Functional Phenotypic Screenings using Neuronal Network Cultures on Microelectrode Arrays

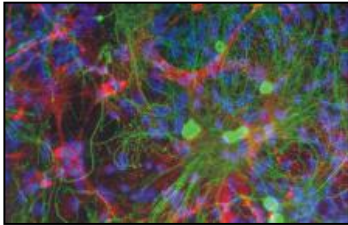**NeuroProof Systems GmbH**, Rostock, Germany
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phone: +49 381 54345660
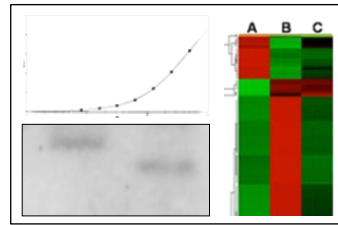
NeuroProof

Cell Culture Support and Services

Primary mouse neurons
- Cortex
- Hippocampus
- Midbrain
- Cerebellum
- Hypothalamus
- Spinal cord, Dorsal root ganglia
- Striatum
- Microglia
- Astrocytes

hiPSC neurons
- Cortical
- Glutamatergic
- GABAergic
- Dopaminergic
- Motor neurons
- Sensory neurons
- +/- human astrocytes
- +/- human microglia

Validation & Assays

Target validation
- Western blot
- Multiplexing
- Gene expression
- rtPCR, seqRNA,
- Single cell seqRNA
- (phospho)-ELISA
- RNA interference
- Overexpression
- Knockout studies

Vitality assays
- LDH assay
- ATP
- Protease
- ROS
- **Imaging**
- ICC
- Ca^{2+}

Premium MEA recording services and support

Compound profiling
- Neuractive functionality
- Potency comparison
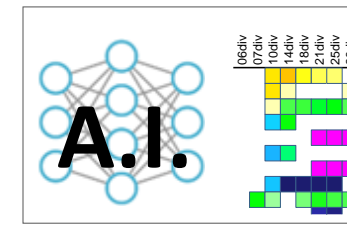- Side effect prediction
- Neuro-development

Disease models
- Alzheimer (APP)
- Parkinson (A35T, MPP+)
- Immunology
- ALS
- SMA
- NPC1
- FragileX
- Epilepsy
- Schizophrenia

Disease model development:
iPSC → neuronal phenotype
AAV transduction

CNS Big Data AI Analytics

Multi-parametric analysis

AI-based analytics
- Classification
- in-house databases
- E/I balance
- Deep learning methods

Compelling data visualisization

Brain data integration into propietary scores
- Effect Score
- Seizure Score
- Sedation Score
- Development Index

Mouse cultures

Frontal cortex

Hippocampus

Hippocampus + Amygdala

Ventral midbrain

Ventral midbrain + frontal cortex

Hypothalamus

Spinal cord

Dorsal root ganglia

Spinal cord + DRG

Cerebellum

Hippocampus (ictal)

Striatum

Microglia

Well-characterized references
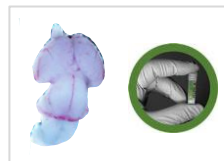
Human culture models

