (hiPSC)-neuronal model assessed with microelectrode array (MEA) to measure electrical activity of cells when challenged with known seizurogenic compounds.
- This study aimed to assess the effects of a selection of sedative compounds (both GABA and non-GABA modulators) on the electrical activity of hiPSC-derived neuronal co-cultures, and the ion flux of several CNS-related ion channels.

METHODS

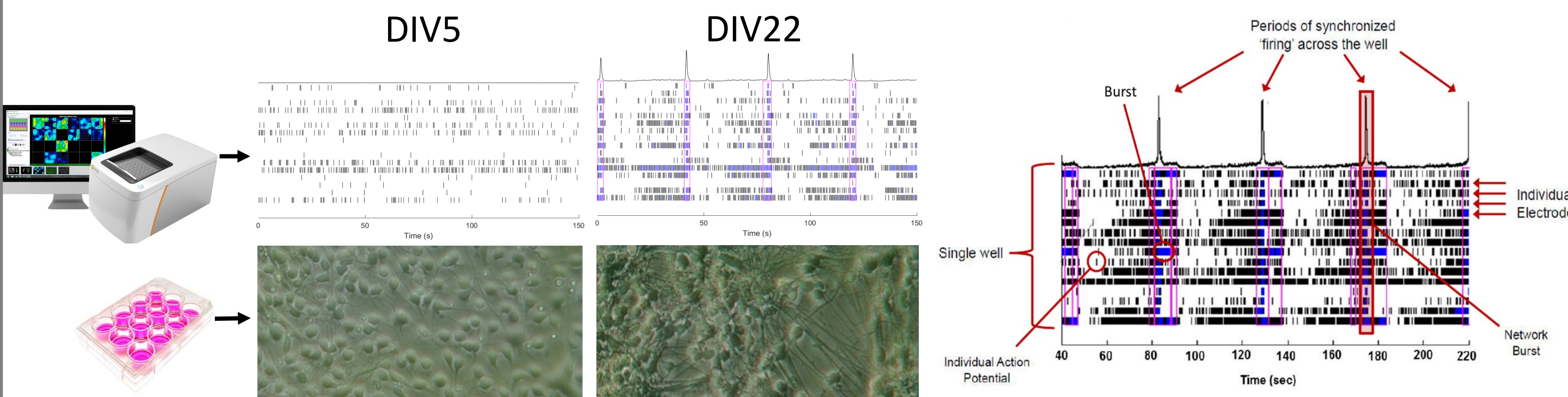
Human ion channel assays

- Compound activity was assessed by automated patch-clamp electrophysiology (QPatch II, Sophion) using CHO or HEK cells stably expressing human K_v1.1, K_v2.1, K_v3.1, K_v7.2/7.3, Na_v1.2, GABA- $\alpha_1\beta_2\gamma_2$, nicotinic- $\alpha_4\beta_2$ or K_{2p}2.1 ion channels.
- 5- or 6-point dose-response curves were produced for all modulators to generate IC₅₀ or EC₅₀ values.

hiPSC-derived neuronal co-cultures

- hiPSC iCell GlutaNeurons (FujiFilm CDI, ~80% glutamatergic/20% GABAergic neurons) were plated with iCell Astrocytes (85%:15%) and monitored using the Maestro MEA system (Axion Biosystems).
- On day *in vitro* (DIV) 21-31, spontaneous electrical activity was recorded for 15 minutes at baseline and 1 hour after exposure to compounds or solvent controls.

