SCALABLE AND WELL DEFINED HUMAN GLUTAMATERGIC AND GABAERGIC CO-CULTURE PLATFORM FOR STUDYING EXCITATORY-INHIBITORY NEURON IMBALANCES AND THE DISCOVERY OF DRUGS TO TREAT ASSOCIATED DISEASES

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Abstract

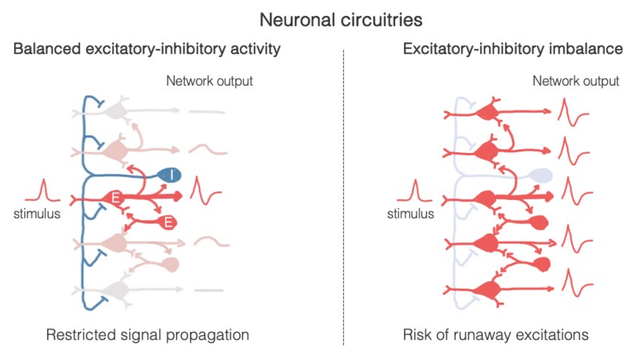
Neuronal circuits in the cortex consist of two main neuronal types, glutamatergic excitatory neurons and GABAergic inhibitory neurons (IN). The inputs of IN provide cortical networks with the ability to balance spontaneous and evoked excitatory activities, preventing runaway excitation. Abnormal IN function is associated with various neurological diseases including autism, epilepsy and schizophrenia. Scalable approaches are needed to generate reliable human in vitro models suitable for high-content drug screening to develop therapeutics to treat these neurological diseases. We have used our deterministic cell programming technology opti-ox* (optimised inducible overexpression) to generate a highly pure (>99%) population of GABAergic neurons, named ioGABAergic Neurons*, from human iPSCs, at scale, within 12 days post-revival.

A deep molecular characterisation by immunocytochemistry, RT-qPCR and single-cell RNA-sequencing revealed that cultures consist of over 99% pure GABAergic neurons expressing the classical markers GAD1, GAD2, VGAT, DLX1, as well as DLX2, and are positive for GABA. Remarkably, SST was the only GABAergic subtype specific marker that was detected, further highlighting the purity of the ioGABAergic Neurons. Moreover, three independently manufactured ioGABAergic Neurons lots displayed highly equivalent transcriptomic profiles, confirming the consistency and scalability of the opti-ox technology. Functional assessment by MEA analysis showed that ioGABAergic Neurons inhibit the excitatory activity of ioGlutamatergic Neurons in a ratio dependent manner, and that the inhibitory and excitatory balance can be further modulated by drugs targeting GABAergic signalling. Thus, the developed opti-ox driven co-culture platform can be used to study the principles underlying excitatory-inhibitory neuron imbalances and for the discovery of new drugs to treat, for example, epilepsy patients.

1. Excitatory-inhibitory imbalances

Inhibitory GABAergic neurons in the cortex form functional networks with the excitatory glutamatergic neurons to restrict signal propagation in the brain. Reduced activity or lack of GABAergic neurons results in excitatory-inhibitory imbalances and can lead to runaway excitations, for example.

Excitatory-inhibitory imbalances are associated with various diseases, including epilepsy, schizophrenia and autism.