GABAergic neurons

Dopaminergic neurons

Glutamatergic neurons

Motor neurons

Sensory neurons

Astrocytes

Microglia

Co-culture and tri-culture systems

In total >20 human cell lines screened

Disease-specific neuron models

Alzheimer's disease, Parkinson's disease, ALS, SMA, Fragile X, Epilepsy, Schizophrenia, Autism, Depression
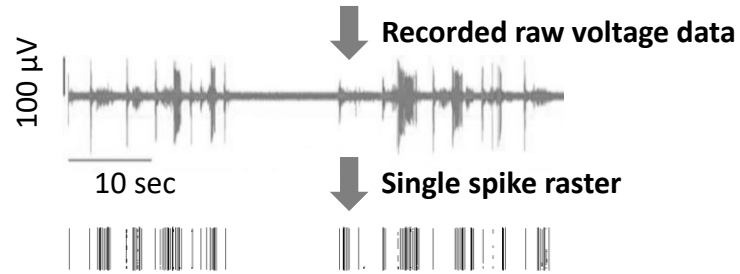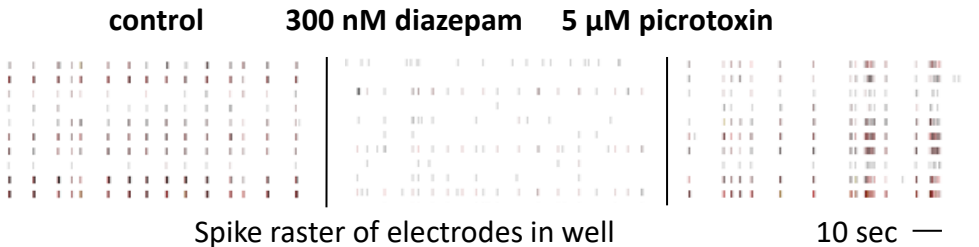
Multielectrode Array Recordings - MEA
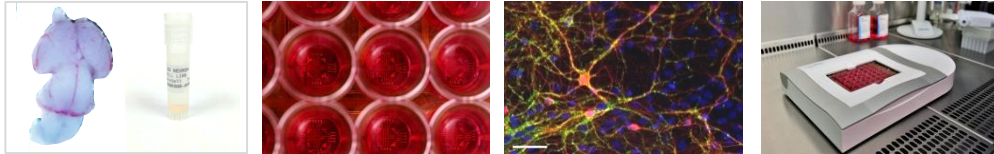
Embryonic brain or human iPSC neurons

MEA, 48 wells, 16 electrodes

Neuronal network

Axion MAESTRO recording station

Recorded raw voltage data

100 µV

10 sec

Single spike raster

Effects of compounds

control   300 nM diazepam   5 µM picrotoxin

Spike raster of electrodes in well   10 sec ⎯

Each spike train is described by more than 200 activity parameters:

Burst Plateau

Burst Area

Burst Duration   Burst IBI   Burst Duration

Burst Period   Burst ISI
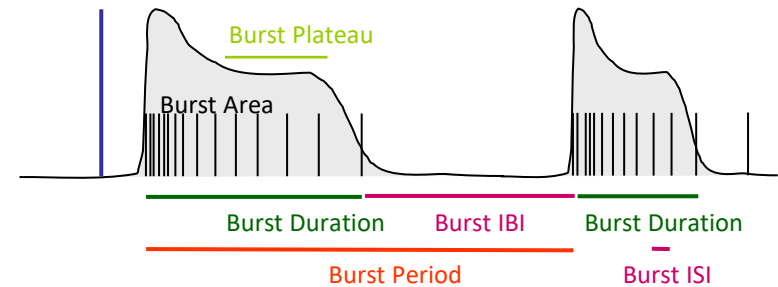
1 General Activity
e.g. spike rate, burst rate, burst period, percent of spikes in burst

2 Burst Structure
e.g. number, frequency and ISI of spikes in bursts; burst duration, amplitude, area, plateau position, plateau duration
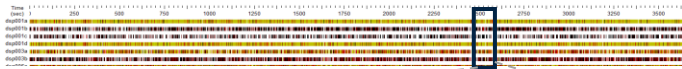
3 Oscillation
e.g. variation over time as an indicator for the strength of the oscillation; Gabor function fitted to autocorrelograms

4 Synchronicity / Connectivity
e.g. variation within the network as an Indicator for the strength of the synchronization; simplex synchronization, percent of units in synchronized burst
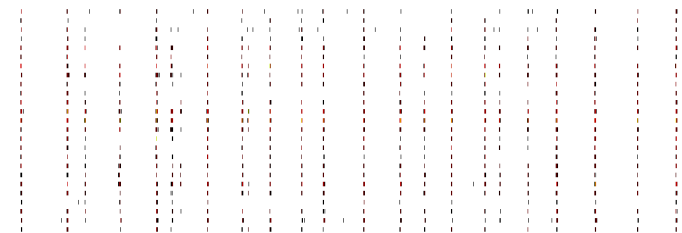
Functional Phenotype = Fingerprint

