
NeuCyte
Translatable Neuroscience
Highly Functional
iPSC-Derived
Induced Neurons
Drug Discovery and
Safety Assessment
Services

Bridging the Drug Discovery Path with Translatable Neuroscience

The high attrition rate of novel CNS drugs during clinical development has been a major challenge to the pharmaceutical industry. This is largely attributed to the lack of biologically relevant models to study functional links between target and phenotype. NeuCyte's mission is to accelerate and optimize CNS drug discovery by developing more predictive assays and platforms for phenotypic screening.

Based on the advantageous SynFire® technology for generating human induced pluripotent stem cell (iPSC)-derived induced neuronal cells (iNs), NeuCyte has developed a proprietary *in vitro* human neural platform for complex electrophysiological and morphological readouts suited for target identification and validation, efficacy testing and neurotoxicity assessment. Using patient-derived and genetically engineered defined neural cell types, NeuCyte builds unique cell-based assays for modeling neurological and neurodegenerative disorders.

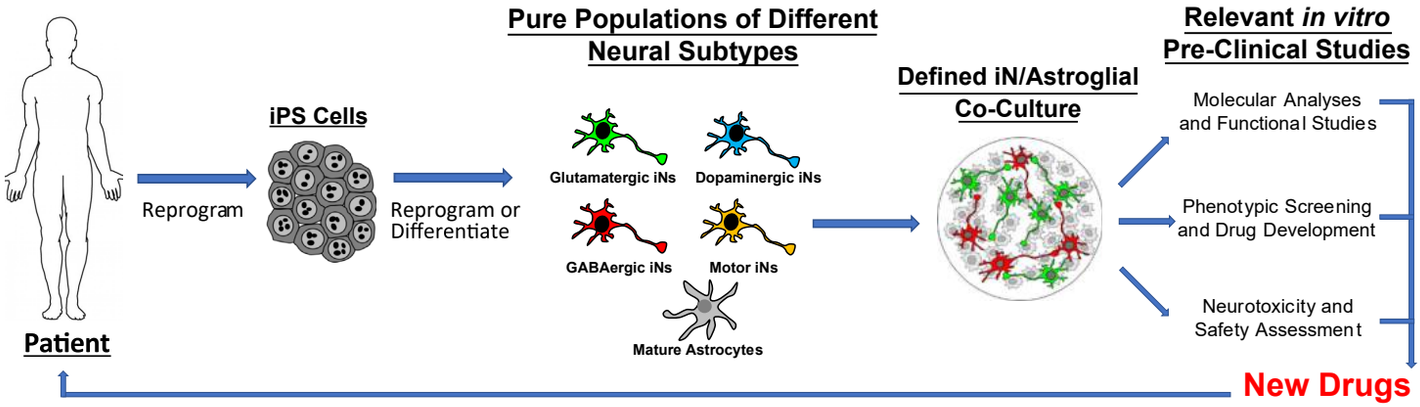


Figure 1. How NeyCyte can support neurological drug discovery and pre-clinical studies

Why work with NeuCyte?

Highly functional products: NeuCyte provides pure and ready-to-use iPSC-derived glutamatergic or GABAergic induced neurons (iNs) and astroglia. This platform most closely resembles real human neurobiology observed in primary cultures, providing the ability to effectively study the function of human neurons *in vitro*.

Extensive neuroscience expertise: NeuCyte has put together an outstanding scientific team. Our extensive knowledge of the biology behind human neurological disorders allows us to introduce advancements in *in vitro* disease modeling, particularly for phenotypic and target-based drug screens. As our client, you always work directly with the neuroscientists who developed our technology platform, with no barrier in between.

Personalized approach towards each project: Our versatile *in vitro* cell system is suitable for compound screening and nonclinical neurotoxicity-based safety assessment for drugs and environmental chemicals. Our goal is to support our clients' needs using our technology platform. We always start with the questions you are trying to answer and design our work around your project.