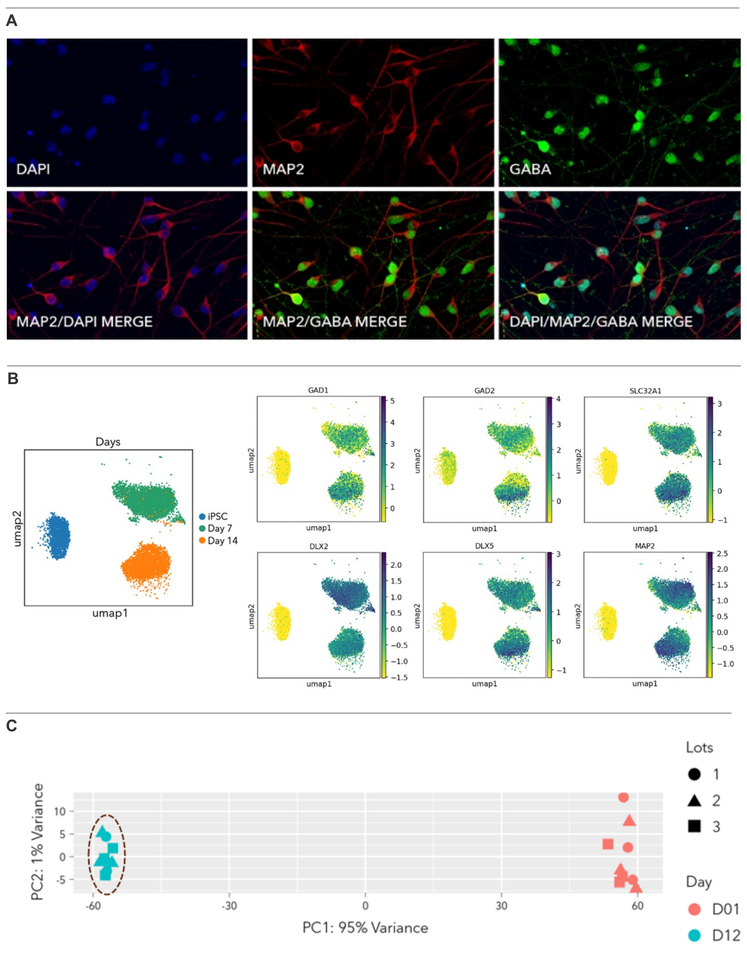
2. Consistent manufacturing of a highly pure population of GABAergic neurons

Molecular characterisation of ioGABAergic Neurons shows that opti-ox-driven deterministic cell programming results in consistent manufacturing of a pure population of GABAergic neurons.

(A) Immunocytochemistry showing that ioGABAergic Neurons consist of a pure population of GABA positive, MAP2 expressing neurons.

(B) UMAP plot representing 3 timepoints of a continuous culture of ioGABAergic Neurons, namely iPSC-stage, day 7 and 14 after the start of cell programming of the ioGABAergic Neurons. The cells from each time point form tight clusters, which are clearly separated from each other. The day 7 and 14 clusters are highly positive for key GABAergic makers, including GAD1, GAD2, SLC32A1, DLX1 and 5 and for the neuronal marker MAP2.

(C) Bulk RNA-seq analysis of three independent manufactured lots of ioGABAergic Neurons. While the D1 and D12 timepoints are separated far away from each other along Principal Component 1 (PC1; 95% Variance), the three lots tightly cluster along PC2 (only 1% Variance). Remarkably, the maximum number of differentially expressed genes between lots at D12 is only 5 genes (|logFC| > 0.5 and FDR < 0.01).

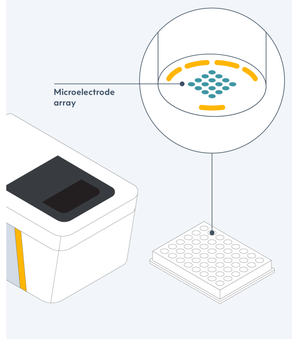


3. Co-culture platform to study excitatory-inhibitory imbalances

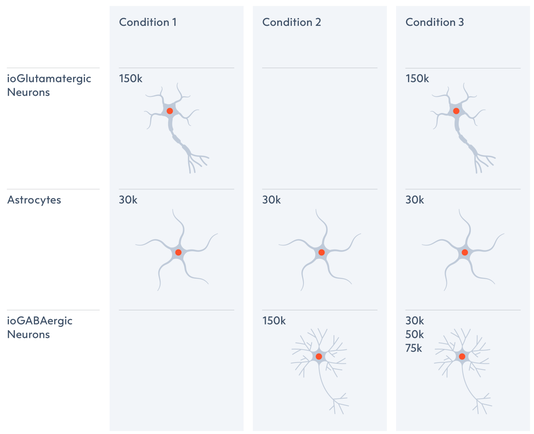
Platform

Micro-/Multielectrode array (MEA)

- Analysis was performed on an Axion Maestro Pro MEA platform.
- Cells cultured on MEA plate.
- Plate measures extracellular electrical activity, and microelectrodes detect neuron firing in real time.

