



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

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# 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









## microBrain<sup>®</sup> 3D



## **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)

![](_page_3_Picture_8.jpeg)

## microBrain 3D spheroids are homogenous

#### 384w microBrain 3D plate

![](_page_4_Picture_2.jpeg)

![](_page_4_Figure_3.jpeg)

![](_page_4_Picture_4.jpeg)

microBrain 3D spheroid

Features:

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

![](_page_4_Picture_11.jpeg)

## Gene expression profile of microBrain 3D

#### Neurotransmitter profiling

![](_page_5_Figure_2.jpeg)

![](_page_5_Figure_3.jpeg)

![](_page_5_Figure_4.jpeg)

## microBrain 3D neural composition

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

![](_page_6_Figure_4.jpeg)

![](_page_6_Figure_5.jpeg)

## 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<sup>2+</sup> measurements with high-speed confocal microscopy (IXM-C)

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![](_page_7_Figure_3.jpeg)

![](_page_7_Picture_4.jpeg)

## microBrain 3D activity can be monitored in high throughput format

 Neuronal activity is monitored as calcium oscillations detected by high throughput kinetic fluorescence (FLIPR<sup>®</sup> Tetra System)

#### Spontaneous neuronal activity

![](_page_8_Picture_3.jpeg)

#### **FLIPR Tetra System**

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

![](_page_8_Figure_7.jpeg)

mm

![](_page_8_Picture_10.jpeg)

![](_page_8_Picture_11.jpeg)

## 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

![](_page_9_Figure_3.jpeg)

#### **MOLECULAR** DEVICES

![](_page_9_Picture_5.jpeg)

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## microBrain 3D showcase

Drug Discovery / Screening:

- Epilepsy Drug Discovery
- High Throughput Screening of LOPAC<sup>®</sup>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)

![](_page_10_Picture_10.jpeg)

![](_page_10_Picture_11.jpeg)

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## **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.

![](_page_11_Picture_5.jpeg)

![](_page_11_Picture_6.jpeg)

## **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.

![](_page_12_Picture_3.jpeg)

![](_page_12_Picture_4.jpeg)

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## **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.

![](_page_13_Figure_3.jpeg)

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![](_page_13_Picture_4.jpeg)

![](_page_13_Picture_5.jpeg)

## microBrain 3D activity modulation

• Representative FLIPR Tetra System recordings of spontaneous neuronal activity

#### Calcium oscillation traces recorded by the FLIPR Tetra System:

![](_page_14_Figure_3.jpeg)

Sirenko et al. 2018

![](_page_14_Picture_5.jpeg)

![](_page_14_Picture_6.jpeg)

![](_page_14_Picture_7.jpeg)

## microBrain 3D activity is reproducible

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

![](_page_15_Figure_3.jpeg)

#### Inter-plate variability in vehicle controls

Inter-plate compound response comparison

Sirenko et al. 2018

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![](_page_15_Picture_7.jpeg)

![](_page_15_Picture_8.jpeg)

## microBrain 3D activity modulation

Representative FLIPR Tetra System recordings of spontaneous neuronal activity

![](_page_16_Figure_2.jpeg)

#### Legend:

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- 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<sub>A</sub> agonist
- Phenytoin: Blocker of voltage gated Na<sup>+</sup> channels
- Baclofen: GABA<sub>B</sub> agonist
- Ifenprodil: non-competitive NMDA antagonist

Sirenko et al. 2018

![](_page_16_Picture_14.jpeg)

## microBrain 3D activity modulation

• IC<sub>50</sub> determination and comparison with the literature of some control compounds

Compound	Mechanism of Action	IC <sub>50</sub> (μM), This Study	Reference IC <sub>50</sub> (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 <sub>A</sub> receptor	0.021	0.47
(R)-Baclofen	Agonist of GABA <sub>B</sub> 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 <sup>+</sup> channel blocker	9.47	5
Phenytoin (dilantin)	Voltage-gated Na <sup>+</sup> channel blocker	9.41	16
Lamotrigine isothionate	Voltage-gated Na <sup>+</sup> channel blocker	34.1	66

![](_page_17_Picture_3.jpeg)

![](_page_17_Picture_4.jpeg)

![](_page_17_Picture_5.jpeg)

## microBrain 3D is amenable to HCS

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

![](_page_18_Figure_2.jpeg)

Nuclei (Hoechst): Blue / Viability (Calcein AM): Green / Mitochondria (MitoTracker Orange): Red Sirenko et al. 2018

![](_page_18_Picture_4.jpeg)

![](_page_18_Picture_5.jpeg)

Yalproic acid sodium salt Fluorouracil Thalidomide Acetaminophen (4-hydroxyacetanilic

## microBrain 3D is amendal to HCS

0.3μΜ	1μM	ЗμМ	10µM	30µM	100µM
102		115	106	105	103
	93	101	108	97	
97		114	99	102	102
100		111	114	99	
	93	109			
97		112	116		100
94		113	108		
99		104	101		
97		117			106
	94	113	116	99	104
			103	100	
103					
98		115	111		
99		118	116		
101					
		117	107		
	97	105			97
97	100		106		
			90		
			115		97
	99	101	110		
	103	104			
	100		99		
97	94	100			
	94	97	106		
	101				91
97	97	109	113		
99		111			
	97				
		111	109		4
98		101	99		67
97		105	98		
		95	95		
	89	101	92		
96	93	92	95	63	
		110	1.1.0	1.00	
93		113	112		
101	83	107			69
97	100	116	89	90	104
94	95	107	111	104	
105	100	115	111		
		106	108		
		100	114		105
		106	114		
		102			
			95		
92	82	102		102	125
92 98	82 103	102 106	105	102	125

