CNS Capabilities at Axcelead

Axcelead Drug Discovery Partners, Inc.

May 2024

Overview of Axcelead capability in CNS

Drug discovery phase	Target identification	Lead generation	Lead optimization	Study for IND filing	Study for NDA
Biology	☐Pharmacological validation of concept (Proof of concept)	☐Evaluation of lead compounds on target molecule and / or cells (in vitro screening)	☐Evaluation of compounds on target molecule and in disease model in vivo (in vivo/ex-vivo screening, Target engagement, efficacy study)	☐Pharmacological evaluation of candidate on disease model ☐Differentiate from competitor(s) (Create pharmacological data package for IND/NDA filing)	

Target ID / validation

Multi-Omics analysis

BI analysis

Flow cytometry

Phenotypic screening

snRNA-seq/ VISIUM

***In vitro* assay**

iPS cell/ primary cell/ cell line

Gene KO/KI by CRISPR

Second messenger

Downstream signaling (gene/protein expression)

Enzyme assay

***In vivo* assay**

Motor function

Cognitive function

Psychiatric-disease related model

Microdyalysis

Electroencephalogram (EEG)

Translational research

PK/ PD/ efficacy

Tg/KO mouse

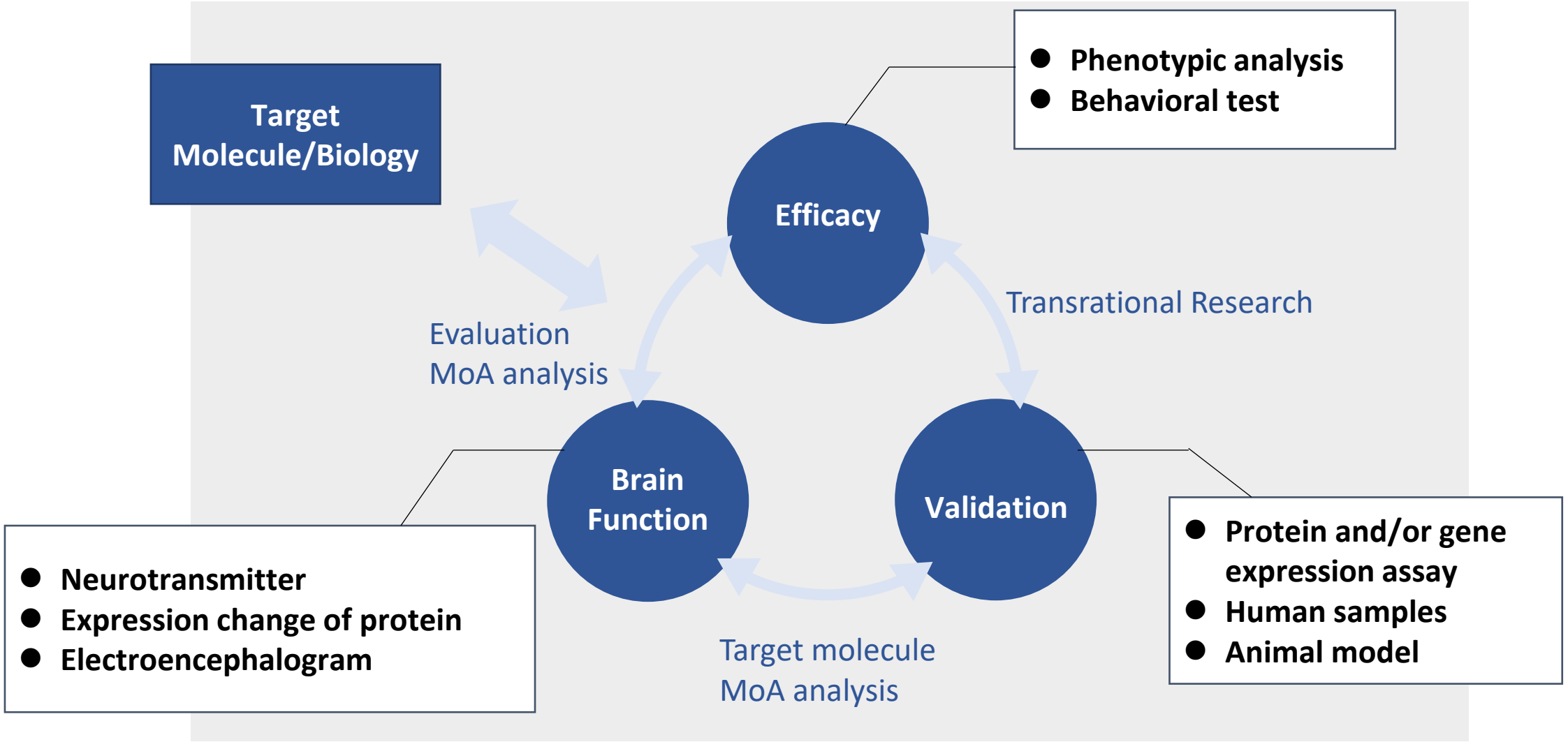
Biomarker analysis

Assay w clinical samples (CSF)