Proprietary Enabling Technology

SynFire® iNs are generated using a patented procedure for direct reprogramming and exhibit the main characteristics of human primary neurons, such as expression of typical pan-neuronal markers and complex electrophysiology, including spontaneous/evoked action potentials and synchronized network activity. Neuronal subtype identities have been confirmed by staining and patch clamping[1,2].

SynFire®iNs are suitable for a variety of functional assays. For example, the effect of compounds on neuronal survival, axonal outgrowth, or dendritic arborization can be measured by standard assessment of viability, or image-based analysis of labeled cells, respectively. When co-cultured with glial cells, effects on synapse formation and composition, transcriptional programs, and electrophysiology can be tested. Neuronal subtypes can be mixed in different ratios for making a defined co-culture for different experimental purposes.

Advantages of SynFire® iNs include:

Real human biology: these cells closely resemble real human biology, resulting in better ability to predict responses to compounds.

Rapid maturation: produced through a direct reprogramming approach leading to rapid and homogeneous maturation, SynFire® iNs exhibit mature synaptic network activity, such as synchronous bursting phenotypes resembling those in rodent primary cultures appearing within three to four weeks.

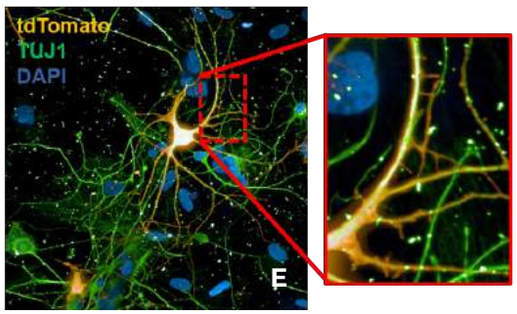
Reliable, robust and ready-to-use: this reprogramming approach also results in lot-to-lot consistency, providing reproducible results.

Flexible modular system: The user can control subtype to subtype relative seeding density and ratio, in order to track, analyze and manipulate specific cell types to fit individual projects.

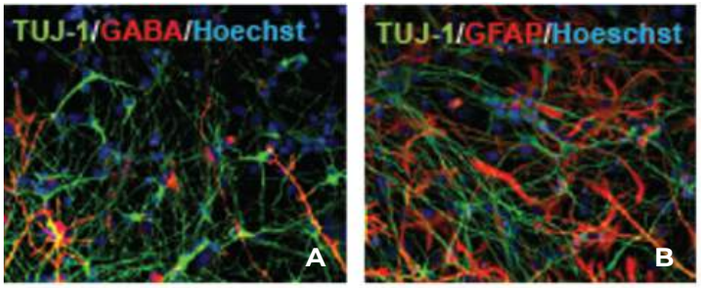
Pure populations of human neural cell types we offer:

- **Glutamatergic excitatory neurons**
- **GABAergic inhibitory neurons**
- **Astroglia**

