StemoniX

MOLECULAR DEVICES

ISSCR

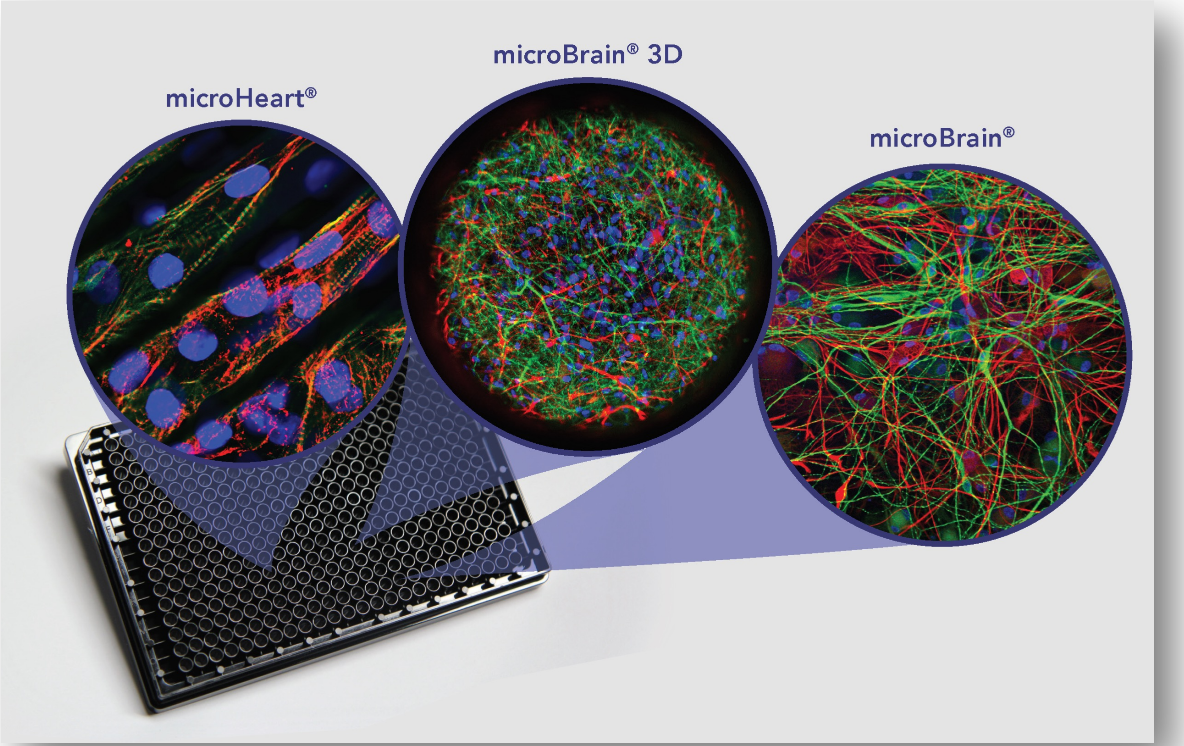
2019 ANNUAL MEETING

Functional and mechanistic neurotoxicity profiling using human iPSC-derived neural 3D cultures

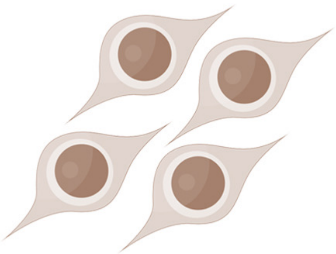
Cassiano Carromeu, PhD - StemoniX

StemoniX®

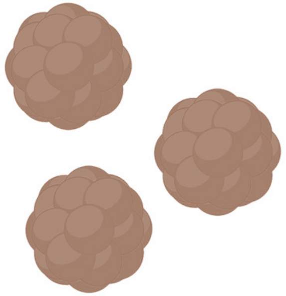
- Expertise in human iPSC-derived neural and cardiac platforms
- Focus on adapting platforms to HTS and producing assay-ready solutions to accelerate drug discovery
- Neural platform: microBrain®