SPONTANEOUS ACTIVITY OF HIPSC NEURONAL CELLS



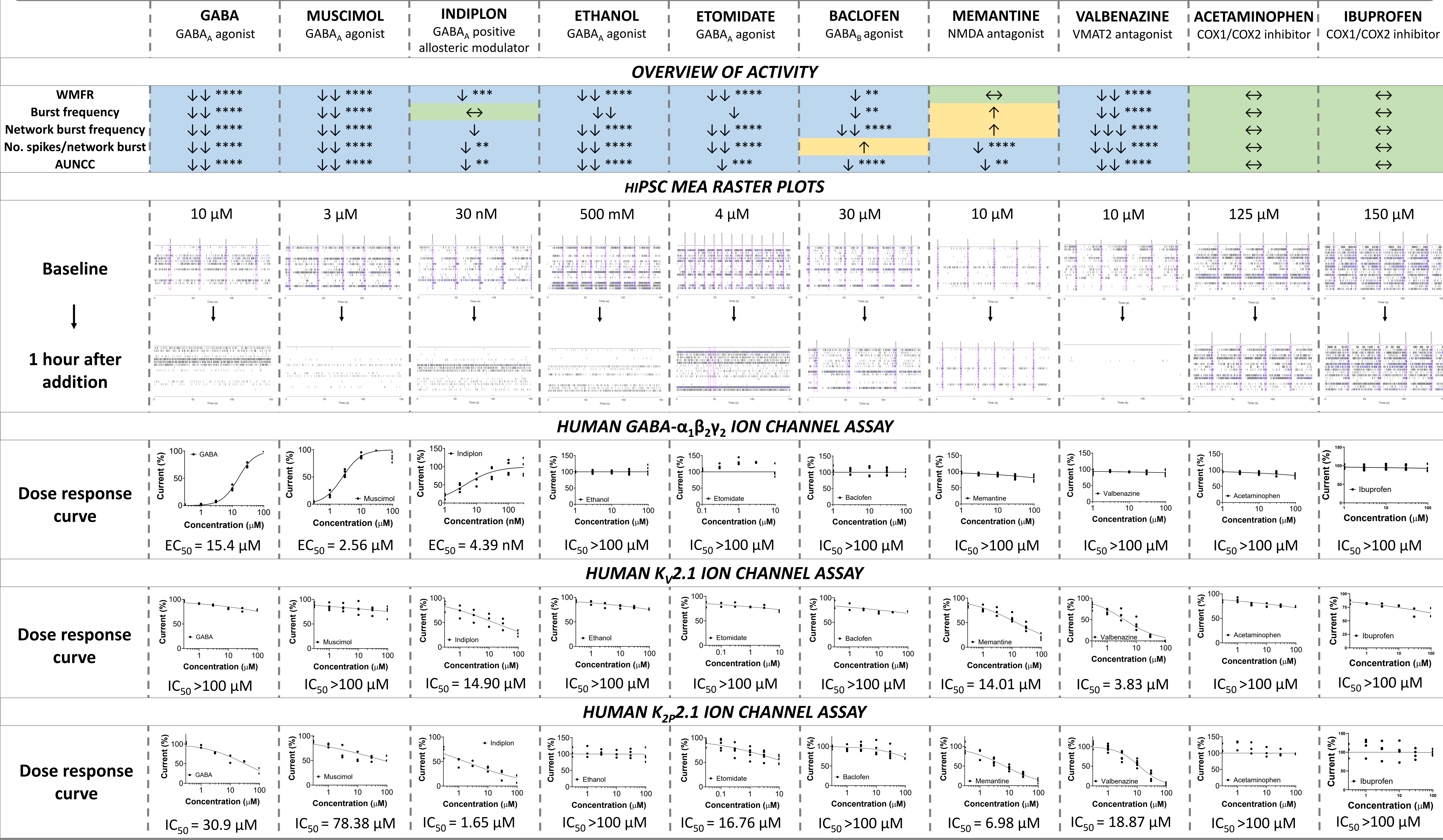
MICROELECTRODE ARRAY PARAMETERS

WMFR: Weighted mean firing rate; based on electrodes with activity $\geq$ minimum spike rate (set by neural statistics calculator).	$\uparrow\uparrow\uparrow$	$\geq 100\%$
Burst frequency: Total number of electrode bursts per recording time.	$\uparrow\uparrow$	50 to 99%
Network burst frequency: Total network bursts per recording time.	$\uparrow$	10 to 50%
No. spikes/network burst: Average number of spikes in a network burst.	$\leftrightarrow$	$\pm 10\%$
AUNCC: Area under normalise cross-correlation; area under well-wide pooled inter-electrode cross-correlation normalised to auto-correlations. High values indicate greater synchronicity of the network.	$\downarrow$	-10 to -50%
	$\downarrow\downarrow$	-50 to -99%
	$\downarrow\downarrow\downarrow$	-100%

*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 (one-way ANOVA with post-hoc Dunnet's test).