60 min

25 neurons, 60 sec, 28 days in vitro

Frontal Cortex (FC)

Hippocampus (HC)
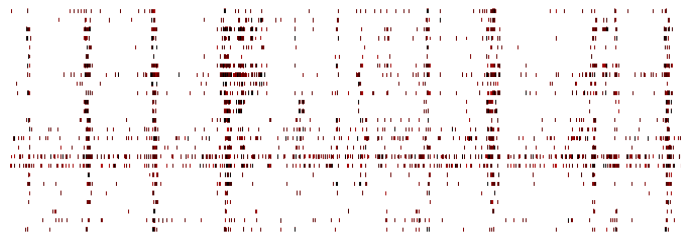
Spinal Cord (SC)
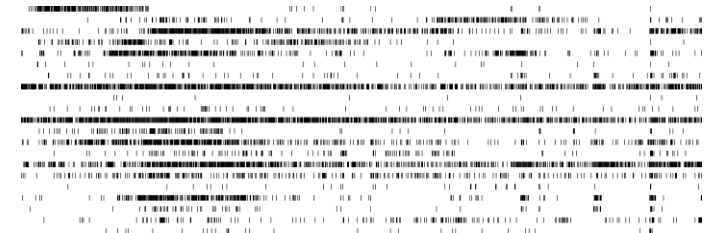
60 sec

Hypothalamus (Hth)
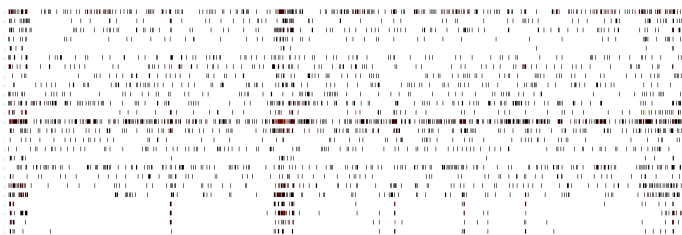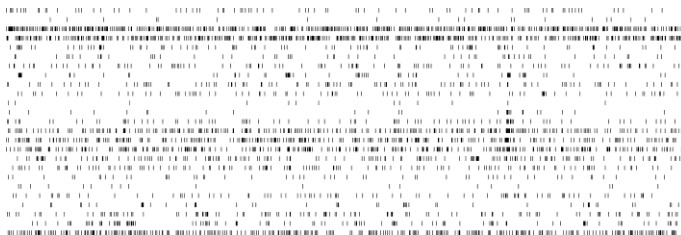
Midbrain (Mb)

Midbrain/Frontal Cortex (Mb + FC)
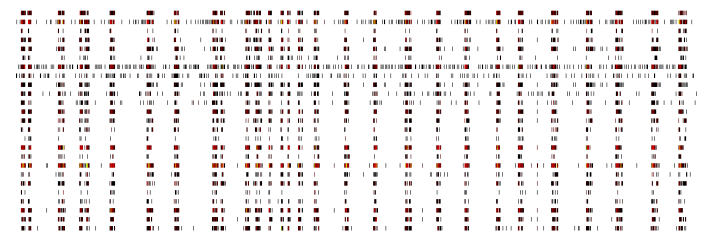
Co-cultures are customizable based on customer wishes

	predicted as					
tissue culture	Hth	Mb	Mb+FC	FC	HC	SC
Hth	88	7	0	0	4	1
Mb	12	66	4	1	3	16
Mb + FC	0	3	79	15	2	3
FC	0	1	13	82	3	1
HC	0	2	2	2	93	1
SC	2	9	2	1	1	86

Cross validation matrix shows highly reproducible tissue-specific activity pattern

