Modulation of $GABA_A$ activity: Investigations in hiPSC-derived neuronal co-cultures and human ion channel assays

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INTRODUCTION

A balance between inhibitory neurotransmission and neuronal excitation is critical for normal brain function. γ-aminobutyric acid (GABA) is the most abundant inhibitory neurotransmitter, which acts on $GABA_A$ receptors. Perturbation of $GABA_A$ signalling by drug-induced inhibition and potentiation are common mechanisms producing seizure and sedation, respectively. The introduction of commercially available human induced pluripotent stem cell (hiPSC-) derived neurons facilitates the *in vitro* study of neuronal function and, in our work, the detection of seizure liability during drug discovery. It is known that $GABA_A$ antagonists such as picrotoxin increase neuronal firing and induce a seizure-like phenotype in hiPSC-derived neurons, however further characterisation of $GABA_A$ modulation within these cell models is lacking. This study aimed to address this by assessing the effects of a selection of GABA modulators on the electrical activity of hiPSC-derived neuronal co-cultures, and the ion flux of $\alpha_1\beta_2\gamma_2$-$GABA_A$.

METHODS

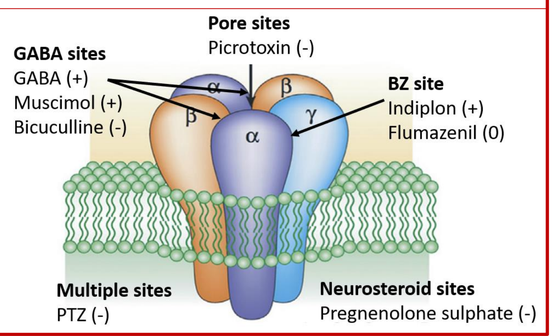
hiPSC-DERIVED NEURONAL CO-CULTURES

- iCell Glutaneurons (80% glutamatergic/20% GABAergic neurons) were plated with astrocytes (85%:15%) and monitored using a microelectrode array (MEA) system (Maestro Edge, Axion).
- On DIV22 and DIV23, spontaneous electrical activity was recorded at baseline and 1 hour after exposure to $GABA_A$ modulators and solvent controls.
- Cells exposed to agonists were subsequently challenged with antagonists and spontaneous electrical activity was measured 15 minutes after application.

HUMAN $\alpha_1\beta_2\gamma_2$-GABAA ION CHANNEL ASSAYS

- The activity of GABA modulators was assessed by automated patch-clamp (QPatch II, Sophion) using a CHO $\alpha_1\beta_2\gamma_2$-$GABA_A$ cell line.
- All modulators except for ligands were co-applied with 30μM GABA.
- 6-point dose-response curves were generated for all modulators.
- For agonists, a 5-point dose-response curve plus subsequent antagonist challenge was generated.

COMPOUND SELECTION



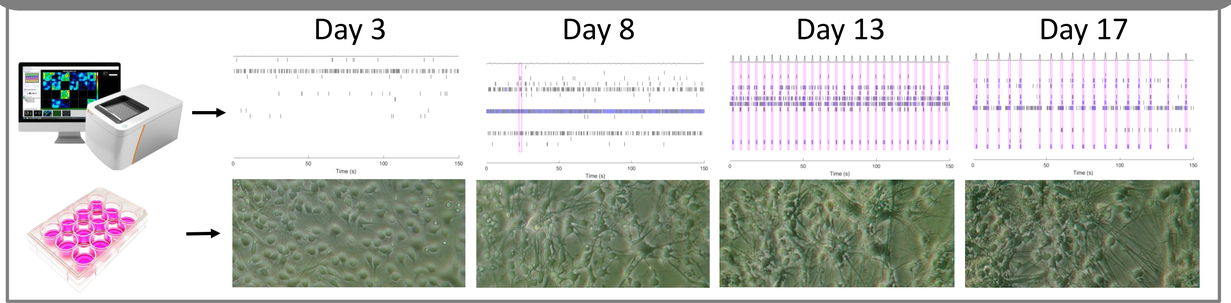
RESULTS

MEA PARAMETERS

Firing rate - Weighted mean firing rate based on electrodes with activity greater than minimum spike rate, set by the neural statistics calculator.
Burst duration - Average time between the first and last spike in a burst.
Network burst freq. - Total number of electrode bursts divided by recording time.
Network burst duration - Average time between the first and last spike in a network burst.
No. spikes per network burst - Average number of spikes in a network burst.