Models available for Neuroscience research

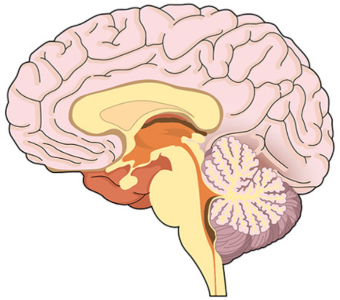


Cellular models



Organoids

Animal models

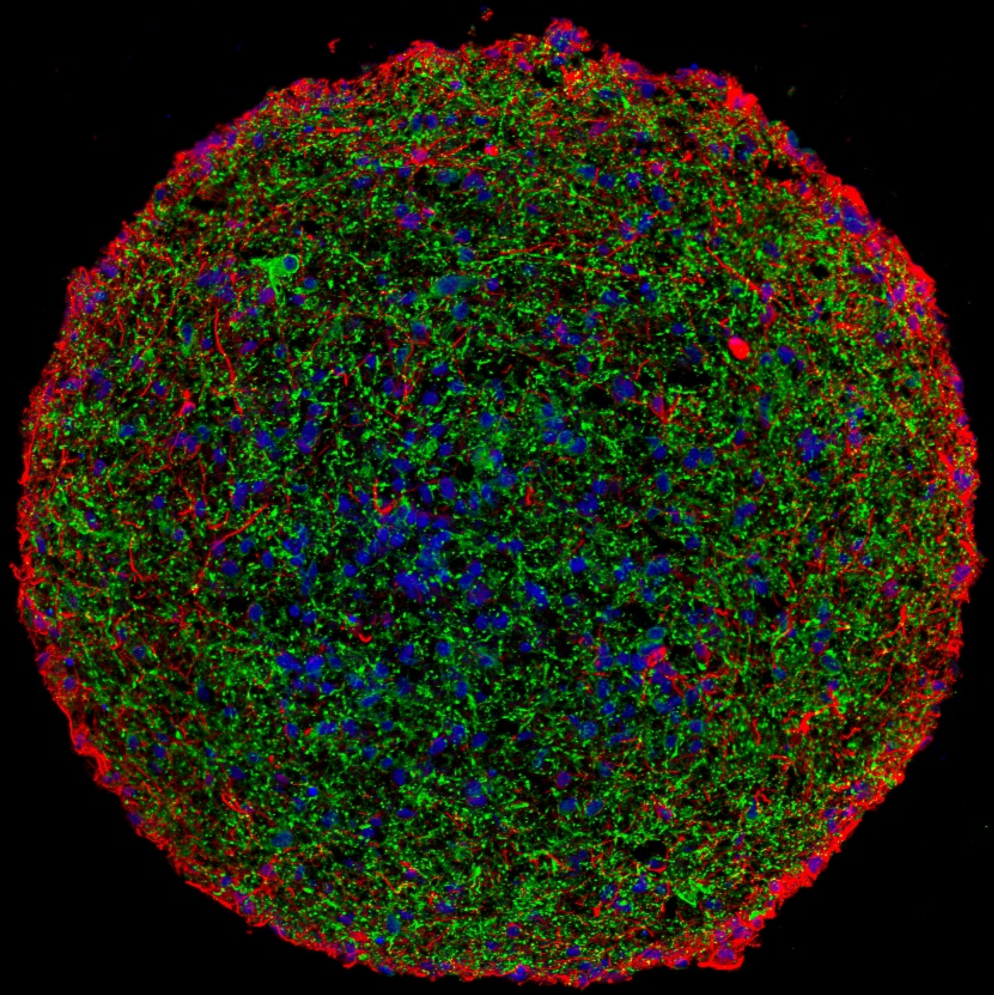


Brain

Tissue Complexity

Throughput

microBrain® 3D



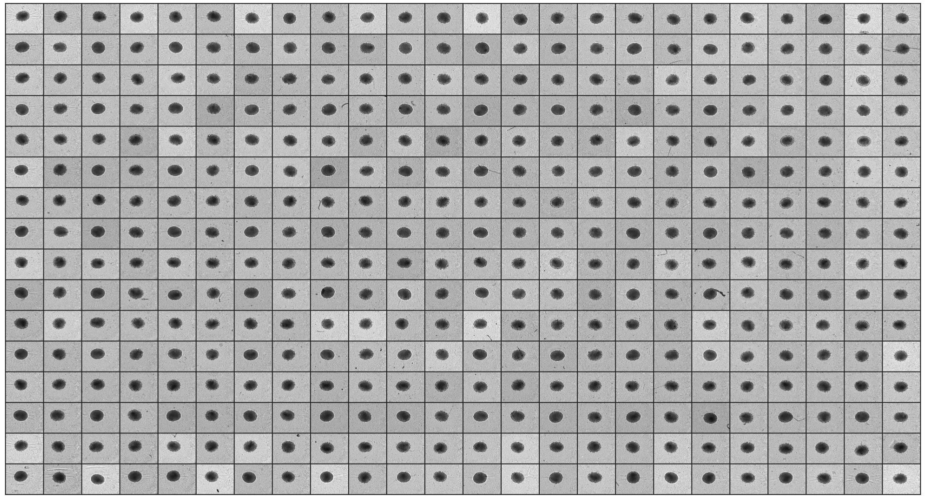
GFAP/MAP2

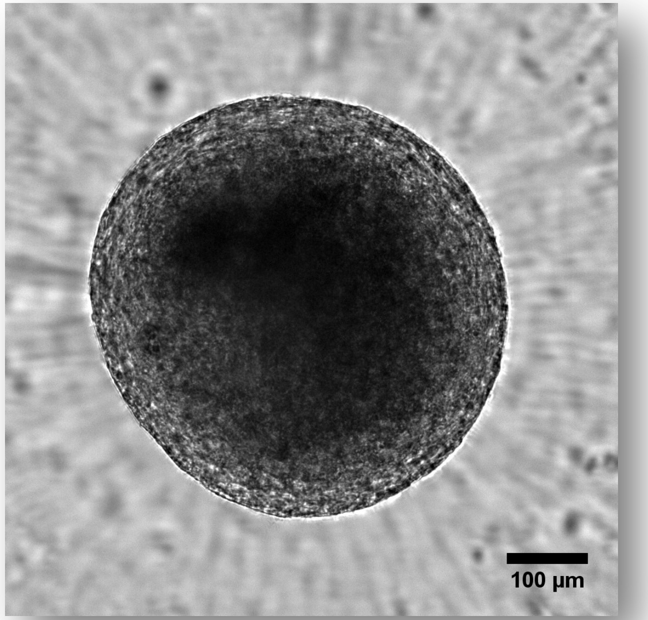
Main Features

- Single donor human iPSC line
- Balanced co-culture of neurons and astrocytes
- Display key neuronal and astrocytes markers
- Spontaneous synchronized activity
- Amenable to High Content Screening and High Content Imaging (384-well format)

microBrain 3D spheroids are homogenous

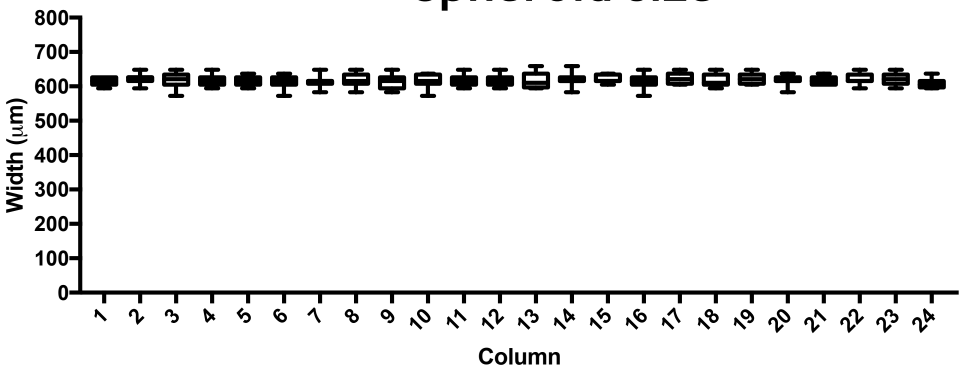
384w microBrain 3D plate





microBrain 3D spheroid

Spheroid size

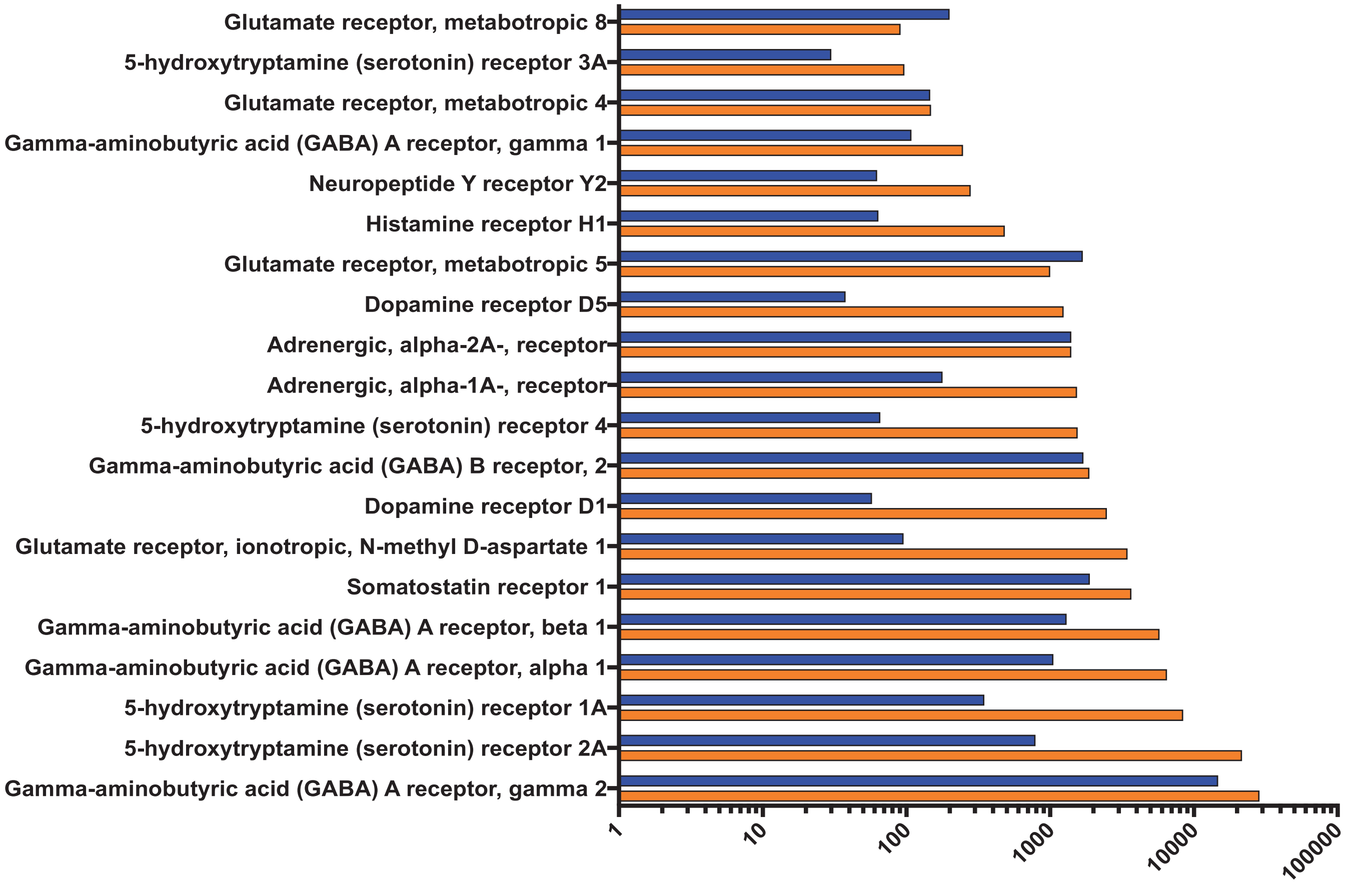


Features:

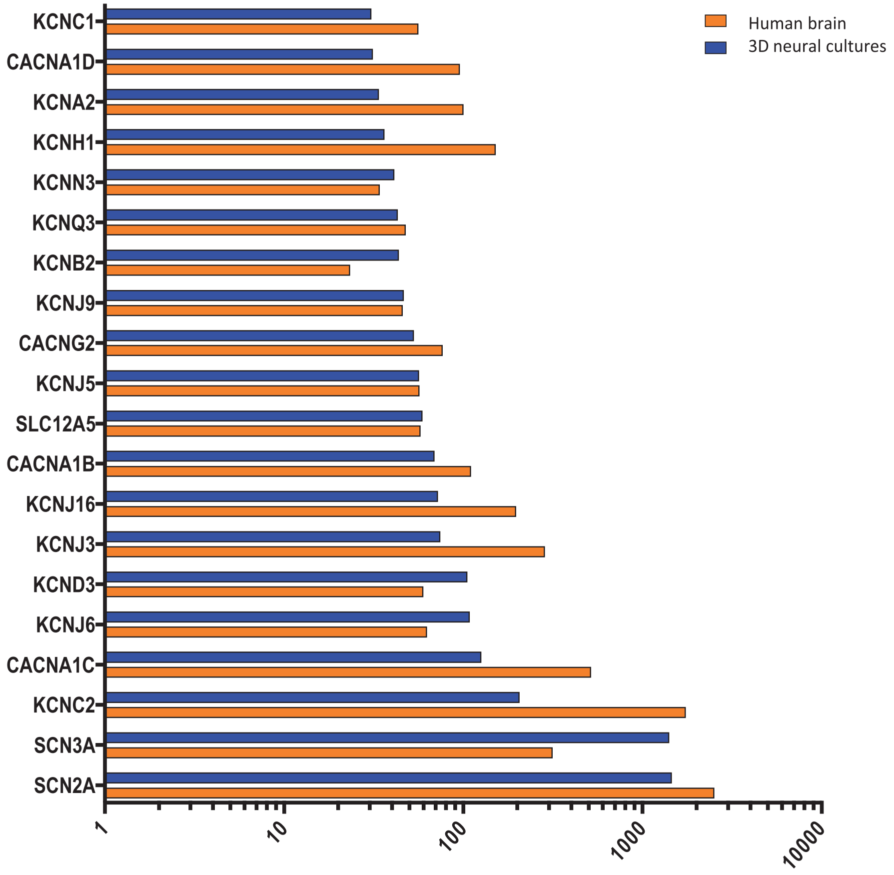
- 1 spheroid per well
- Consistent size across the plate
- Able to culture for weeks

Gene expression profile of microBrain 3D

Neurotransmitter profiling



Ion channel profiling



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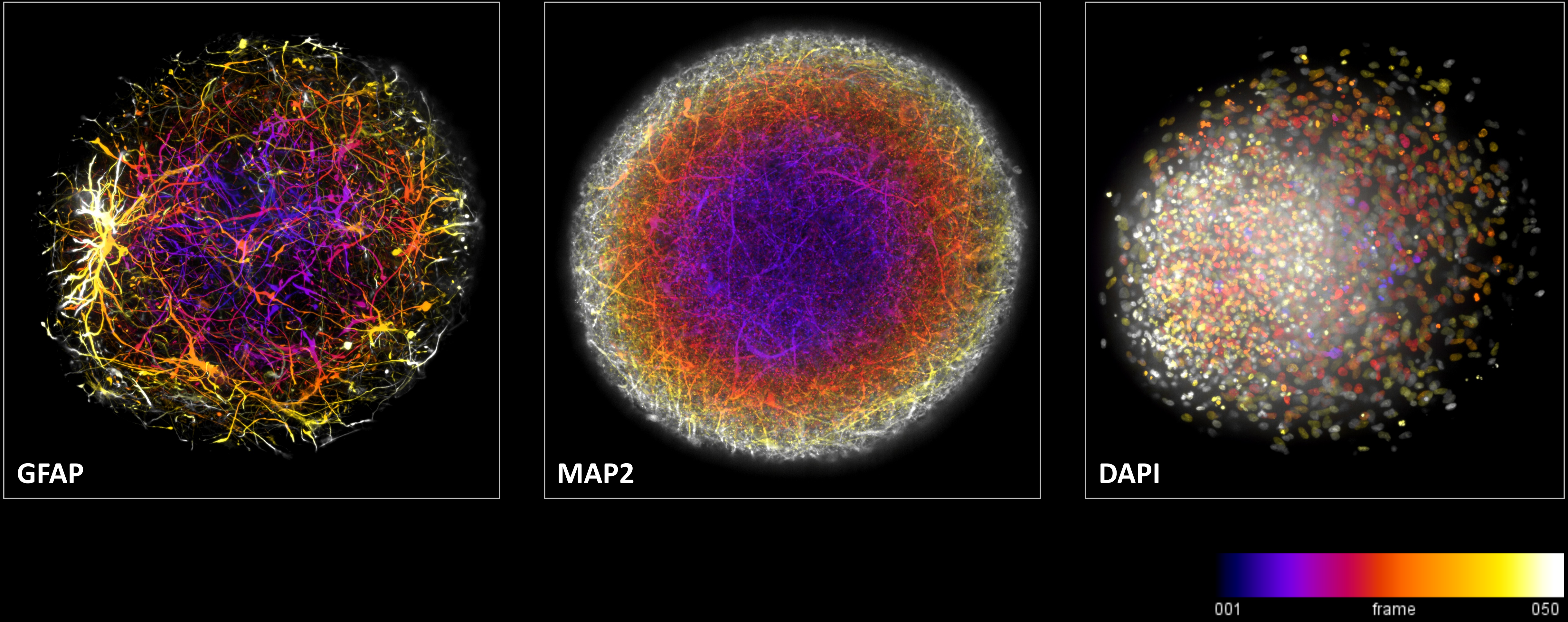
microBrain 3D neural composition

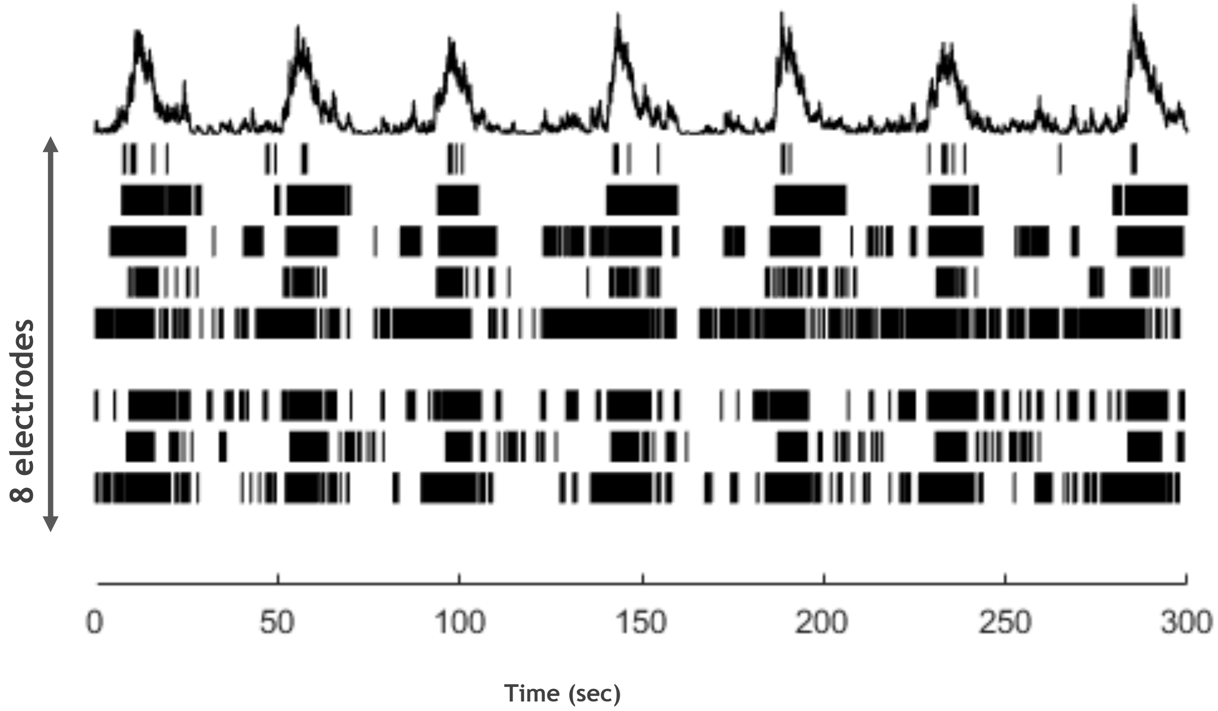
- Immunostaining with neuronal and glial markers on microBrain spheroids
- Confocal images using ImageXpress® Micro Confocal system (IXM-C)
- Optical clearing protocol applied after staining



