



Drug safety and discovery with Human iPSC-Neuron and Artificial Intelligence

TechnoPro Inc.
info-rd@technopro.com
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Research services: Biotechnology and chemistry
 Research centers: Tokyo area - Kashiwa, Saitama, Chiba
 Shonan iPark for office
 Osaka area - Kobe, Kyoto

Established: October, 2014

Leadership:

- Gaku Shimaoka: President and Representative Director, TechnoPro Inc.
- Masami Hayafune: Representative director, TechnoPro R&D Company, TechnoPro Inc.

Gene/Cell

Expression vector
transduced cell
line, gene analysis

Protein

Expression of
recombinant protein
Protein identification
Enzyme assay

Drug screening

System validation
Biochemical assay
Cell-based assay

Cell culture

Toxicology
Skin-Lightening,
Moisturizing, Sebum

Material

compound,
polymer synthesis

Peptide

synthesis,
bio-activity assay

Analytical chemistry

Animal models

■ Neuroscience and Drug Discovery in public private partnership

- Revealing neural function in drug discovery
with integrated research of neuroscience and electronics



Neuroscience and Drug Discovery

MEA assessment system and applications with ;

- **iPS cell-neuron**, human
- **MEA** : Microelectrode array for detection of extracellular action potential
- **AI** : Proprietary Artificial intelligence and multivariate analysis for toxicity and pharmacology

(1) Drug phenotypic screening

- Toxicity and efficacy
- Phenotypic select ➡ Prioritization for lead compounds

(2) Mechanism of action

- Ion channels and receptors for target molecule on neurons
- Assessment with AI and multivariate analysis
- Disease model assay (MEA, qPCR, Immunostaining)

(3) Pain study and neuromuscular disease

- Human iPSC-sensory/motor neuron
- Ion channels and receptors assessment and diseases models

(4) Relativity to animal study

- Solid and tangible assessment in vitro for the relativity
- Evidence for toxicity in human cells but no rodent



**Pharmaceuticals
Biotech
Academia
Research
Institute
Food, Agricultural**

Platform Technology based on Reports (Selected)

■ **CNS : Seizure, Addiction**

- Principal Component Analysis to Distinguish Seizure Liability of Drugs in Human iPSC-Cell-Derived Neurons (Toxicological Sciences, 184:265, 2021)
- Can we panelize seizure? (Toxicological Sciences, 179:3, 2021)
- Toxicological evaluation of convulsant and anticonvulsant drugs in human induced pluripotent stem cell-derived cortical neuronal networks using an MEA system (Scientific Reports, 8:10416, 2018)
- Evaluation of drug addiction using human iPSC-dopamine neuron (Japanese Society of Toxicology, 2022)

■ **Sensory neuron: Pain assessment**

- In vitro pain assay using human iPSC-derived sensory neurons and microelectrode array (Toxicological Sciences, 188:131, 2022)

■ **Motor neuron: Neuromuscular disease** (In preparation for publication)

- ALS and related diseases models with human iPSC-motor neurons
- Efficacy for mutated neurons by gene-editing of a disease gene
- Inhibition effect for accumulation of mutated proteins in patient motor neurons

■ **Axon propagation : Electrical footprint** Versatile live-cell activity analysis platform for characterization of neuronal dynamics at single-cell and network level (Nature Communications, 11:4854, 2020)

■ **Artificial intelligence** : Raster plots machine learning to predict the seizure liability of drugs and to identify drugs (Scientific Reports, 12:2281, 2022)

■ **Drug response** : Physiological maturation and drug responses of human induced pluripotent stem cell-derived cortical neuronal networks in long-term culture (Scientific Reports, 6:26181, 2016)



■ Cell Culture

Analysis

Record

Culture

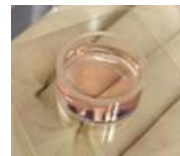
Neural network (CNS)

Cell	CNS	Sensory/Moto
Human iPSC-neuron	✓	✓
Rodent, primary	✓	✓

- Co-culture of iPSC-neuron with astrocyte
- Selected human iPSC-neuron from 50+ neuron lines
- Optimized iPSC-neuron balance at excitation-inhibition ratio