Complex Morphologies



Pan-Neuronal and Subtype Specific Markers



Elaborate Networks

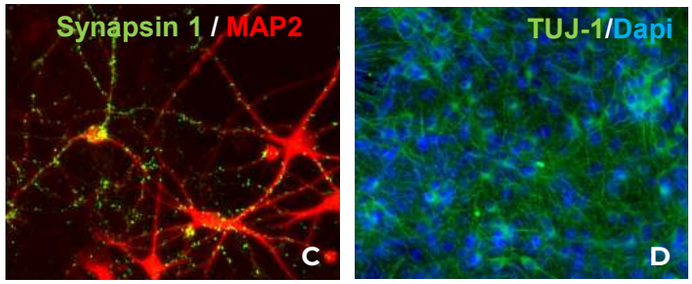


Figure 2. SynFire® iNs exhibit mature neuronal characteristics through immuno-staining
NeuCyte's SynFire® iNs express pan-neuronal and subtype specific markers, rapidly mature to form complex networks and cellular morphologies. The modular aspect of SynFire® neural cells allow for defined co-culture conditions and specific ratios of mixed neuronal subtypes, including inhibitory GABAergic neurons. (A) Pan-neuronal marker β3-Tubb (Tuj1) / Inhibitory neuron GABA-A receptor, α1 / Nuclear staining Hoeschst. (B) Pan-neuronal marker Tuj1 / Astroglia marker GFAP / Nuclear staining Hoeschst. 3-4 week old co-cultures exhibit complex neuronal networks, morphologies and show mature synaptic markers. (C) Pan-neuronal marker Map2 / Synaptic marker Synapsin1 / Nuclear staining Dapi. (D) Pan-neuronal marker Tuj1 / Nuclear staining Dapi. (E) Zoom in of spine-like formations on tdTomato and Tuj1 labeled glutamatergic excitatory neuron.

Highly Functional, Robust SynFire® iNs

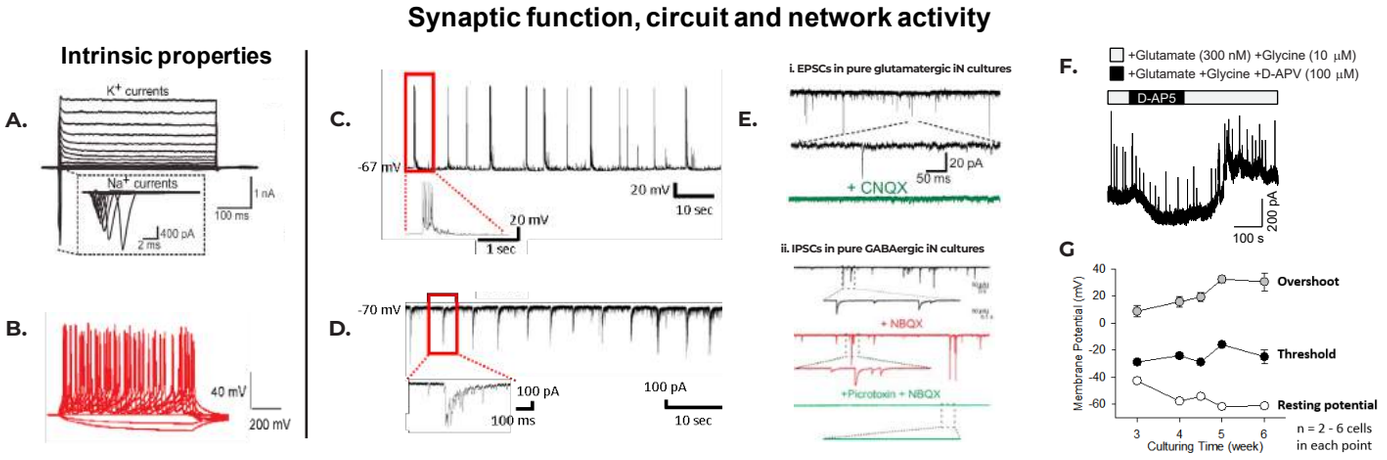


Figure 3. NeuCyte's iNs demonstrate principal neurophysiological properties
Patch-clamp studies show mature intrinsic and extrinsic properties in SynFire® neural cultures including (A) voltage-dependent K+ and Na+currents, (B) action potential firing (evoked). SynFire® iNs show, (C) bursting of single neurons, and (D) large postsynaptic currents indicating advance synaptic competence. Pure SynFire® subtype cultures (E) of either (i) only excitatory iNs or (ii) only inhibitory iNs exclusively show glutamate mediated excitatory postsynaptic currents (EPSCs) or GABA-mediated inhibitory postsynaptic currents (IPSCs), respectively. SynFire® iNs are suited for studying short and long term plasticity and show (E) robust NMDA currents starting at five weeks in culture. Moreover, extra-synaptic NMDA currents can be specifically analyzed by (F) co-application of activating glutamate and glycine in the presence of the NMDA inhibitor D-AP5. SynFire® neural cultures rapidly mature within five weeks (G) reaching a resting membrane potential <-60 mV and showing stable excitability (action potential threshold and overshoot).