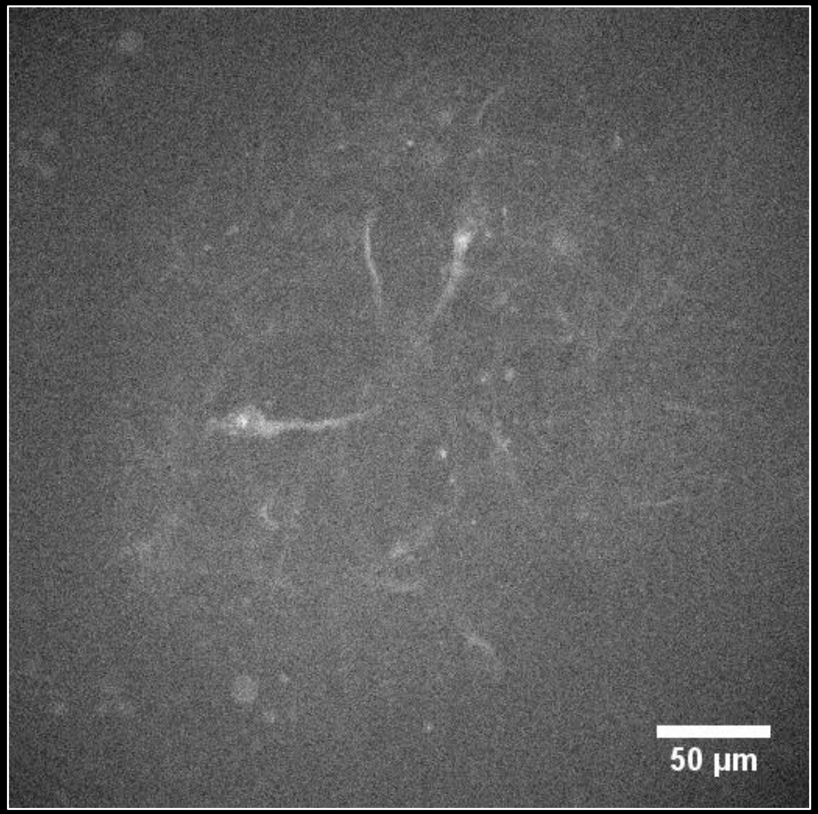
microBrain 3D demonstrates spontaneous synchronized neural activity

- Spheroid electrical activity is confirmed via MEA recordings
- Neuronal origin of activity is confirmed via synapsin-targeted Ca^{2+} measurements with high-speed confocal microscopy (IXM-C)

Plated microBrain 3D on MEA (Maestro)



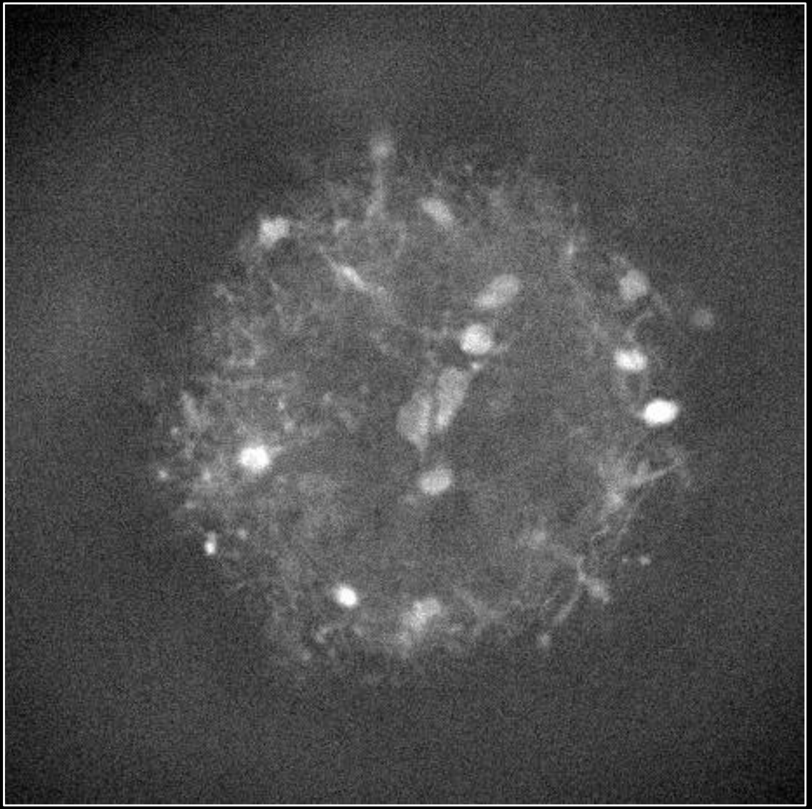
Synapsin driven Ca^{2+} indicator



microBrain 3D activity can be monitored in high throughput format

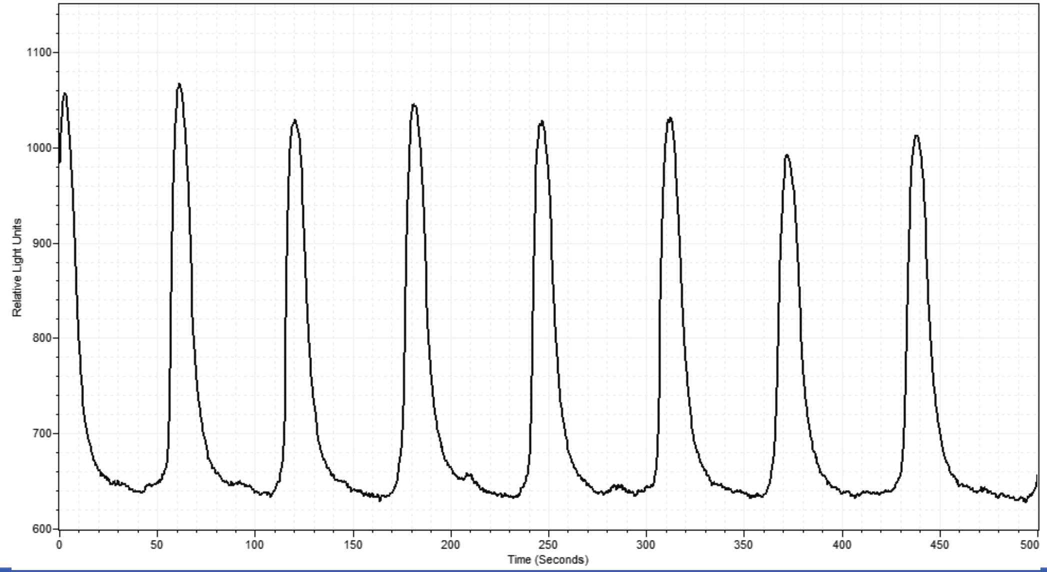
- **Neuronal activity is monitored as calcium oscillations detected by high throughput kinetic fluorescence (FLIPR® Tetra System)**

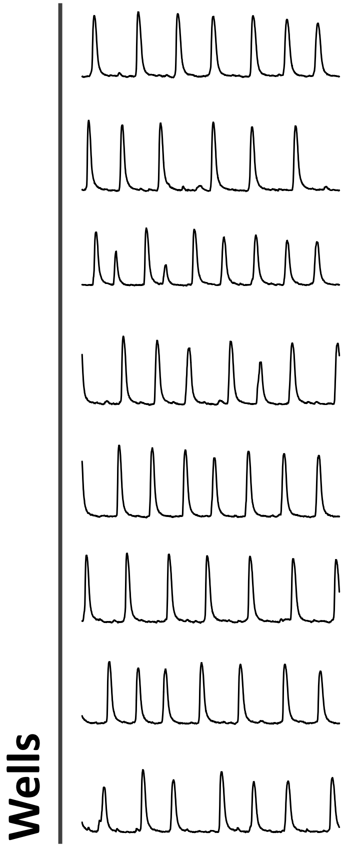
Spontaneous neuronal activity



FLIPR Tetra System

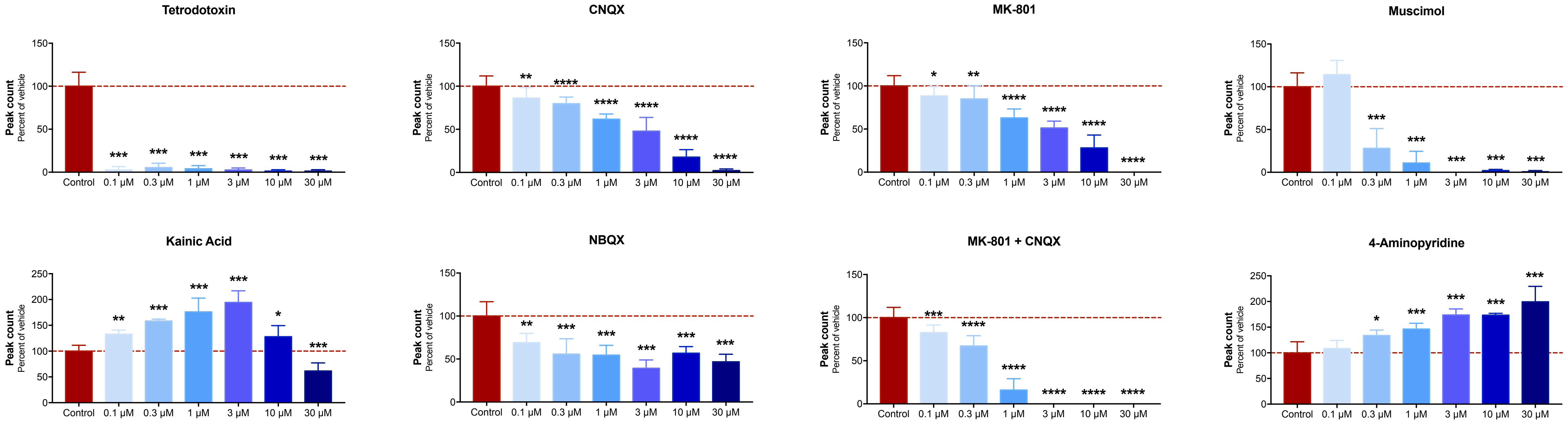
- Spontaneous activity on one well
- Detected oscillations correspond to synchronized calcium oscillation occurring on the sphere





microBrain 3D activity respond to known neuromodulators

- Spontaneous neural activity can be monitored by high throughout calcium flux analysis (FLIPR Tetra System)
- Graphs show modulation on spontaneous oscillations 30min after adding compounds