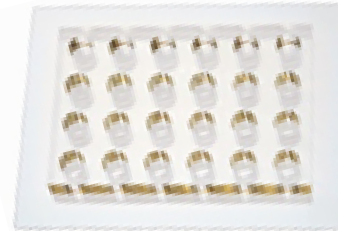


26400 electrodes

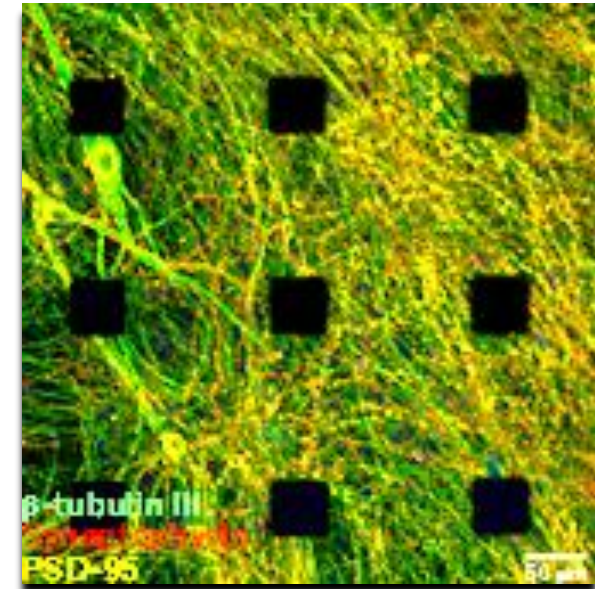


64electrodes

24-well/384 electrodes
(16 electrodes/well)



Modality: Small Molecule,
Oligonucleotide, Antibody,
Protein, Cell, Tissue



G Tubulin beta III/Tuj1, Neuron
R Synaptophysin, Presynaptic vesicles
Y PSD-95, Synaptic region



■ Recording of Response

Analysis

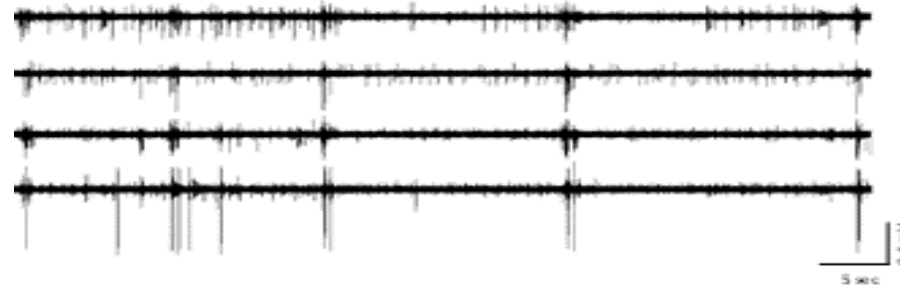
Record

Culture

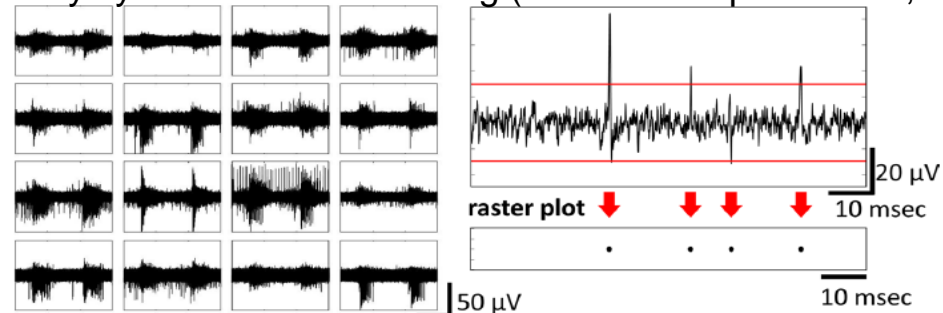
- Measurement of extracellular **action potential** in neural network and sensory/motor neurons in vitro by the MEA **system** adding test compounds
- Recording **spikes** and synchronized **burst** firings

Recording Action potential of ion influx on an extracellular field

Typical action potential waveform at electrodes, and periodically synchronized burst firing



Periodically synchronized burst firing (Scientific Reports 2022, 12:2281)