% of datasets, 500 datasets each

Reproducible spontaneous tissue-specific activity pattern

➔ **Allows similarity analysis to unknown activity pattern**

- ✓ **Proprietary compound databases for fast phenotypic screening.**
- ✓ **Compound combinations.**
- ✓ **Repositioning.**
- ✓ **Toxicity tests (e.g. seizure).**
- ✓ **Phenotypic profiling against marketed drugs.**
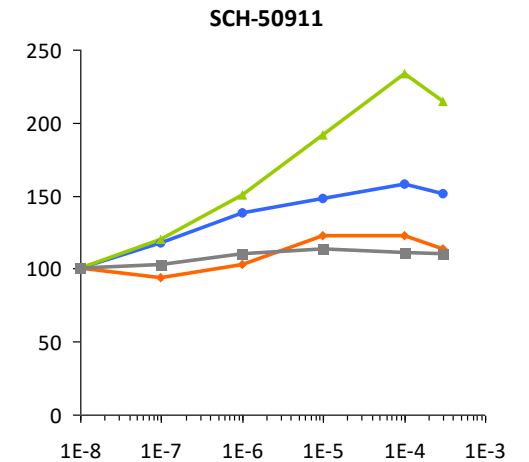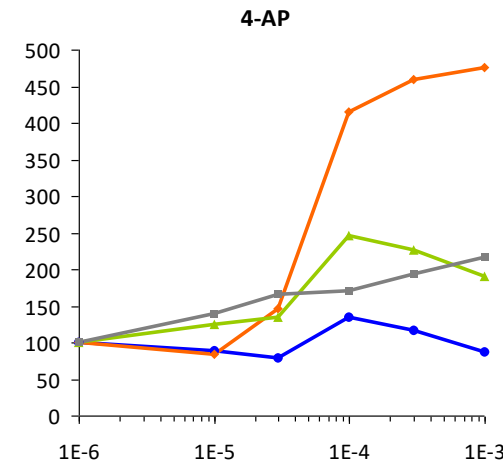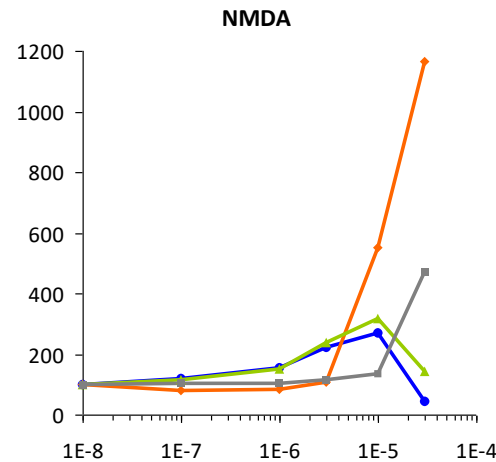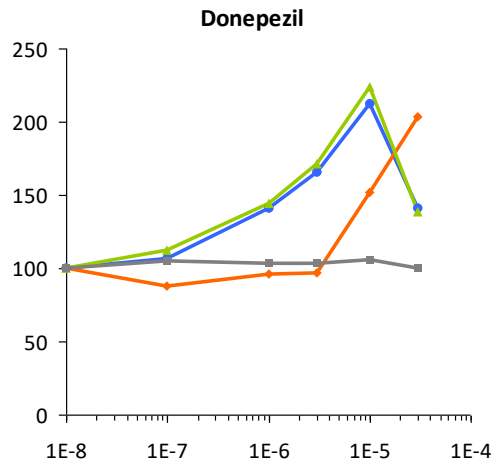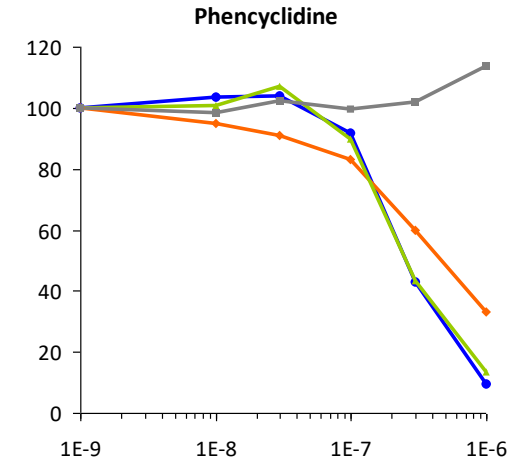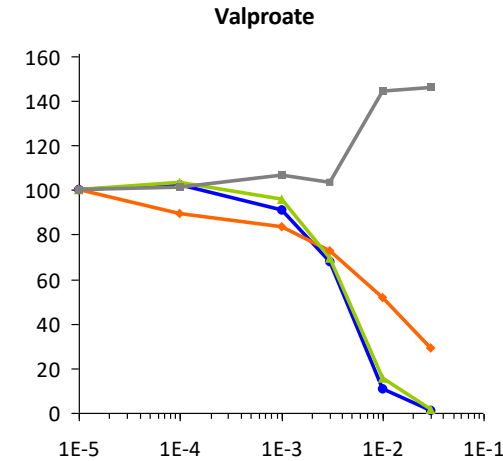
Charts are % control — general activity: Spike rate (SR) — burst structure: Burst duration — regularity over time: SR SD — synchronization: SR CVnet