Tetrodoxin: sodium channel blocker (potent neurotoxin)

Kainic Acid: Kainate receptor agonist

MK-801: NMDA receptor antagonist

CNQX: competitive AMPA/kainate receptor antagonist

NBQX: AMPA receptor antagonist

Muscimol: potent GABA agonist

4-Aminopyrydine: Non-selective K_V channel blocker

microBrain 3D showcase

Drug Discovery / Screening:

- Epilepsy Drug Discovery
- High Throughput Screening of LOPAC®1280
- Thousands of compounds screened as a service with high reproducibility among replicates

Safety Pharmacology and Investigative Toxicology:

- Neurotoxicity Screen of Drug Candidates to Treat Zika Infection
- Screening of Environmental Toxins for Neurotoxic Effects

Disease Modeling:

- Neurodevelopmental disorders (Rett Syndrome)

Screening of Environmental Toxins for Neurotoxic Effects

- Neurotoxicity is a major reason for attrition in the final stages of a drug development pipeline.
- Current neurotoxic evaluation of chemicals rely mostly on animal models.
- Among hiPSC neurotoxicity assays, neurite outgrowth and multi-electrode arrays (MEAs) have been used as tool for evaluation of toxicity on the Central Nervous System (CNS).
- There is a need to more predictive and scalable human *in vitro* models to test compounds for their toxicological effects on the CNS.

Screening of Environmental Toxins for Neurotoxic Effects

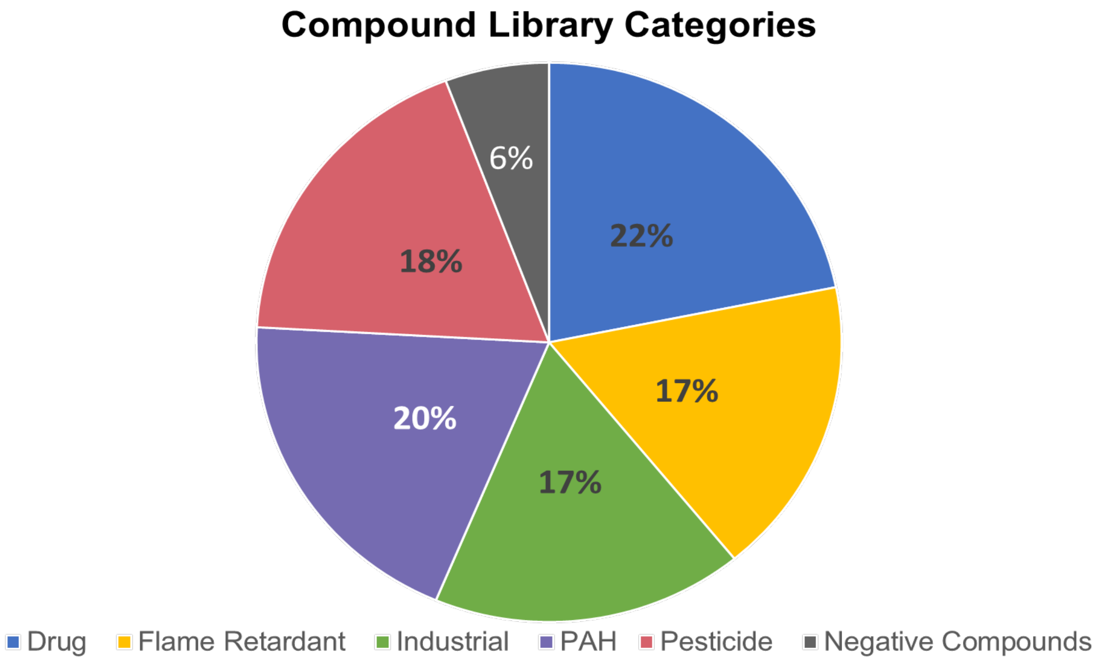
- Goal:

  We used microBrain 3D platform to characterize the phenotypic responses to various compounds by monitoring the impact on the frequency and pattern of the spontaneous calcium oscillations.

Screening of Environmental Toxins for Neurotoxic Effects

- Method:

We screened a diverse library of 91 compounds comprised of representative examples of compounds from various environmentally relevant and potentially neurotoxic groups using an HTS (FLIPR Tetra System) and HCS (ImageXpress Micro Confocal system) workflow.

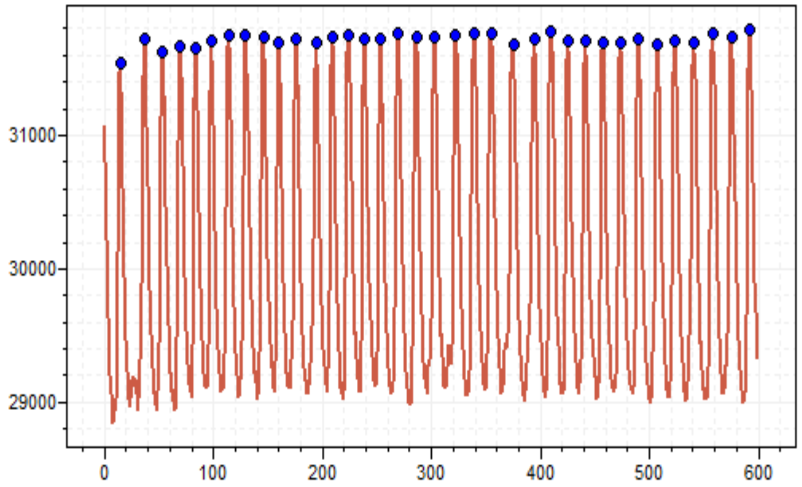


microBrain 3D activity modulation

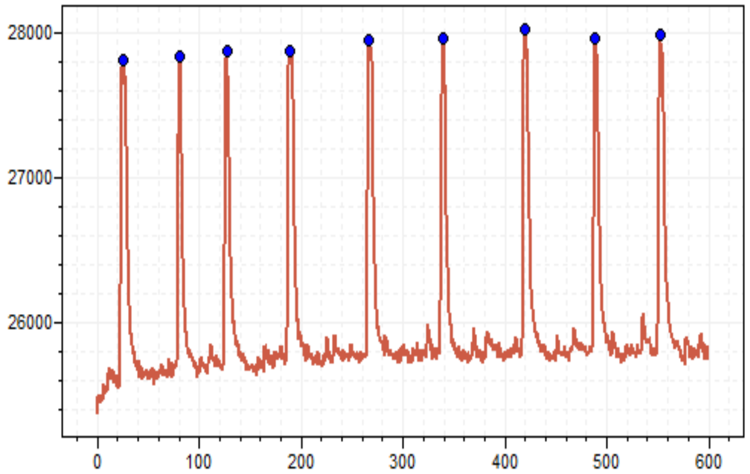
- Representative FLIPR Tetra System recordings of spontaneous neuronal activity

Calcium oscillation traces recorded by the FLIPR Tetra System:

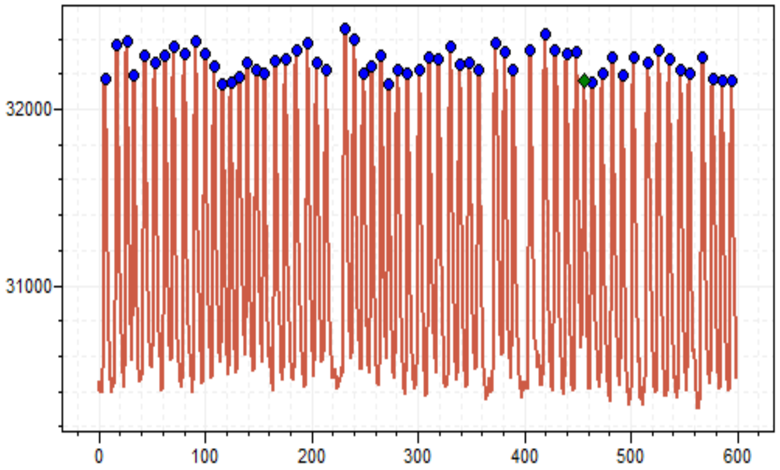
Control



GABA



AMPA



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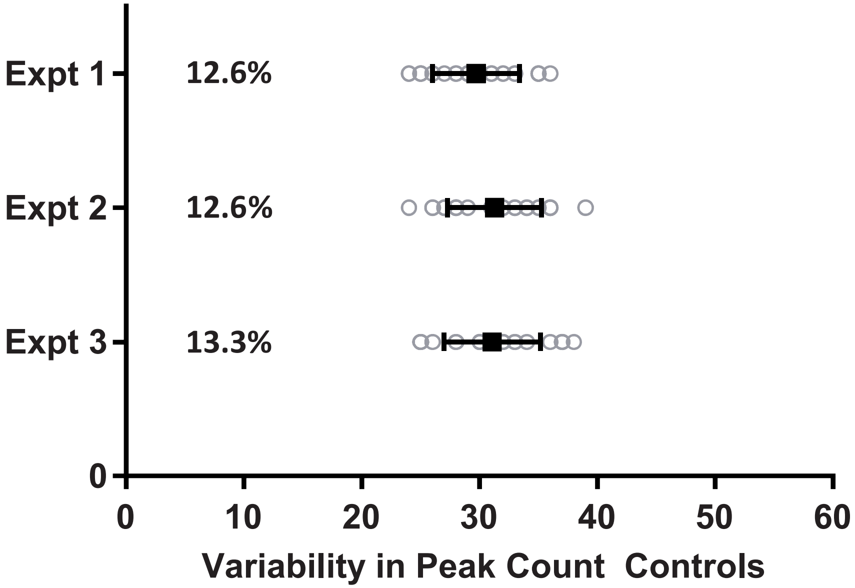
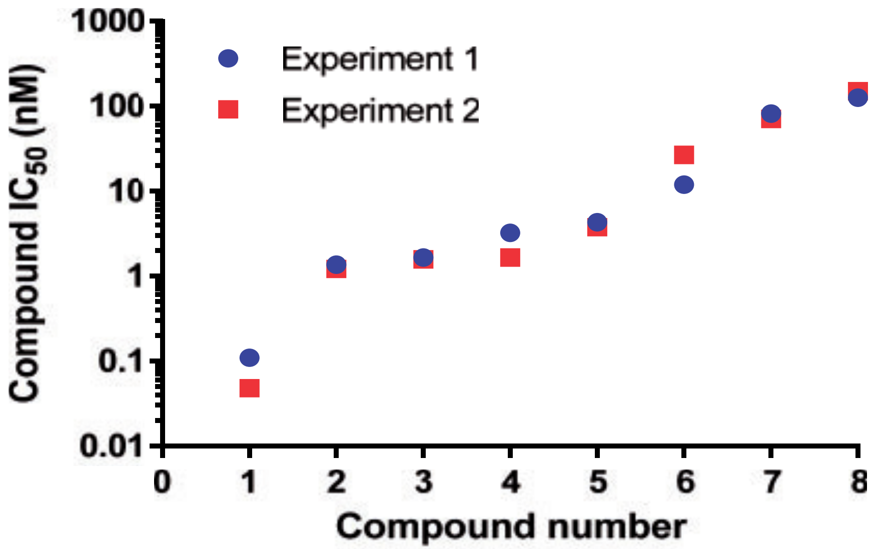
microBrain 3D activity is reproducible

- Inter-plate variability of spontaneous neuronal activity
- Each experiment number correspond to a different microBrain 3D plate

Inter-plate variability in vehicle controls



Inter-plate compound response comparison



Compound Legend:

[1]Rotenone
[2]2-Ethylhexyl diphenyl phosphate
[3]Methyl mercuric (II) chloride
[4]Isodecyl diphenyl phosphate
[5]2,2',4,4',5-Pentabromodiphenyl ether
[6]2,2',4,4'-Tetrabromodiphenyl ether
[7]Lead (II) acetate trihydratre
[8] 1-Ethyl-3-methylimidazolium diethylphosphate

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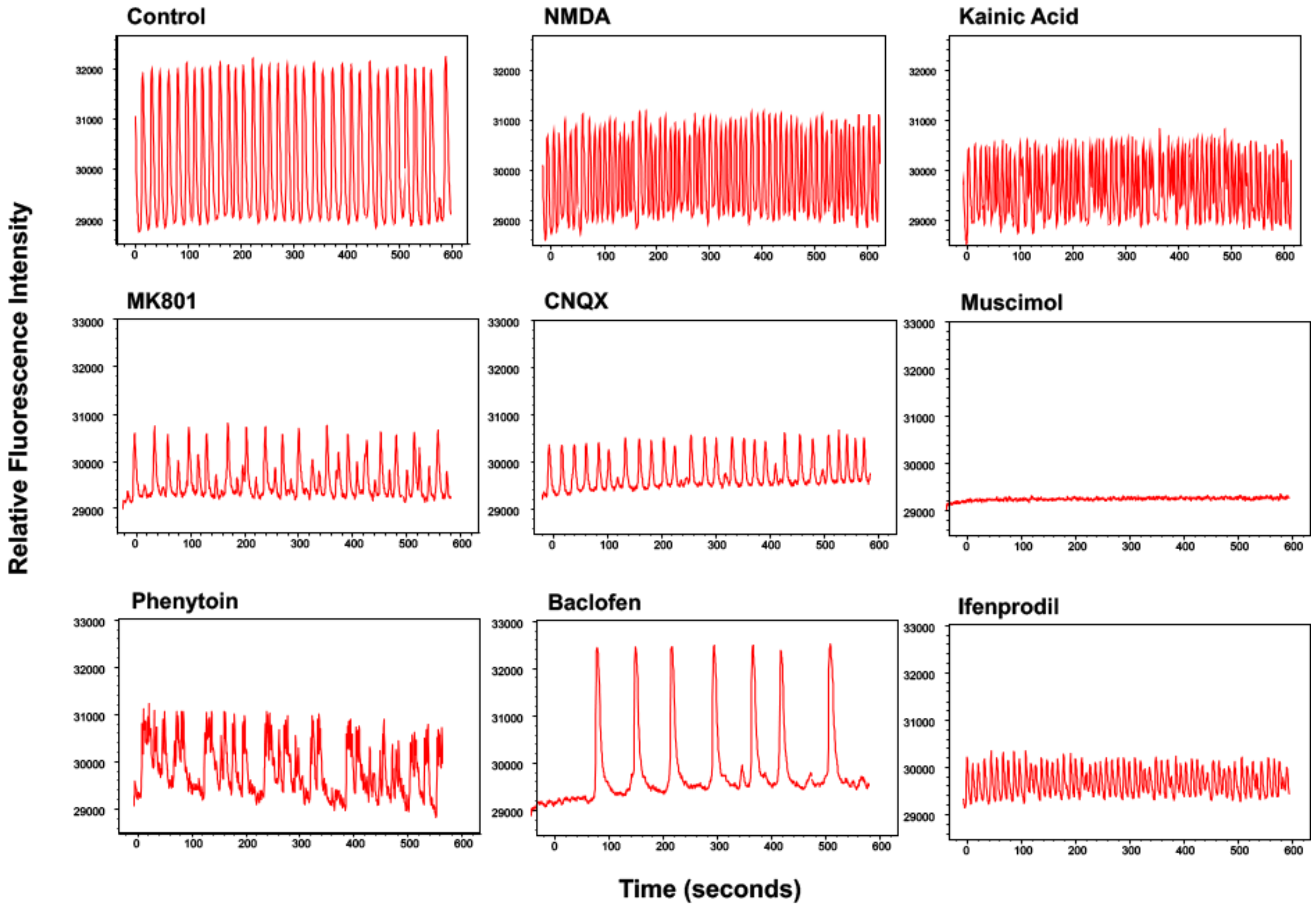
microBrain 3D activity modulation

- **Representative FLIPR Tetra System recordings of spontaneous neuronal activity**



Legend:

- Control: Vehicle DMSO control
- NMDA: NMDA receptor agonist
- Kainic Acid: Kainate receptor agonist
- MK-801: NMDA receptor antagonist
- CNQX: AMPA/Kainate receptor antagonist
- Muscimol: $GABA_A$ agonist
- Phenytoin: Blocker of voltage gated Na^+ channels
- Baclofen: $GABA_B$ agonist
- Ifenprodil: non-competitive NMDA antagonist

