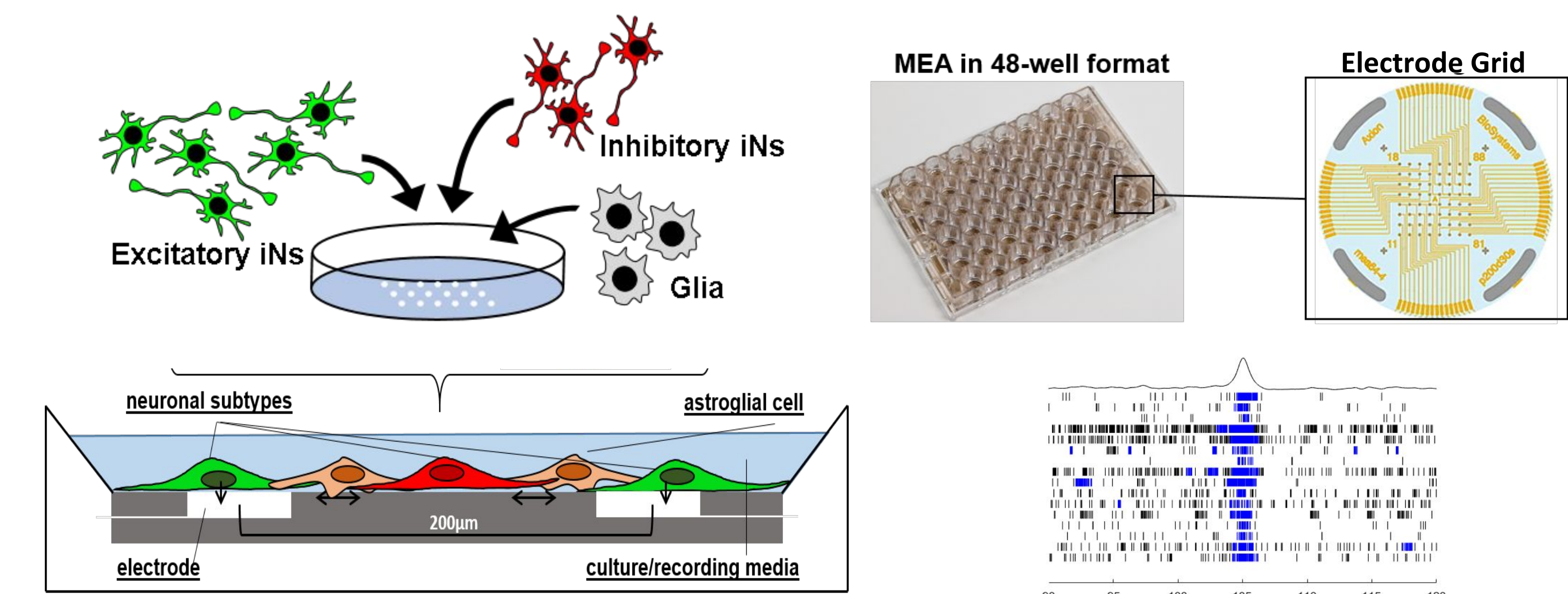


1. Abstract

The increasing amount of chemicals with human exposure and accumulating evidence for interspecies differences regarding adverse effects on the CNS call for scalable in vitro neurotoxicity screening tools using human cells. Generating neuronal cells through differentiation from induced pluripotent stem cells (iPSCs) carries a great potential to overcome the inaccessibility of human primary tissue and accelerate cell-based assays. In combination with multi-electrode array (MEA) readouts, neural activity in response to chemical compounds can be quantified allowing neurotoxicity screening. However, the assessment of neuroactive effects of in human-derived cell-based assays remains challenging due to cell type variability and poorly defined baseline physiology.