Antidepressants, Neuroleptics

Amisulpride
Amitriptyline
Aripiprazole
Chlorpromazine
Clozapine
Duloxetine
Fluoxetine
Haloperidol
Lithium chloride
Nortriptyline
Olanzapine
Quetiapine
Risperidone
Sertraline

Anesthetics, Analgesics

Acetaminophen
Acetylsalicylic acid
Ibuprofen
Ketamine
L-Polamidone
Morphine
Propofol
Sufentanil

Anticonvulsants, Sedatives

Carbamazepine
Clobazam
Clonazepam
Diazepam
Gabapentin
Flunitrazepam
Lamotrigine
Levetiracetam
Phenobarbital
Phenytoin
Pregabalin
Thiopental
Valproate
Zolpidem

Cognition Enhancers

Caffeine
Donepezil
D-Cycloserine
Galanthamine
Memantine
Modafinil
Piracetam

GABAA Receptor

Bicuculline
DS1
DS2
Etomidate
Flumazenil
GABA
L-655,708
Methaqualone
Muscimol
Pentylenetetrazole
Picrotoxin
Propofol
SB-205384
Thio-THIP
THIP
Xli 093

GABAB Receptor

Baclofen
CGP 7930
GS 39783
SCH 50911
SKF 97541

Glutamate Receptor

AMPA
LY-341,495
LY-354,740
LY-393,558
MK 801
NBQX
NMDA

Acetyl Choline System

Atropine methyl bromide
Epibatidine
Eserine
Ipratropium
Oxotremorine
Pilocarpine

Dopamine System

Apomorphine
Dopamine
GBR 12935
Indatraline

Serotonine and somatostatin receptors

Cp 93129
MK-4256
NAD 299
Ondansetron
Palonosetron
Segletide
WAY 100 635

Opioid System

DPDPE
Dermorphine
Endomorphin I + II
Enkephaline
Nalorphine
Naloxone
L-Polamidone

Gap Junctions

Carbenoxolone
Mefloquine
1-Octanol
Sodium Propionate

Frontal Cortex
Release: 06/2020
Clinical drugs

Growth factors

BDNF
Nerve growth factor (NGF)
GDNF

Solvents

DMSO
Ethanol
Cyclodextrine

Sugars

Saccharin
D-sorbitol
Aspartam

Others

Agmatine
Benzoquinone
Chlorpyrifos Oxon
Clonidine
Corticosterone
Cortisol
Cyclosporin A
DOI
Glyphosate
K252a
Mefloquine
Neuropeptide Y
Neurotensin
Orexin A
Ouabain
PCP
Pilocarpine
Tacrolimus
Tetrodotoxin
Wortmannin

Complete concentration-response „fingerprints" available

Pattern recognition allows similarity analysis between novel compounds and known reference compounds

Pattern recognition returns a similarity matrix, i.e. two substances with similar effects have a greater similarity index.