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microBrain 3D activity modulation

- IC_{50} determination and comparison with the literature of some control compounds

Compound	Mechanism of Action	IC_{50} (μM), This Study	Reference IC_{50} (μM)
Kainic acid	Agonist of kainate receptor	2.66	3; 20
MK-801	Antagonist of NMDA receptor	0.033	0.037
CNQX	Antagonist of AMPA/kainate receptor	2.05	0.3–1.5
Muscimol	Agonist of $GABA_A$ receptor	0.021	0.47
(R)-Baclofen	Agonist of $GABA_B$ receptor	0.45	0.70
GABA	Endogenous agonist of GABA receptor	5.93	7; 27
Haloperidol	Antagonist of D2 dopamine receptor	0.13	0.037
Lidocaine	Voltage-gated Na^+ channel blocker	9.47	5
Phenytoin (dilantin)	Voltage-gated Na^+ channel blocker	9.41	16
Lamotrigine isothionate	Voltage-gated Na^+ channel blocker	34.1	66

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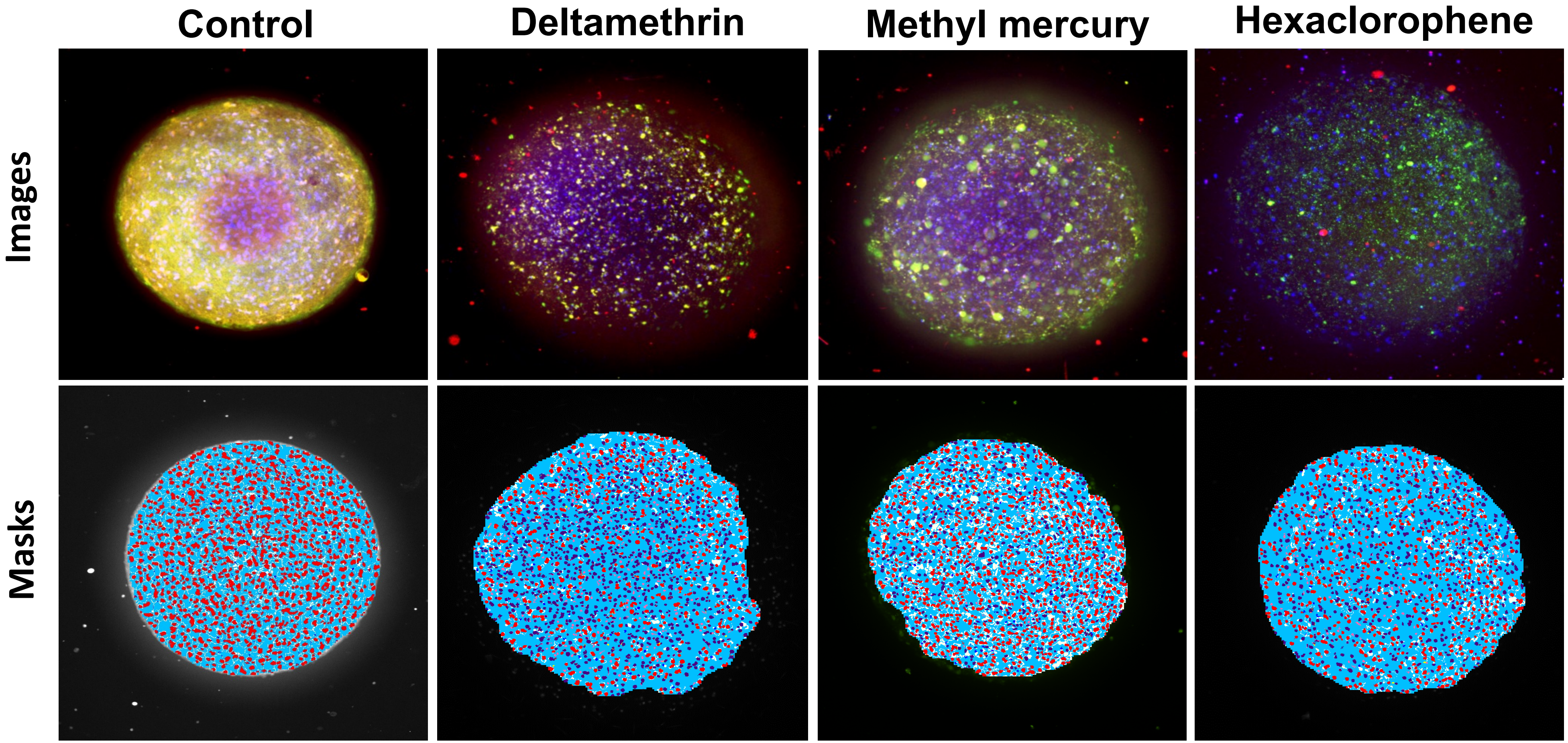
microBrain 3D is amenable to HCS

- Representative images of Cell Viability quantification using ImageXpress Micro Confocal system



Nuclei (Hoechst): Blue / Viability (Calcein AM): Green / Mitochondria (MitoTracker Orange): Red

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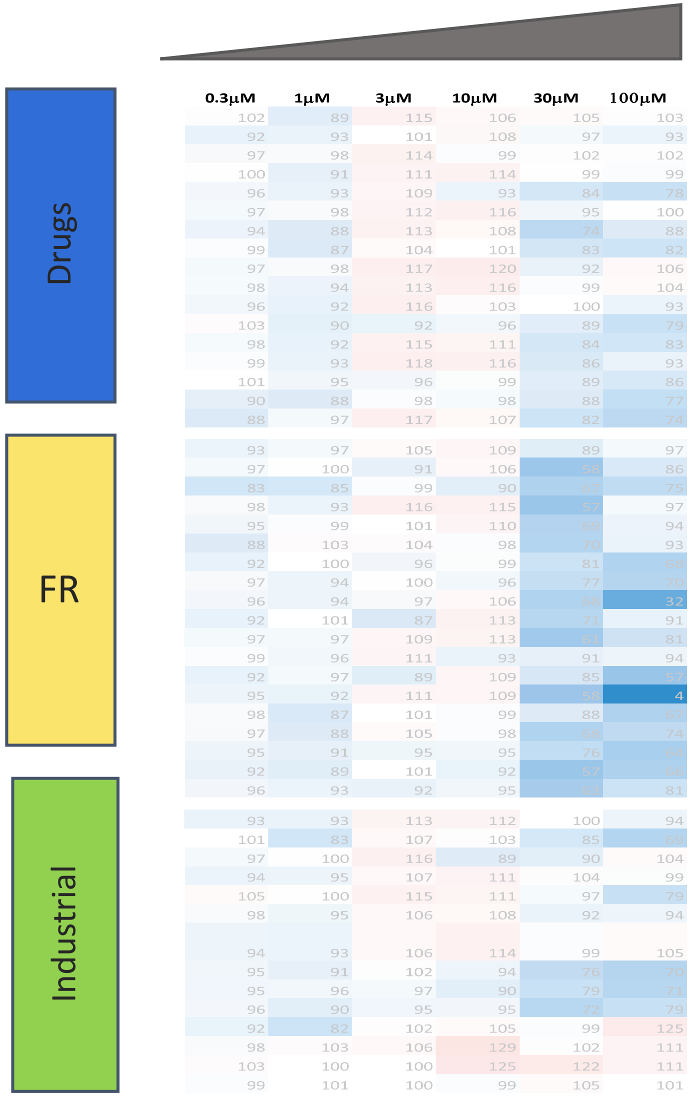
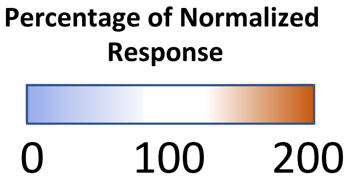
microBrain 3D is amenable to HCS



Legend:

- Drugs: Pharmaceutical compounds (n=17)
- FR: Flame retardants (n=19)
- Industrial (n=15)
- PAH: Polycyclic Aromatic Hydrocarbons (n=20)
- Pesticides (n=17)
- Controls (n=11)