Here, we describe a new screening platform using highly functional neural cultures with defined cell ratios consisting of excitatory and inhibitory neurons that were separately generated by direct conversion from human iPSCs (NeuCyte SynFire®) as well as primary human astroglial cells. The reduced complexity of this iPSC-derived co-culture system, compared to primary rodent cultures, however, requires a detailed molecular and functional characterization to define its applicability domain and develop screening assays. Therefore, we conducted comprehensive transcriptome profiling at different maturation time points of the co-cultures and tested altered neuroactivity in response to a set of 8 agonistic and 8 antagonistic tool compounds targeting neuronal signaling (e.g. GABAAR, AMPAR, NMDAR, AChR, D1/2R) on MEAs. We then correlated dose-dependent responses with expression patterns at different co-culture time points to determine specificity, sensitivity and potential assay windows for interference with these pathways. Furthermore, we confirmed specific responses and distinguished synaptic from cell-intrinsic effects by patch clamping of matched neuronal/glia co-cultures. This yielded a detailed pharmacological characterization of NeuCyte's human iPSC-derived neural co-cultures as baseline and reference for multiple neurotoxicity testing applications.

2. SynFire® iPSC-Derived Neural (iN) Cell Technology



We combined our induced neuron (iN) technology with human primary glial supporter cells on 48-well MEA plates to develop a pure human neural co-culture system consisting of glutamatergic excitatory neurons (140K cells/well), GABAergic inhibitory neurons (60K cells/well), and astrocytes (70K cells/well). Reproducible formation of spontaneous synchronized neuronal network activity can be detected 3-4 weeks after plating.

3. Gene Expression Profiling of iN/Glia Co-Cultures

We performed transcriptome profiling using RNA-seq at different maturation time points to characterize which neuronal signaling pathways are represented in our SynFire® neuronal/glia co-cultures. Genes coding for GABA and AMPA receptors were highly expressed early on with broad subunit representation. Moreover, decent expression levels were detected for transcripts coding for cholinergic, dopaminergic, cannabinoid and NMDA receptors.