RESULTS

Sedative compounds decreased hiPSC population activity and modulated ion channel activity



Compounds exhibited mixed activity across CNS ion channels

Table 1. Activity of 19 compounds (14 with reported sedative activity and 5 negative controls) at 9 neuronal ion channels implicated in central nervous system function. Compounds are grouped by primary neuronal target. Key depicts compound activity. Abbreviations: GABA (γ -aminobutyric acid), GPCR (G-protein coupled receptor), NMDA (N-methyl-D-aspartate), THC (tetrahydrocannabinol), VGCC (voltage-gated calcium channel) and VGSC (voltage-gated sodium channel).

		Key								
		IC ₅₀ >100 μ M			IC ₅₀ 30 - 100 μ M			IC ₅₀ <30 μ M		
		IC ₅₀ >30 μ M (agonist)								
Primary target	Compound	K _v 1.1	K _v 2.1	K _v 3.1	K _v 7.2/7.3	K _{Ca} 1.1	Na _v 1.2	GABA- $\alpha_1\beta_2\gamma_2$	nicotinic- $\alpha_4\beta_2$	K _{2p} 2.1
GABA	Baclofen	>100 μ M	>100 μ M	>100 μ M	87.97 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
	Ethanol	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
	Etomidate	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	8.6 μ M	>100 μ M	21.42 μ M	16.76 μ M
	GABA	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	15.40 μ M	>100 μ M	30.93 μ M
	Indiplon	>100 μ M	14.90 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	4.39 μ M	65.34 μ M	1.652 μ M
	Muscimol	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	2.56 μ M	>100 μ M	78.38 μ M
NMDA	Dextromethorphan	>100 μ M	1.639 μ M	>100 μ M	4.923 μ M	>100 μ M	>100 μ M	52.26 μ M	1.694 μ M	6.851 μ M
	Memantine	34.61 μ M	14.01 μ M	>100 μ M	34.38 μ M	>100 μ M	56.63 μ M	>100 μ M	4.269 μ M	6.981 μ M
VGSCs	Carbamazepine	>100 μ M	22.34 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	9.805 μ M
	Primidone	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
VGCCs	Mirogabalin	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	39.37 μ M	>100 μ M	>100 μ M	64.53 μ M
	D9-THC	>100 μ M	5.791 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	18.87 μ M
GPCRs	L-DOPA	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
	Valbenazine	5.574 μ M	3.832 μ M	>100 μ M	26.56 μ M	82.16 μ M	36.72 μ M	>100 μ M	9.274 μ M	12.01 μ M
Negative controls	Acetaminophen	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
	Glucose	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
	Ibuprofen	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
	Vitamin B6	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M
	Vitamin D	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M	>100 μ M

DISCUSSION AND CONCLUSIONS

- Most sedative compounds induced a clear sedation-like phenotype in hiPSC-derived neuronal co-cultures (decreased firing rate and network burst activity), whilst others (e.g. baclofen and memantine) produced a more mixed response.
- GABA, muscimol and indiplon increased GABA- $\alpha_1\beta_2\gamma_2$ current, confirming their agonistic effects.
- Most sedative compounds inhibited activity of at least one ion channel in our *in vitro* screening panel, including K_v2.1, nicotinic- $\alpha_4\beta_2$ and K_{2p}2.1 suggesting ion channels may play a role in drug-induce sedative liabilities.
- However, some sedative compounds (ethanol, L-DOPA) did not modulate any ion channels screened, suggesting they may act through other mechanisms.
- These studies have further characterised modulation of GABA activity within hiPSC-derived neuronal co-cultures by recapitulating expected clinical outcomes. This further validates the model as a translationally relevant screen which shows promise for sedation.

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