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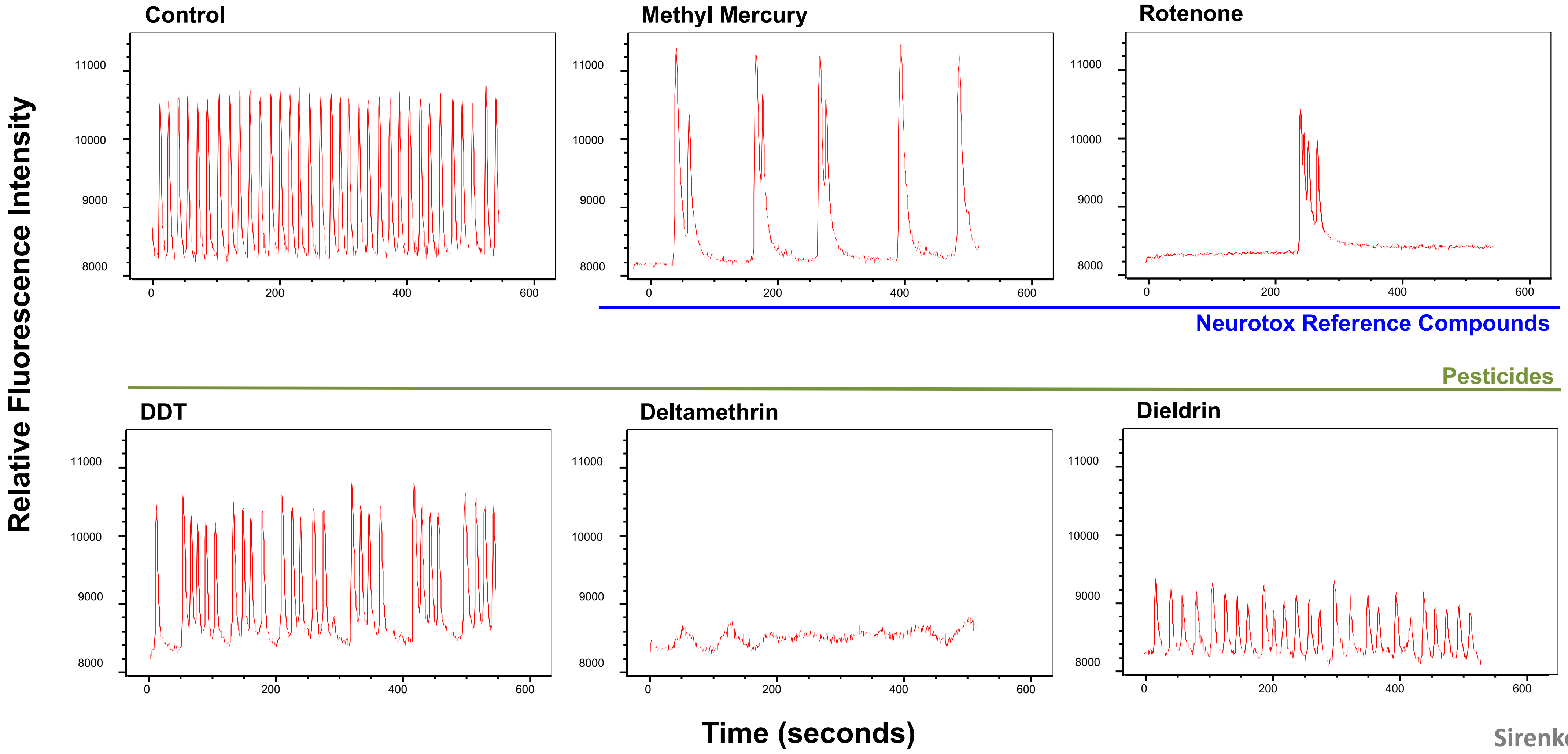
Neurotoxic compounds interfere with microBrain 3D activity

- Representative traces of spontaneous activity recorded on FLIPR Tetra System



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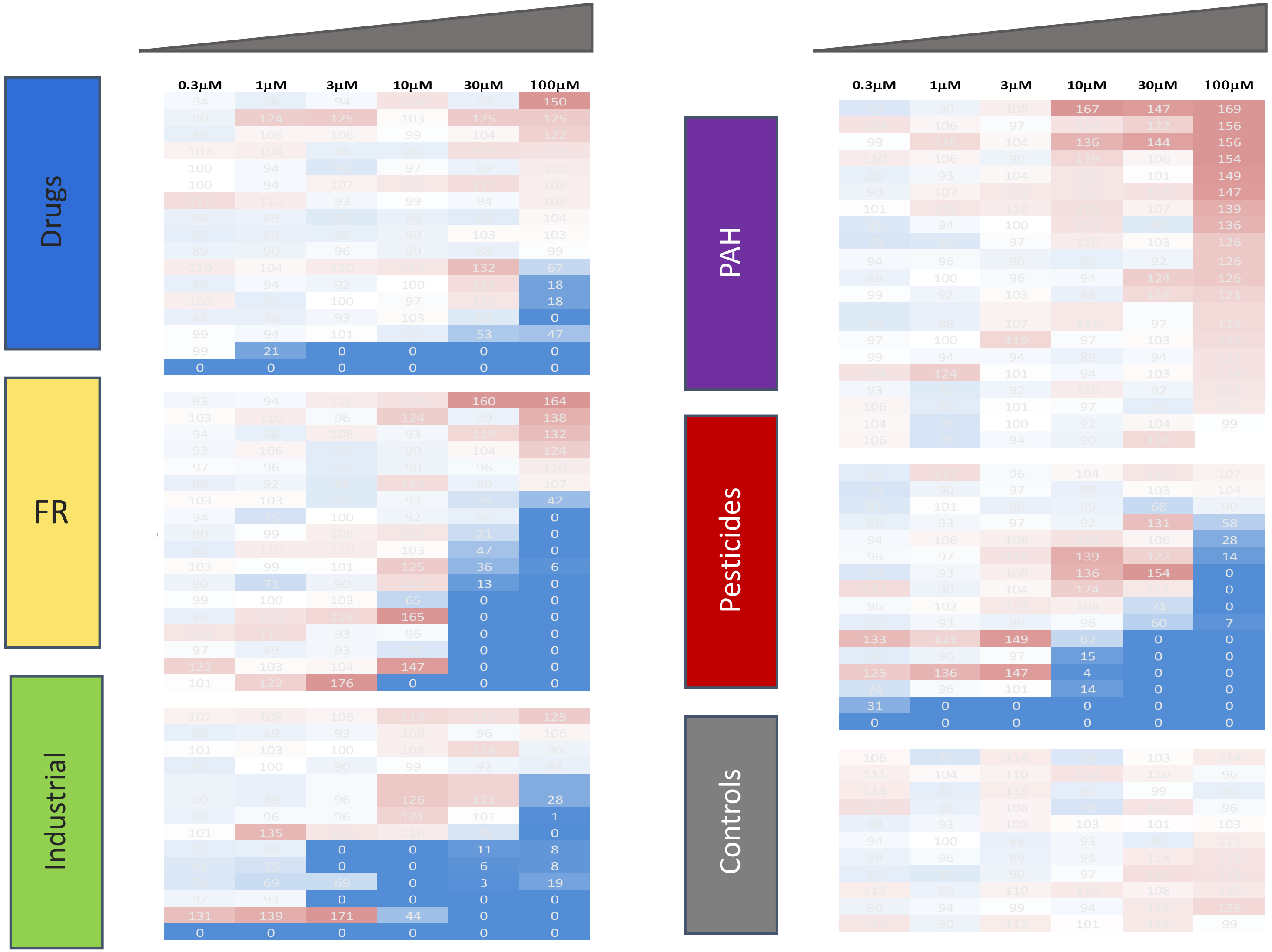
Neurotoxic compounds interfere with microBrain 3D activity



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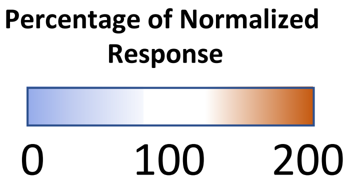

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Conclusions

- microBrain 3D is a very homogenous human-based tool for CNS interrogation *in vitro*.
- The platform was successfully used for HCS and HTS when paired with FLIPR Tetra System and ImageXpress Micro Confocal system.
- A functional phenotype (Ca^{2+} spontaneous activity) was more sensitive to capture toxicity of compounds than cell viability.
- Many compounds investigated on this study presented a detrimental effect on the neuronal activity of microBrain 3D, which could be used as a phenotype to investigate CNS toxicity.

Acknowledgements

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Ryan Gordon, PhD

Fabian Zanella, PhD

Poster:

Multiplexed Automated Imaging Assays for Compound Testing Using Induced Pluripotent Stem Cell-Derived Cells

Poster W-3210

Date: 06/28/2019

Time: 7pm

https://bit.ly/2WXNS3W

For additional information:

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Read More:

Sirenko, O. et al. Functional and Mechanistic Neurotoxicity Profiling Using Human iPSC-Derived Neural 3D Cultures, *Toxicological Sciences*, Volume 167, Issue 1, January 2019, Pages 58–76, https://doi.org/10.1093/toxsci/kfy218

microBrain® 3D Neurotoxicity Profiling Application Note http://bit.ly/2YstGc1