Gene	7 days	14 days	21 days	28 days	35 days	42 days
ionotropic glutamate (AMPA/kainate)						
GR1A1	5.70	5.17	7.37	6.27	8.35	9.50
GR1A2	0.00	0.00	0.00	0.00	0.00	0.00
GR1A3	5.76	10.70	18.90	18.48	10.64	23.15
GR1A4	9.26	12.16	15.07	13.02	13.52	20.24
GR1A5	2.11	2.14	2.78	2.54	1.37	1.52
GR1A6	0.45	0.56	0.90	0.96	1.13	1.24
GR1A7	7.75	7.02	7.75	6.84	6.01	5.75
GR1A8	6.92	7.17	7.21	7.49	6.81	6.32
GR1A9	3.95	3.18	2.78	2.46	2.59	2.36
GR1A10	1.70	1.75	1.76	1.94	2.83	2.90
GR1A11	19.26	12.16	12.07	18.42	18.73	18.80
GR1A12	0.76	0.38	0.52	0.27	0.21	0.11
GR1A13	0.46	0.11	0.14	0.22	0.40	0.32
GR1A14	22.50	12.32	10.98	10.61	12.04	12.27
GR1A15	6.83	6.32	6.78	7.81	6.20	6.17
GR1A16	1.44	1.44	1.44	1.44	1.44	1.44
GR1A17	2.44	1.77	1.13	1.13	1.13	1.13
GR1A18	0.47	0.75	1.29	1.80	1.78	2.44
GR1A19	1.98	1.26	1.40	1.41	1.45	1.39
GR1A20	6.57	14.67	10.26	10.45	10.26	10.26
GR1A21	5.66	7.65	7.13	7.64	7.24	6.50
GR1A22	6.83	5.84	4.93	4.82	5.35	5.05
GR1A23	0.25	0.25	0.25	0.25	0.25	0.25
GR1A24	10.05	8.91	10.71	10.42	10.33	9.29
GABA						
GABRA1	0.00	0.00	0.00	0.00	0.00	0.00
GABRA2	0.00	0.00	0.00	0.00	0.00	0.00
GABRA3	0.00	0.00	0.00	0.00	0.00	0.00
GABRA4	0.00	0.00	0.00	0.00	0.00	0.00
GABRA5	0.00	0.00	0.00	0.00	0.00	0.00
GABRA6	0.00	0.00	0.00	0.00	0.00	0.00
GABRA7	0.00	0.00	0.00	0.00	0.00	0.00
GABRA8	0.00	0.00	0.00	0.00	0.00	0.00
GABRA9	0.00	0.00	0.00	0.00	0.00	0.00
GABRA10	0.00	0.00	0.00	0.00	0.00	0.00
GABRA11	0.00	0.00	0.00	0.00	0.00	0.00
GABRA12	0.00	0.00	0.00	0.00	0.00	0.00
GABRA13	0.00	0.00	0.00	0.00	0.00	0.00
GABRA14	0.00	0.00	0.00	0.00	0.00	0.00
GABRA15	0.00	0.00	0.00	0.00	0.00	0.00
GABRA16	0.00	0.00	0.00	0.00	0.00	0.00
GABRA17	0.00	0.00	0.00	0.00	0.00	0.00
GABRA18	0.00	0.00	0.00	0.00	0.00	0.00
GABRA19	0.00	0.00	0.00	0.00	0.00	0.00
GABRA20	0.00	0.00	0.00	0.00	0.00	0.00
GABRA21	0.00	0.00	0.00	0.00	0.00	0.00
GABRA22	0.00	0.00	0.00	0.00	0.00	0.00
GABRA23	0.00	0.00	0.00	0.00	0.00	0.00
GABRA24	0.00	0.00	0.00	0.00	0.00	0.00
GABRA25	0.00	0.00	0.00	0.00	0.00	0.00
GABRA26	0.00	0.00	0.00	0.00	0.00	0.00
GABRA27	0.00	0.00	0.00	0.00	0.00	0.00
GABRA28	0.00	0.00	0.00	0.00	0.00	0.00
GABRA29	0.00	0.00	0.00	0.00	0.00	0.00
GABRA30	0.00	0.00	0.00	0.00	0.00	0.00
GABRA31	0.00	0.00	0.00	0.00	0.00	0.00
GABRA32	0.00	0.00	0.00	0.00	0.00	0.00
GABRA33	0.00	0.00	0.00	0.00	0.00	0.00
GABRA34	0.00	0.00	0.00	0.00	0.00	0.00
GABRA35	0.00	0.00	0.00	0.00	0.00	0.00
GABRA36	0.00	0.00	0.00	0.00	0.00	0.00
GABRA37	0.00	0.00	0.00	0.00	0.00	0.00
GABRA38	0.00	0.00	0.00	0.00	0.00	0.00
GABRA39	0.00	0.00	0.00	0.00	0.00	0.00
GABRA40	0.00	0.00	0.00	0.00	0.00	0.00
GABRA41	0.00	0.00	0.00	0.00	0.00	0.00
GABRA42	0.00	0.00	0.00	0.00	0.00	0.00
GABRA43	0.00	0.00	0.00	0.00	0.00	0.00
GABRA44	0.00	0.00	0.00	0.00	0.00	0.00
GABRA45	0.00	0.00	0.00	0.00	0.00	0.00