■ Analysis 1: Action potential



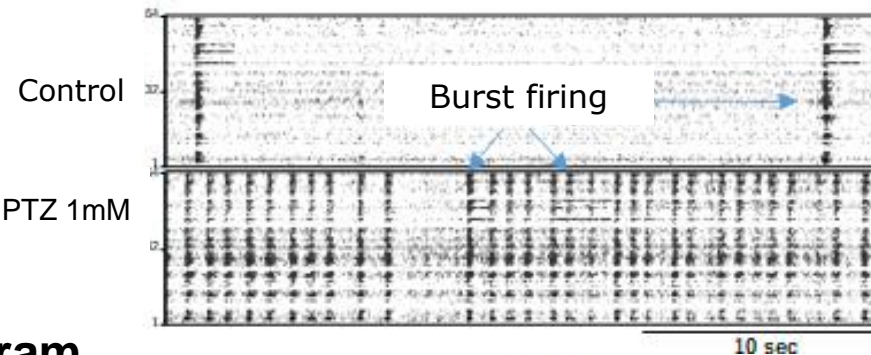
Analysis
1

Record

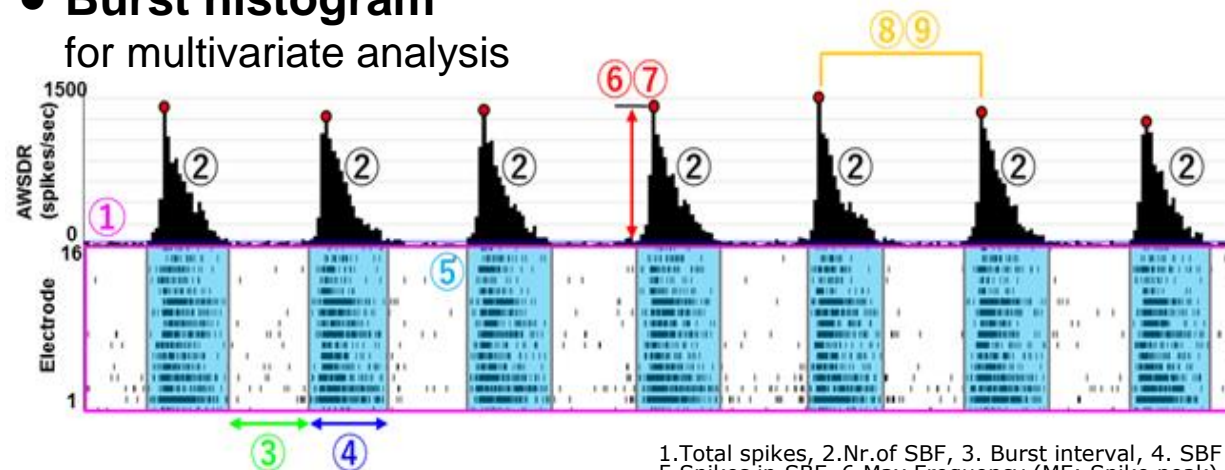
Culture

- **Raster plot** generated by spikes data ➡ Visualization of neural response
- **Burst histogram** ➡ Detection of neural activity
- Extracted effective **parameters** from 90+ for analysis of test compound profiles (Fine evaluation completed for physiologically active substances over 200)

- **Raster plot**
for AI analysis



- **Burst histogram**
for multivariate analysis



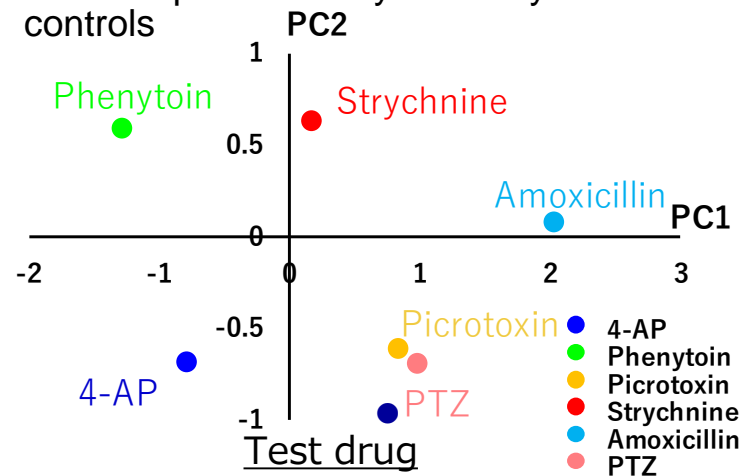
1.Total spikes, 2.Nr.of SBF, 3. Burst interval, 4. SBF Duration, 5.Spikes in SBF, 6.Max Frequency (MF; Spike peak), 7. CV coefficient of variation of MF, 8.Inter MF Interval (IMFI), 9.CV of IMFI