	AMPA	Baclofen	CGP-7930	Clozapine	Fluoxetine	GABA	GS-3978	Indatraline	LY 341,495	LY 354,740	NBQX	NMDA	Olanzapine	SCH 50911	SKF-97541
AMPA															
Baclofen	1														
CGP-7930	8	0													
Clozapine	4	2	9												
Fluoxetine	7	5	6	26											
GABA	7	3	10	3	1										
GS-39783	8	3	12	6	7	10									
Indatraline	7	1	11	11	8	4	14								
LY 341,495	2	3	5	9	16	6	7	16							
LY 354,740	1	13	7	3	5	16	11	6	8						
NBQX	3	6	2	11	6	8	2	7	3	21					
NMDA	19	0	7	5	9	5	4	1	10	4	3				
Olanzapine	11	1	9	18	14	2	7	26	11	3	1	7			
SCH 50911	1	0	11	1	2	21	7	6	18	7	1	11	4		
SKF-97541	2	41	6	9	6	5	11	7	1	10	6	0	8	2	

Above: 2D representation of the similitarities using a phylogenetic tree. Similar substances are grouped, agonists are opposite their antagonists.

Assay and Readout	Application	Cell Culture
Primary Screening		
Compound characterization	Neuroactivity	Mouse, human
EC50, Hill coefficient	Potency comparison	Mouse, human
Database comparison	Similarity/difference to marketed drugs	Mouse, human
	Toxicology	
Seizure		
Classification	HESI Neutox Assay for Safety Pharmacology	Mouse, human
Sedation		
Classification	Sedation score	Mouse
E/I Balance		
E/I Balance ratio	Validation of disease models	Mouse

Readout	Application	Cell Culture
Development		
Early Brain Development Index Classification	Delay and promotion of functional development	Mouse, human
iPSC neuron characterization		
Multivariate analysis, synchronization index	Similarity to mouse brain tissue culture	Human
	lot-comparison	
Combination		
Phenotypic multi-parametric compound characterization in combination matrix	Synergy	Mouse, human
	Additive	
Electrical stimulation		
Phenotypic multi-parametric compound characterization	Synaptic plasticity	Mouse, human
	Connectivity	

Model Type	Cell Culture
Alzheimer's Disease	
Amyloid beta addition	Mouse, human iPSC neurons
Streptozotocin addition	Mouse, human iPSC neurons
Parkinson's Disease	
MPP+ addition	Mouse, human dopa neurons
Alpha-synuclein induction with AAV	In Development
5-HT2A	
Depression, PTSD	Mouse, human iPSC neurons
Epilepsy	
4-AP addition	Mouse
Bicucullin addition	Mouse
Pentylene tetrazole addition	Mouse
Developmental model	Mouse
Pruritus and Neuropathic Pain	
Perturbation model	Mouse – sensory neurons

Model Type	Cell Culture
Bipolar disorder	
Ouabain addition	Mouse
Schizophrenia	
Ketamine addition	Mouse
PCP addition	Mouse
Developmental model	Mouse, human iPSC neurons
Tachycardia	
Cardiomyocyte	In Development
Patient material and isogenic control	Human iPSC neurons
Spinal Muscular Atrophy	
Patient material, isogenic control	Human iPSC motor neurons
ALS	
Patient material, healthy control	Human iPSC motor neurons
Fragile X Syndrome	
Patient material	Human iPSC neurons

NeuroProof offers full technology access to CNS related projects from start to market as Fee for Service or integrated projects on a Full Time Equivalent business.
We provide complete support for every stage - from research to clinical.

- Specialized CRO partner for neuro-pharmacology and nutrition

- More than 20 years of experience with MEA technology, highly qualified experts

- A leader in quality standards, cell culture applications and data analysis

- Our USP: Proprietary data analysis platform including artificial intelligence
  (e.g. classification of compounds, side effect prediction, disease scores, deep learning methods)

- Established CNS assays and experience in assays development
  (for neurodegenerative, psychotic and rare disorders)

- Experienced partner in hiPSC neuron MEA screening
  (associated with IMI-EBISC and StemBancc, screening of numerous hiPSC neuron lines)

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Thank you!

We appreciate your interest in this presentation.

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