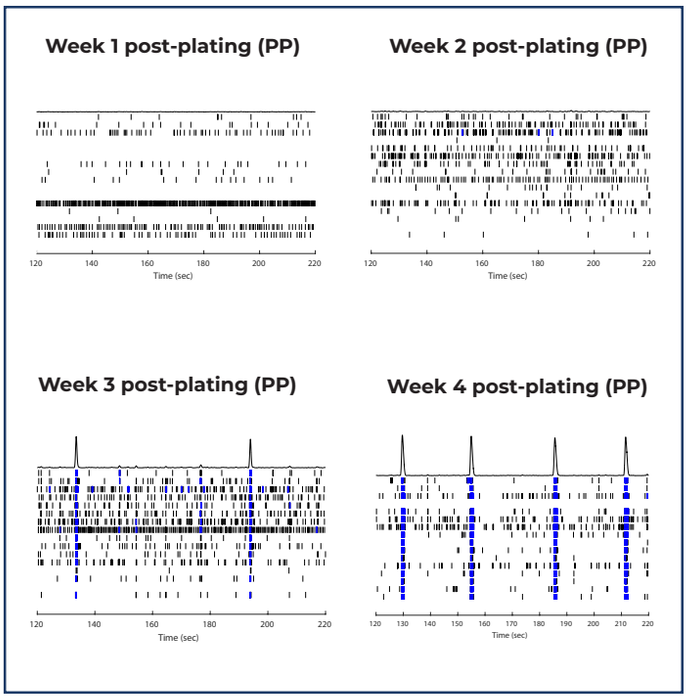


Figure 4. Ontogeny of neural network activity maturation of SynFire co-cultures
These co-cultures contain 70% Glutamatergic, 30% GABAergic neurons and human astrocytes. Representative raster plots from MEA recordings at weeks 1-4. Axion 48 well MEA plates were used to assess activity.

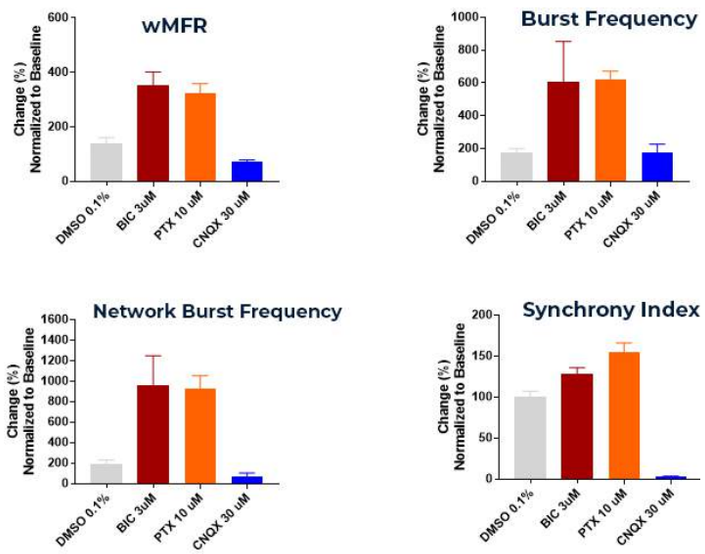


Figure 5. SynFire® co-culture responsiveness to GABA and AMPA modulators
Neuronal firing and network activity were assessed in SynFire® co-cultures after dosing with the GABA-A blockers Bicuculline (BIC 3 μM) and Picrotoxin (PTX 10 μM) or the AMPA blocker CNQX (30 μM). Changes in weighted mean firing rate (wFMR), burst frequency, network burst frequency and synchrony index were measured using Axions' MEA plates. GABA blockers have an organizing effect on the network firing. Meanwhile, AMPA blockers cause a break-down in synchronous firing.