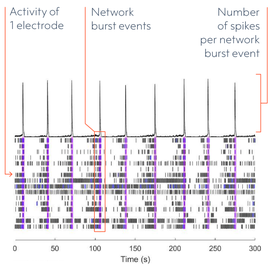


Experimental plan



Results

Raster plots

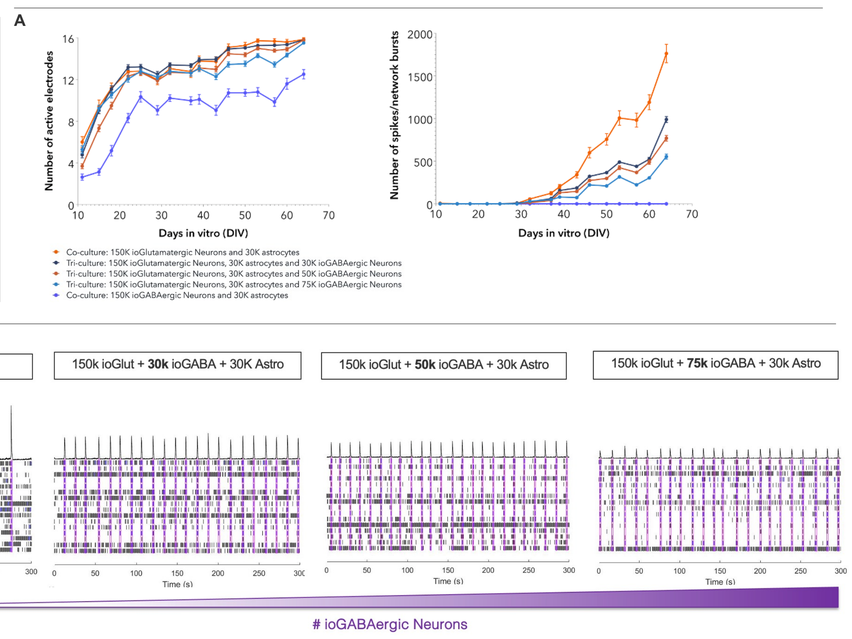


4. ioGABAergic Neurons inhibit excitatory neurons

ioGABAergic Neurons form functional networks with the excitatory ioGlutamatergic Neurons and inhibit their activity by reducing the number of spikes per network burst in a cell number dependent manner.

(A) Graphs showing the average number of active electrodes and number of spikes per condition.

(B) Raster plots of representative well of four distinct experimental conditions.



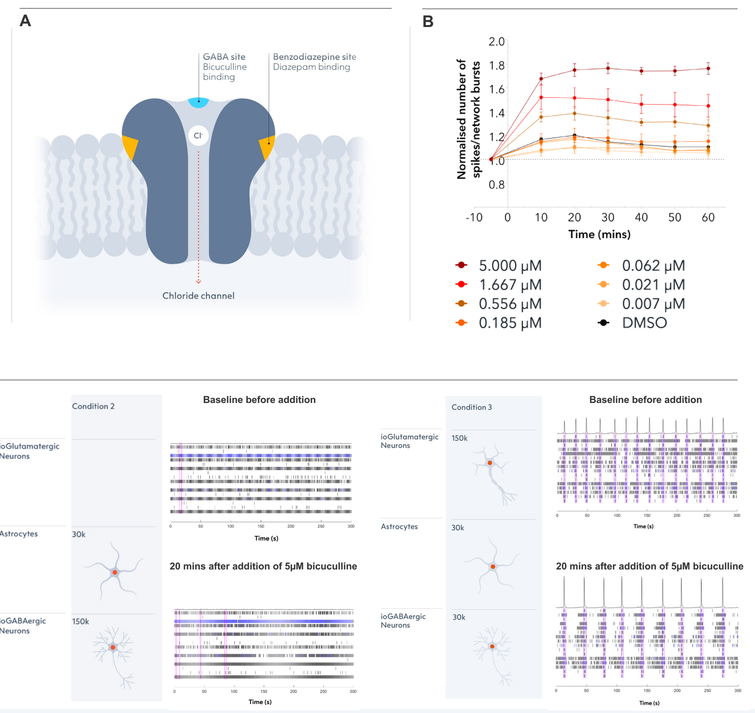
5. Bicuculline releases ioGABAergic Neuron inhibition

The competitive antagonist bicuculline releases the inhibition by ioGABAergic Neurons in a dose-dependent fashion.