Valinomycin		0	0 0	0	0	o l
tris(Chloropropyl) phosphat	e, TCPP				160 1	.64
Tric/2 chloroothyl) phorpha	to			6 124		38
Methoxyethanol	ice -					24
Tetrachlorodibenzo-p-dioxi Iminodipropionitrile	n					10
Tetrabromobisphenol A					75	42 IM
Triphenyl phosphate Ethylhexyl-2.3.4.5-tetrabro					86	0
Tetrabromodiphenyl ether			94			
Hexabromodiphenyl ether Triphenyl phosphate			107		38	
Tricresyl phosphate			106	95		
Pentabromodiphenyl ether			105	101	68	50
tert-Butylphenyl diphenyl p	93	99	100	92		17
Phenol, isopropylated, pho				96	52	71
Ethymexyr diplienyr phospi			105	111	90	
Bisphenol S Acculamido			107	110		78
Lead (II) acetate trihydrate	99	97	107	111	104	103
Di(2-ethylhexyl) phthalate		97				
methyl-2,4-cyclopentadien		94	102	99		
Bisphenol A Bisphenol AF						
Methyl mercuric (II) chloric	100		103	101	32	
Methyl mercuric (II) chloric Bis(tributyltin)oxido						
Acetic acid, manganese (24			108	109		29
Auramine O	99	91	99	97		102
Hexachiorophene	101	101	109	97		105
Benzo(b)fluoranthene	95			106		103
Anthracene Benz(a)anthracene	102	96	104	92		60
Benzo(a)pyrene						
Acenaphthene Dibenz[a,c]anthracene	101	103		97	99	100
Cyclopenta(d,e,f)phenanth	100	99		101	101	104
Benzo(k)fluoranthene Benzo[g,h,i]perylene			117	103	61	
Propyl-2-thiouracil	100		101	104		99
Phenanthrene Fluorene		91	109	115	95	94
Bis(2-ethylhexyl) 3,4,5,6-			115	111	106	100
tetrabromophthalate (TBP Benzo(e)pyrene			112	114		
Toluene	107	94	114	107	105	100
Naphthalene	100		100	105		
Acenaphthylene	95		98	95		
n-Hexane Pyrene	99	101			104	
Chausan				114		.9
Captan	102	99	116	104		0
Aldicarb	98		115	115		9
Chlorpyrifos (Dursban)			112	107	112	
Lindane	104		107			
Tebuconazole	100		105	1.01	100	
Heptachlor			106	101	103	99
Isodecyl diphenyl phosphat			109	112		104
Dichlorodiphenyltrichloroe			107	112	99	103
Permethrin			100	105		
Deltamethrin	100	103	100	114		
Rotenone	92	95		109	99	102
DMSO	100		111	110		103
DMSO		103	113		99	102
PBS			108			
PBS Acetylsalicylic acid	99	102	110	120	97	99
Amoxicillin						
D-Glucitol L-Ascorbic acid						
Saccharin Sodium Salt hydra	ate					
saccharin Sodium Salt hydra	ate					99

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#### Legend:

Percentage of Normalized Response

100

0

- 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)

200

#### **MOLECULAR** DEVICES

Drugs

FR

Industrial

### Sirenko et al. 2018

![](_page_19_Picture_13.jpeg)

## Neurotoxic compounds interfere with microBrain 3D activity

• Representative traces of spontaneous activity recorded on FLIPR Tetra System

![](_page_20_Figure_2.jpeg)

## Neurotoxic compounds vinter er ferrer with microBrain 3D activity

Phenobarbital Hydroxyurea Valproic acid sodium salt Fluorouracil

0.3µM	1µM	ЗμМ	10µM	30µM	100µM
					150
					67
					18
					18
					0
99	94	101	85	53	47
99					
0	0	0	0	0	0
				160	164
				89	
					42
					0
					0
					0
			65		
			165		
			79		
		104	147		
		176	0	0	0
					105
			99		88
					Mangar
					methyl-
89	96				1
101		0	0	11	0
				11	
		0	0		
		69	0		19
92	93	0	0		
131	139	1/1	44		

![](_page_21_Figure_2.jpeg)

#### Legend:

Percentage of Normalized

Response

100

N

- 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)

200

Sirenko et al. 2018

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![](_page_21_Figure_10.jpeg)

Drugs

FR

Industrial

![](_page_21_Picture_11.jpeg)

### 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<sup>2+</sup> 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.

![](_page_22_Picture_5.jpeg)

![](_page_22_Picture_6.jpeg)

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### Acknowledgements

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#### **Poster:**

Multiplexed Automated Imaging Assays for Compound Testing Using Induced Pluripotent Stem Cell-Derived Cells Poster W-3210 Date: 06/28/2019 Time: 7pm

![](_page_23_Picture_11.jpeg)

#### https://bit.ly/2WXNS3W

![](_page_23_Picture_13.jpeg)

![](_page_23_Picture_14.jpeg)

![](_page_23_Picture_15.jpeg)

### 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, <u>https://doi.org/10.1093/toxsci/kfy218</u>

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

Stemoni**X** 

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![](_page_24_Picture_10.jpeg)

![](_page_24_Picture_11.jpeg)