Reliable Predictive System for Drug Efficacy and Safety Assessment

NeuCyte has a focus on enabling the advancement of initial phases of CNS drug discovery programs for lead optimization as well as the investigation of mechanism of action for experimental compounds. Our capacity to make large lots of cryopreserved specific neuronal subtypes is ideal for drug discovery and screening.

With the advantages of the SynFire® technology, such as rapid maturation and synaptic competence, our human neural *in vitro* platforms are uniquely suited for assessing relevant complex electrophysiology readouts, which allows better prediction of drug efficacy and potential CNS safety/toxicology than other systems.

SynFire® iN cells represent a versatile *in vitro* cell system for basic research and disease modeling, including *in vitro* gain-of-function and loss-of-function genetic studies[3,4]. The technology can be used to develop *in vitro* disease models for several neurological disorders with genetic drivers. It also enables the evaluation of human specific neural phenotypes that might not be identifiable in standard animal models. These cells can also be used for compound screening as well as nonclinical safety assessment and chemical neurotoxicity studies.

NeuCyte's Platform is Ideal for a Wide Range of Applications

Drug discovery and pre-clinical testing

Custom *in vitro* neural disease modeling

Development of neural cell based assays

Phenotypic and targeted drug screening

Neural subtype specific biochemistry

Target identification and validation in biologically relevant tissues

CNS safety/ Neurotoxicity

Cell death, apoptosis, autophagy and mitochondrial activity assays

Cell stress tests

Neural network physiology assessment (MEA)

Compound seizurogenic potential testing

Neurite outgrowth and morphology evaluations

Mechanism of action prediction by gene expression profiling

References

1. Zhang, Y. et al. Rapid single-step induction of functional neurons from human pluripotent stem cells. Neuron, 78(5): 785-98, 2013.
2. Yang, N et al. Generation of pure GABAergic neurons by transcription factor programming. Nat Methods, 14(6): 621–628, 2017.
3. Pak, C. et al. Human Neuropsychiatric Disease Modeling using Conditional Deletion Reveals Synaptic Transmission Defects Caused by Heterozygous Mutations in NRXN1. Cell Stem Cell, 17(3): 316-28, 2015.
4. Yi, F. et al. Autism-associated SHANK3 haploinsufficiency causes Ih channelopathy in human neurons. Science, 352(6286): aaf2669, 2016.

Diverse Platform for Endless Possibilities

NeuCyte's platform is suitable for developing a broad range of functional assays, neurite outgrowth and seizure liability are two examples below.