GABRA46	0.00	0.00	0.00	0.00	0.00	0.00
GABRA47	0.00	0.00	0.00	0.00	0.00	0.00
GABRA48	0.00	0.00	0.00	0.00	0.00	0.00
GABRA49	0.00	0.00	0.00	0.00	0.00	0.00
GABRA50	0.00	0.00	0.00	0.00	0.00	0.00
GABRA51	0.00	0.00	0.00	0.00	0.00	0.00
GABRA52	0.00	0.00	0.00	0.00	0.00	0.00
GABRA53	0.00	0.00	0.00	0.00	0.00	0.00
GABRA54	0.00	0.00	0.00	0.00	0.00	0.00
GABRA55	0.00	0.00	0.00	0.00	0.00	0.00
GABRA56	0.00	0.00	0.00	0.00	0.00	0.00
GABRA57	0.00	0.00	0.00	0.00	0.00	0.00
GABRA58	0.00	0.00	0.00	0.00	0.00	0.00
GABRA59	0.00	0.00	0.00	0.00	0.00	0.00
GABRA60	0.00	0.00	0.00	0.00	0.00	0.00
GABRA61	0.00	0.00	0.00	0.00	0.00	0.00
GABRA62	0.00	0.00	0.00	0.00	0.00	0.00
GABRA63	0.00	0.00	0.00	0.00	0.00	0.00
GABRA64	0.00	0.00	0.00	0.00	0.00	0.00
GABRA65	0.00	0.00	0.00	0.00	0.00	0.00
GABRA66	0.00	0.00	0.00	0.00	0.00	0.00
GABRA67	0.00	0.00	0.00	0.00	0.00	0.00
GABRA68	0.00	0.00	0.00	0.00	0.00	0.00
GABRA69	0.00	0.00	0.00	0.00	0.00	0.00
GABRA70	0.00	0.00	0.00	0.00	0.00	0.00
GABRA71	0.00	0.00	0.00	0.00	0.00	0.00
GABRA72	0.00	0.00	0.00	0.00	0.00	0.00
GABRA73	0.00	0.00	0.00	0.00	0.00	0.00
GABRA74	0.00	0.00	0.00	0.00	0.00	0.00
GABRA75	0.00	0.00	0.00	0.00	0.00	0.00
GABRA76	0.00	0.00	0.00	0.00	0.00	0.00
GABRA77	0.00	0.00	0.00	0.00	0.00	0.00
GABRA78	0.00	0.00	0.00	0.00	0.00	0.00
GABRA79	0.00	0.00	0.00	0.00	0.00	0.00
GABRA80	0.00	0.00	0.00	0.00	0.00	0.00
GABRA81	0.00	0.00	0.00	0.00	0.00	0.00
GABRA82	0.00	0.00	0.00	0.00	0.00	0.00
GABRA83	0.00	0.00	0.00	0.00	0.00	0.00
GABRA84	0.00	0.00	0.00	0.00	0.00	0.00
GABRA85	0.00	0.00	0.00	0.00	0.00	0.00
GABRA86	0.00	0.00	0.00	0.00	0.00	0.00
GABRA87	0.00	0.00	0.00	0.00	0.00	0.00
GABRA88	0.00	0.00	0.00	0.00	0.00	0.00
GABRA89	0.00	0.00	0.00	0.00	0.00	0.00
GABRA90	0.00	0.00	0.00	0.00	0.00	0.00
GABRA91	0.00	0.00	0.00	0.00	0.00	0.00
GABRA92	0.00	0.00	0.00	0.00	0.00	0.00
GABRA93	0.00	0.00	0.00	0.00	0.00	0.00
GABRA94	0.00	0.00	0.00	0.00	0.00	0.00
GABRA95	0.00	0.00	0.00	0.00	0.00	0.00
GABRA96	0.00	0.00	0.00	0.00	0.00	0.00
GABRA97	0.00	0.00	0.00	0.00	0.00	0.00
GABRA98	0.00	0.00	0.00	0.00	0.00	0.00
GABRA99	0.00	0.00	0.00	0.00	0.00	0.00
GABRA100	0.00	0.00	0.00	0.00	0.00	0.00
Serotonin						
HTR1A	0.00	0.00	0.00	0.00	0.00	0.00
HTR1B	0.00	0.00	0.00	0.00	0.00	0.00
HTR1D	0.00	0.00	0.00	0.00	0.00	0.00
HTR1E	0.00	0.00	0.00	0.00	0.00	0.00
HTR1F	0.00	0.00	0.00	0.00	0.00	0.00
HTR2A	0.00	0.00	0.00	0.00	0.00	0.00
HTR2B	0.00	0.00	0.00	0.00	0.00	0.00
HTR2C	0.00	0.00	0.00	0.00	0.00	0.00
HTR2D	0.00	0.00	0.00	0.00	0.00	0.00
HTR2E	0.00	0.00	0.00	0.00	0.00	0.00
HTR2F	0.00	0.00	0.00	0.00	0.00	0.00
HTR3A	0.00	0.00	0.00	0.00	0.00	0.00
HTR3B	0.00	0.00	0.00	0.00	0.00	0.00
HTR3C	0.00	0.00	0.00	0.00	0.00	0.00
HTR3D	0.00	0.00	0.00	0.00	0.00	0.00
HTR3E	0.00	0.00	0.00	0.00	0.00	0.00
HTR3F	0.00	0.00	0.00			