(A) Bicuculline is a competitive inhibition of GABA-receptors.

(B) Bicuculline increases the number of spikes per network burst in a dose-dependent manner.

(C) Bicuculline increases network activity only in the tri-cultures containing both ioGlutamatergic and ioGABAergic Neurons, suggesting that they function as inhibitory neurons by releasing GABA.



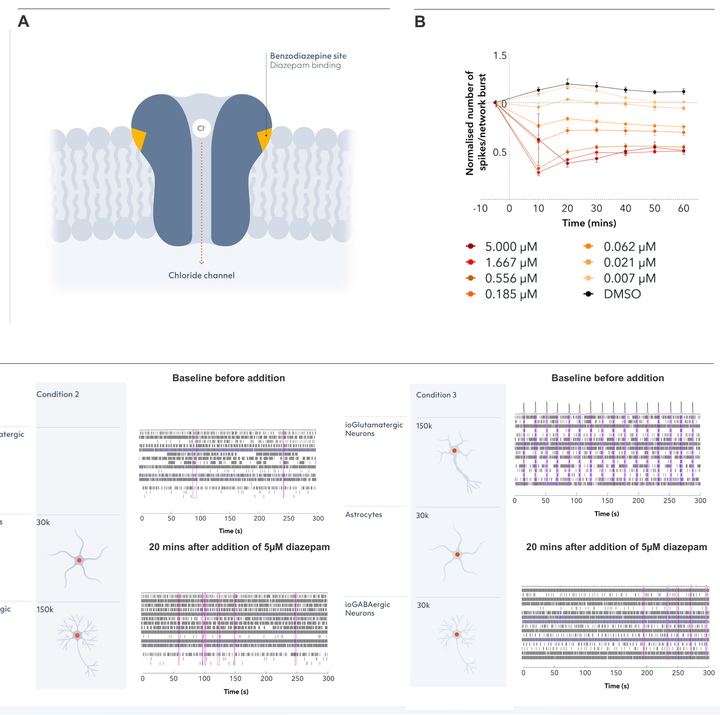
6. Diazepam enhances ioGABAergic Neuron inhibition

Positive allosteric modulator of $GABA_A$ receptors diazepam enhances the inhibition by ioGABAergic Neurons in a dose-dependent fashion.

(A) Diazepam is a positive allosteric modulator of $GABA_A$ receptors.

(B) Diazepam reduces the number of spikes per network burst in a dose-dependent manner.

(C) Diazepam reduces network activity only in the tri-cultures that contain both the ioGlutamatergic and ioGABAergic Neurons, making it a suitable model for the discovery of drugs that treat epilepsy patients.



Summary & conclusions

- Molecular characterisation shows that the ioGABAergic Neurons are a highly pure population (>99%) of neurons expressing key GABAergic marker genes.
- The MEA data demonstrates that ioGlutamatergic Neurons and ioGABAergic Neurons consist of only excitatory and inhibitory neurons respectively, which is in line with the transcriptomics data of both cell types.
- ioGABAergic Neurons function as true inhibitory neurons, and drugs interfering with GABAergic signaling suggest that these neurons release GABA, thereby inhibiting the activity of the excitatory ioGlutamatergic Neurons.
- ioGABAergic Neurons and ioGlutamatergic Neurons form functional circuits.
- In summary, opti-ox deterministic cell programming enables the manufacturing of highly pure (>99%), consistent, and functional GABAergic neurons that can serve as a high-quality human model to study both neurodevelopment and neurological disorders, including epilepsy.