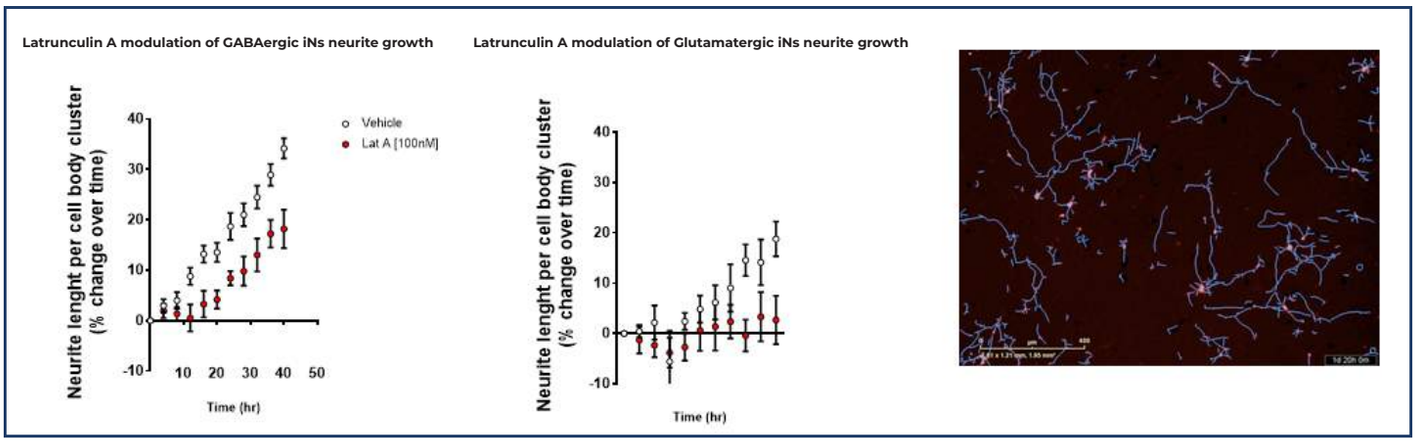


Figure 6. Neurite outgrowth assay using SynFire® iN co-cultures treated with actin filament disruptive toxin
SynFire® neural cultures were treated with the actin filament disruptive toxin Latrunculin A (100 nM). Neurite length was assessed and quantified over a period of 44 hours using a live imaging Incucyte system. Representative images of the neurite traces from both excitatory and inhibitory neurons are included.

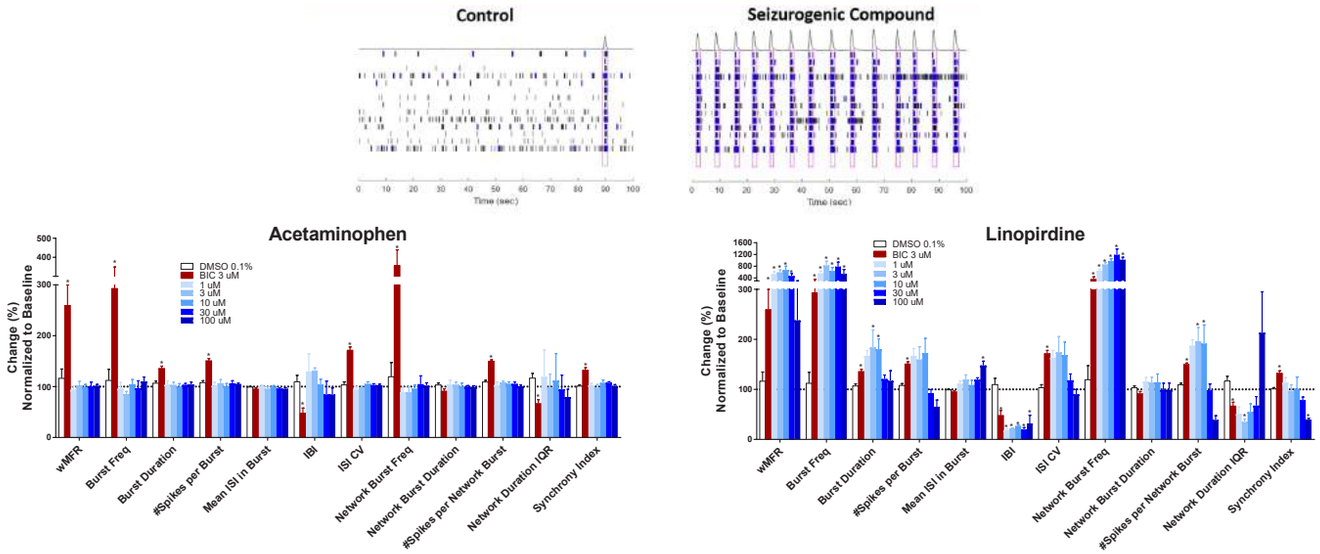


Figure 7. Seizure liability testing with compounds from the HESI NeuroTox MEA seizure prediction initiative using SynFire® iN co-cultures
For each MEA parameter, measurements from vehicle- or compound-treated wells were normalized to their respective baseline values. All parameters are expressed as percent change. Significance for Bicuculline (positive control) relative to DMSO (Vehicle) was determined via Student's T-test (n=4, $p<0.05$). Significance for test compounds relative to DMSO (Vehicle) was determined via One-Way ANOVA (n=4, $p<0.05$). Linopirdine and acetaminophen (negative control) are shown here. **(Data from additional compounds and controls can be found on our website.)**

Please feel free to contact us or visit www.neucyte.com/data for additional data.