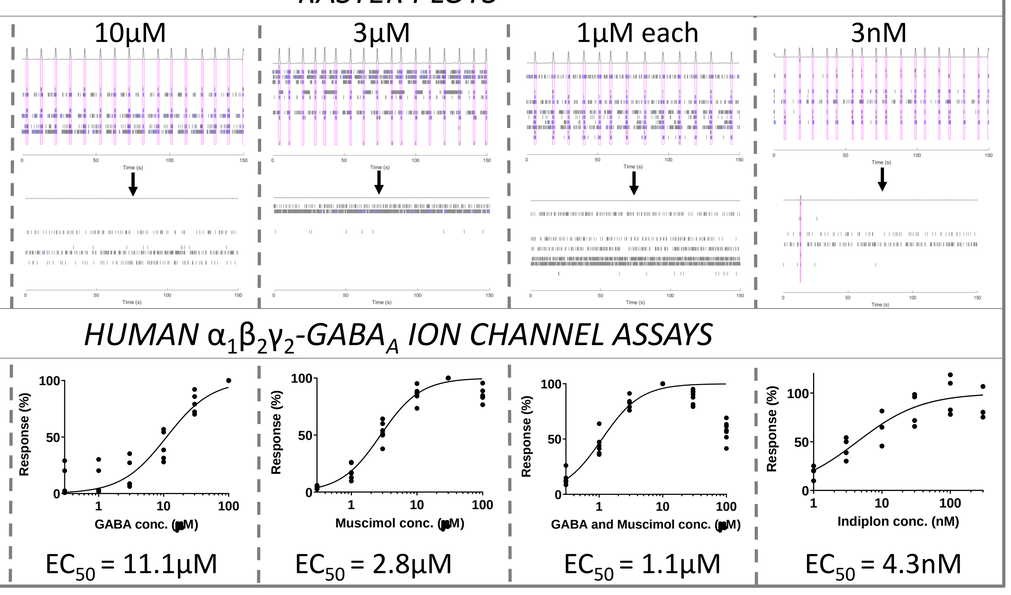
↑↑↑	≥100%
↑↑	50 to 99%
↑	20 to 50%
↔	within +/-20%
↓	-20 to -50%
↓↓	-50 to -99%
↓↓↓	≥-100%
***	p<0.001
**	p<0.01
*	p<0.05

DEVELOPMENT OF SPONTANEOUS ELECTRICAL ACTIVITY



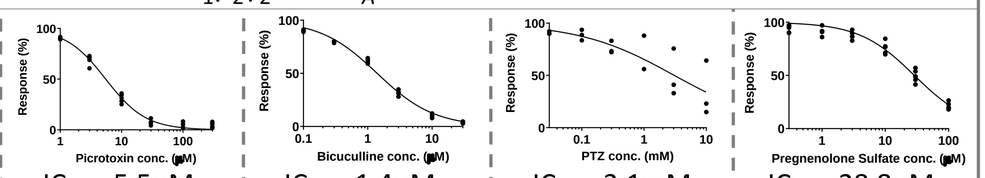
1 AGONISTS *INCREASE $GABA_A$ RESPONSE, DECREASE POPULATION ACTIVITY*

	GABA Agonist, GABA site	**MUSCIMOL** Agonist, GABA site	**GABA & MUSCIMOL**	**INDIPLON** Positive allosteric modulator, BZ site
OVERVIEW OF ACTIVITY				
Mean firing rate	↓↓	↓↓*	↓↓	↓
Burst duration	↔	↓↓*	↓↓*	↓
Network burst freq.	↓↓	↓↓↓*	↓↓↓	↓
Network burst duration	↓↓	↓↓↓*	↓↓↓	↔
No. spikes per network burst	↓↓	↓↓↓*	↓↓↓	↓
RASTER PLOTS				
Baseline ↓ 1 hour after addition	10μM	3μM	1μM each	3nM
HUMAN $\alpha_1\beta_2\gamma_2$-$GABA_A$ ION CHANNEL ASSAYS				
6-point concentration-dose response	EC_{50} = 11.1μM	EC_{50} = 2.8μM	EC_{50} = 1.1μM	EC_{50} = 4.3nM