■ Analysis 2: Multivariate Analysis

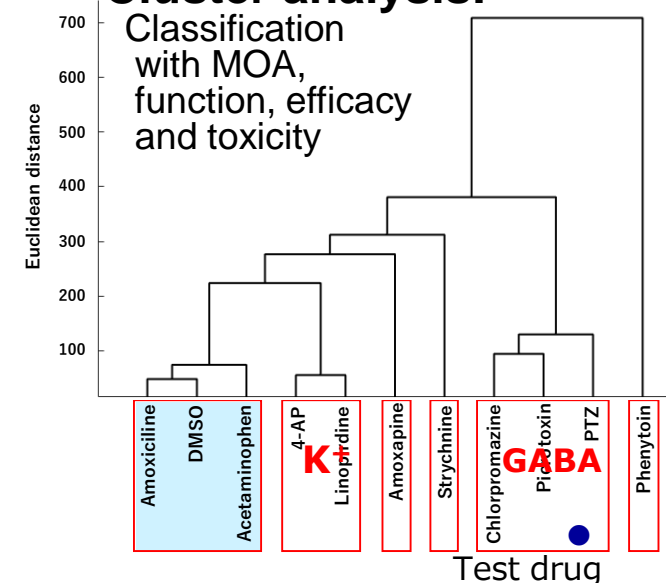
- **PCA**, Principal component analysis,
- **Cluster analysis**,
 - are performed using parameter sets extracted from statistically significant 90+ parameters of burst histogram.

- Phenotypic **screening**
- **Toxicity** assessment
- Prediction of targeting **ion channels and receptors**
- **Prioritization** of compounds
- **Relativity** between human and rodent

PCA is performed by similarity to +ve controls



Cluster analysis:



■ Analysis 3: Artificial Intelligence



Analysis
1,2,3

Record

Culture

【Deep learning】

- Image recognition with raster plot of positive controls
- Weighted pattern recognition extracting feature value
- The AI is created to pile up multi-layers of algorithm.

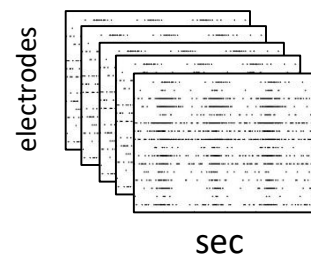
【Identification】

The AI identifies/classifies feature value between control and test compounds to toxicity risk and efficacy.

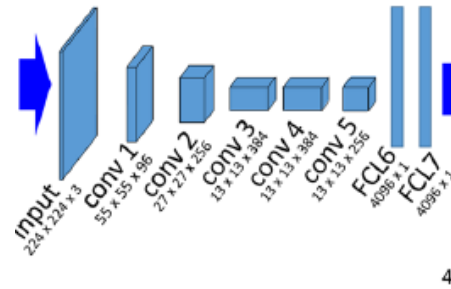
【Advantages】

- A wide range of accurate identification with 4000+ parameters.
- Hi-sensitive evaluation in weak neural firing.

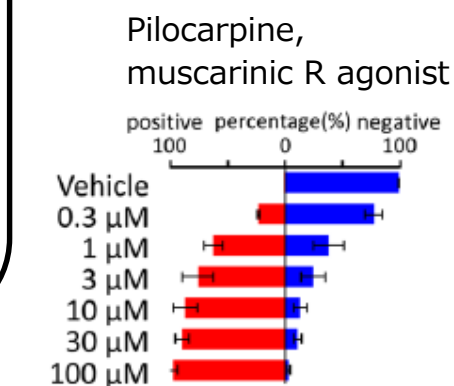
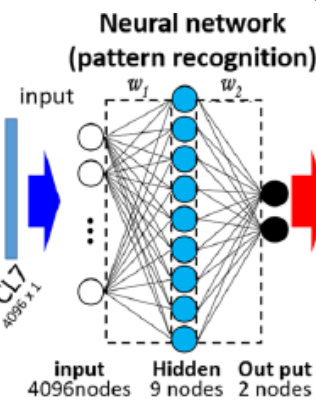
1. Separated spike stamp table



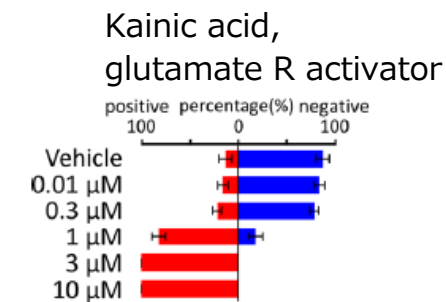
2. Image Recognition (image recognition)



3. Pattern Recognition



4. Identification





info-rd@technopro.com
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[lineup/pharmacological-evaluation/](http://www.technopro.com/rd/services/contract/lineup/pharmacological-evaluation/)