Proven System Supporting Drug Discovery

NeuCyte's SynFire® co-cultures, as an example used in drug discovery, have served to test anti-epileptic drug efficacy and shown better predictive ability than some other iPSC-derived neuronal systems.

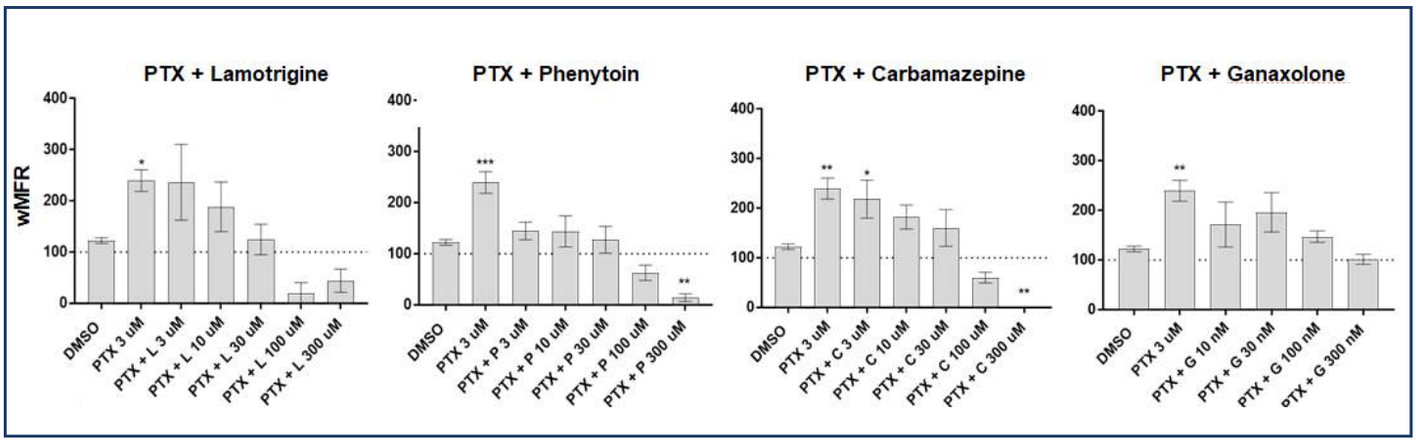


Figure 8. SynFire® neural cultures serve to test anti-epileptic drugs (AED) efficacy
NeuCyte's iNs/MEA platform measures quantifiable effects of drugs on neuronal activity. Chemical induced seizure-like activity can be reversed in a dose dependent manner by several AEDs. Assays performed with mixed excitatory/inhibitory iN co-cultures.