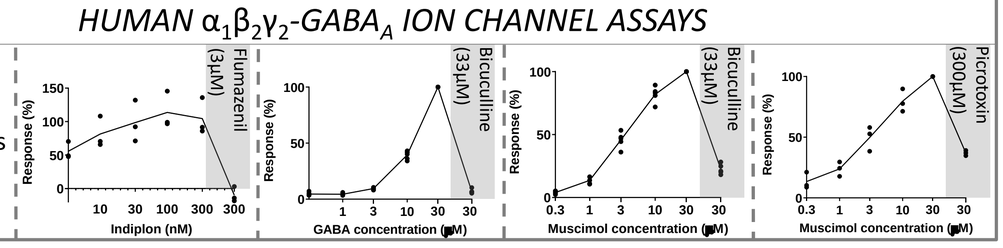
2 ANTAGONISTS *DECREASE $GABA_A$ RESPONSE, MIXED POPULATION ACTIVITY*

	PICROTOXIN Non-competitive antagonist, pore sites	**BICUCULLINE** Competitive antagonist, GABA site	**PTZ** Non-competitive antagonist, multiple modes of action	**PREGNENOLONE SULFATE** Negative allosteric modulator, neurosteroid sites
OVERVIEW OF ACTIVITY				
Mean firing rate	↑	↑	↔	↓
Burst duration	↑↑	↑	↑*	↔
Network burst freq.	↔	↔	↓↓	↔
Network burst duration	↑	↑↑	↔	↔
No. spikes per network burst	↑↑	↑↑↑	↔*	↔
RASTER PLOTS				
Baseline ↓ 1 hour after addition	10μM	3μM	300μM	1μM
HUMAN $\alpha_1\beta_2\gamma_2$-$GABA_A$ ION CHANNEL ASSAYS				
6-point concentration-dose response	IC_{50} = 5.5μM	IC_{50} = 1.4μM	IC_{50} = 3.1mM	IC_{50} = 28.8μM



3 REVERSAL OF AGONIST-INDUCED SEDATION BY ANTAGONISTS

	INDIPLON + FLUMAZENIL silent antagonist, BZ site	**GABA + BICUCULLINE**	**MUSCIMOL + BICUCULLINE**	**MUSCIMOL + PICROTOXIN**
OVERVIEW OF ACTIVITY				
Mean firing rate	↑↑	↑↑↑**	↔	↑↑↑**
Burst duration	↔	↑↑	↑	↑↑↑**
Network burst freq.	↔	↑↑↑	↑ (from 0)	↑** (from 0)
Network burst duration	↓	↑↑↑	↑ (from 0)	↑** (from 0)
No. spikes per network burst	↔	↑↑	↑↑↑	↑↑↑**
RASTER PLOTS				
1 hour after agonist addition ↓ 15 minutes after antagonist addition	3nM → 3nM	10μM → 3μM	3μM → 3μM	3μM → 10μM
HUMAN $\alpha_1\beta_2\gamma_2$-$GABA_A$ ION CHANNEL ASSAYS				
Agonist 5-point concentration-dose response plus Antagonist challenge	Flumazenil (3μM)	Bicuculline (33μM)	Bicuculline (33μM)	Picrotoxin (300μM)



DISCUSSION AND CONCLUSIONS

- Agonists GABA and muscimol induced sedation in hiPSC-derived neuronal co-cultures and increased $\alpha_1\beta_2\gamma_2$-$GABA_A$ current (fig.1) while antagonists bicuculline and picrotoxin induced seizure in hiPSC-neuronal co-cultures and reduced $\alpha_1\beta_2\gamma_2$-$GABA_A$ current (fig.2).
- PTZ is used *in vivo* to induce seizure. This often involves chronic repeat-dose application, suggesting PTZ may not translate well to single-dose *in vitro* studies (fig.2).
- Pregnenolone sulfate (PS) did not induce seizure in hiPSC-derived neuronal co-cultures, yet inhibited $\alpha_1\beta_2\gamma_2$-$GABA_A$ current. This suggests the expression of other subtypes in neuronal cells, possibly $GABA_C$ which is considerably less sensitive to PS than $GABA_A$.
- In ion channel assays, bicuculline blocked GABA- and muscimol-induced current (fig.3). In hiPSC-derived neuronal co-cultures however, muscimol-induced sedation was not reversed by bicuculline. It is known that bicuculline cannot compete with muscimol at $GABA_C$, further suggesting its expression in hiPSC-derived neuronal co-cultures.
- Indiplon, a marketed sleeping aid, induced sedation in hiPSC-derived neuronal co-cultures and increased $\alpha_1\beta_2\gamma_2$-$GABA_A$ current (fig.3). It was competitively antagonized by flumazenil, a clinical antidote to indiplon overdose. As a silent antagonist, Flumazenil was inactive alone in both assays.
- These studies have further characterised modulation of $GABA_A$ activity within hiPSC-derived neuronal co-cultures by recapitulating expected clinical outcomes. This further validates the model as a translationally relevant screen for seizure detection which also shows promise for sedation.