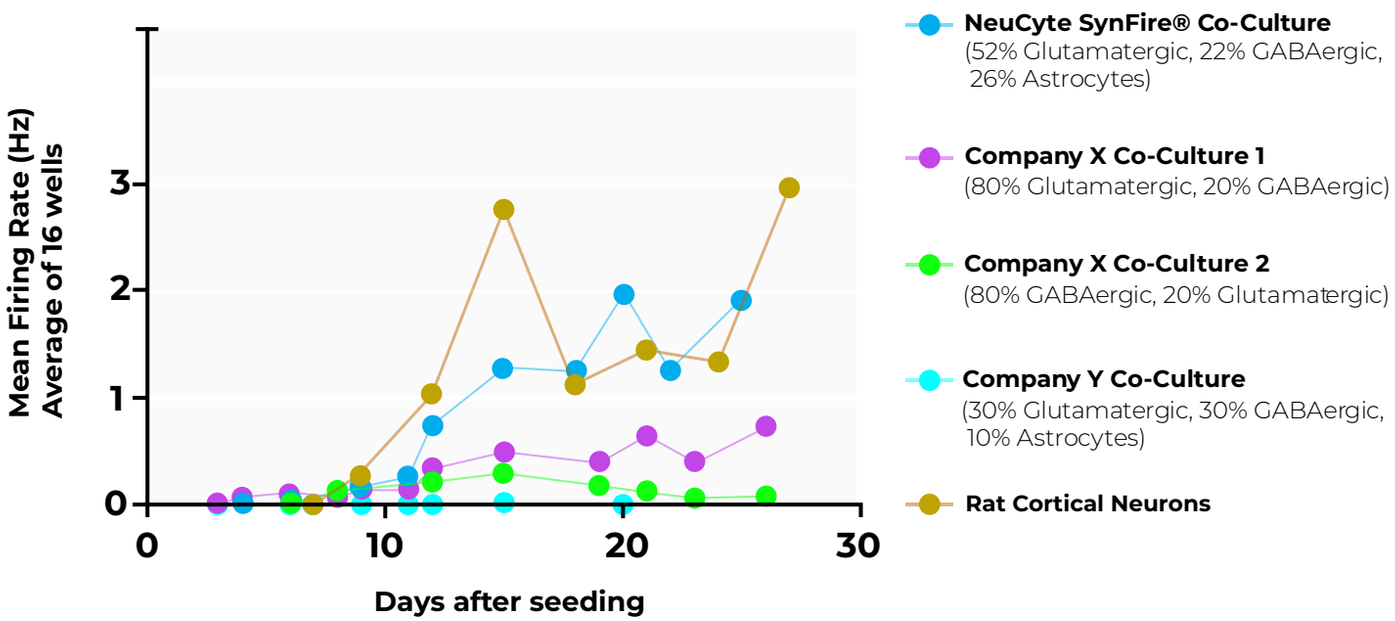


Figure 9. Independent comparison of NeuCyte's SynFire® neural cells to other iPSC derived neurons
Plot shows the mean firing rate (MFR) of SynFire® induced neural co-cultures and other commercially available neurons. MFR was assessed using Axion MEA plates. Axion Maestro Axis software Default setting for spontaneous neuron firing was used (Data provided by customer).

Products, Services & Contact Information

Products

SynFire® Line	Catalog#	Pack size
Glutamatergic Excitatory Neurons	1001	**Various sizes and custom packaging available**
GABAergic Inhibitory Neurons	1002	
Synfire® Co-Culture Kits	1010	
Media (Long-Term Culture)	2003	

Services

Drug Discovery and Pre-Clinical Testing

Development of Neural Cell Based Assays

Phenotypic and Targeted Drug Screening

Electrophysiological Based Readout

Neural Network Physiology Assessment (Multielectrode Arrays)

Traditional Single Cell Patch Clamping Assays

CNS Safety/Toxicology

Cell Death, Apoptosis, Autophagy and Mitochondrial Activity Assays

Cell Stress Tests

Compound Seizurogenic Liability Testing

Our goal is to develop applications, assays and protocols to support clients' needs using our technology platform. We always start with the questions you are trying to answer. We have the suitable infrastructure to support drug discovery and nonclinical safety assessment from low to high throughput based on the needs of the individual project. Please contact us with your unique inquiry.

For more information:
NeuCyte Inc.
1230 Bordeaux Drive
Sunnyvale, CA 94089
Phone : (650) 274-8199
enquiries@neucyte.com
www.neucyte.com

Japanese distributor:
AS ONE INTERNATIONAL, INC.
3350 Scott Blvd, Suite 29
Santa Clara, CA 95054
Phone : 408-638-7415 ext.104
Fax: 408-252-6220
www.asone-int.com

〒550-8527　大阪市西区江戸堀2-1-27
2-1-27 Edobori, Nishi-ku,
Osaka 550-8527 Japan
Phone : 81-6-6447-8641
Fax: 81-6-6447-8642
www.as-1.co.jp