■ **Our strength is a variety of capabilities cultivated in small molecule drug discovery**

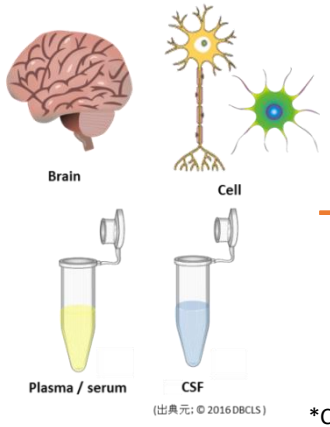
✓ **And now, we are expanding our capability for new modality in CNS including.**

Strong Expertise and Capabilities in CNS field



Overview of Axcelead capability in CNS

Using valuable and rare human samples, validation studies such as MOA analysis can be conducted with following methods.

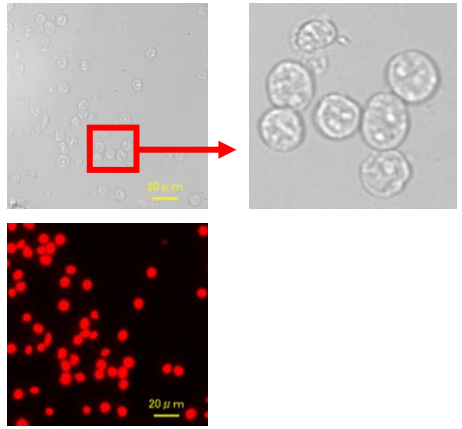


*CSF; cerebrospinal fluid

Plasma, CSF*, Brain/spinal cord sampling

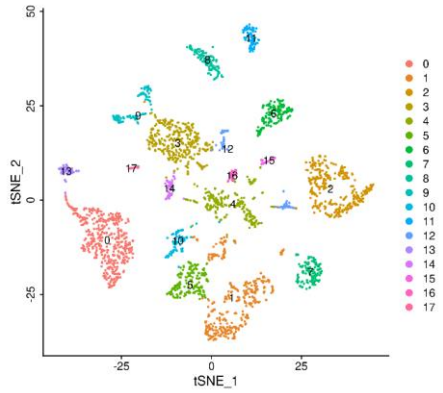
- **Single cell isolation of tissue**
  - **Flow cytometry**
    - Profiling of specific cells, MoA analysis
  - **Single cell / nucleus RNA-seq**
    - Gene expression analysis in specific cells / nuclei, MoA analysis
- **Gene expression / RNA-seq**
- **Omics analysis**
- **IHC / visium**
  - Spatial analysis of protein / gene expression

【Single nucleus RNA-seq】



Obtaining nuclei from frozen brain tissue

Library preparation NGS



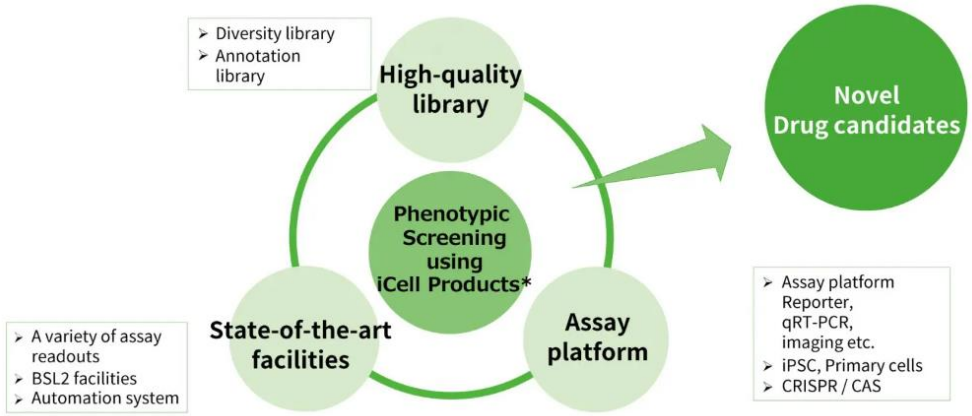
Visualization of cell subpopulations in brain tissue

- Single-nuclei RNA sequencing in human and mice brain
- Bioinformatics

iPS Cell: Target validation, phenotypic screening

iCell® and iCell-DDP® cells

Based on the scientific experience of Axcelead DDP and FCDI collaboration, we offer a wide selection of phenotypic screening services incorporating iCell® products.

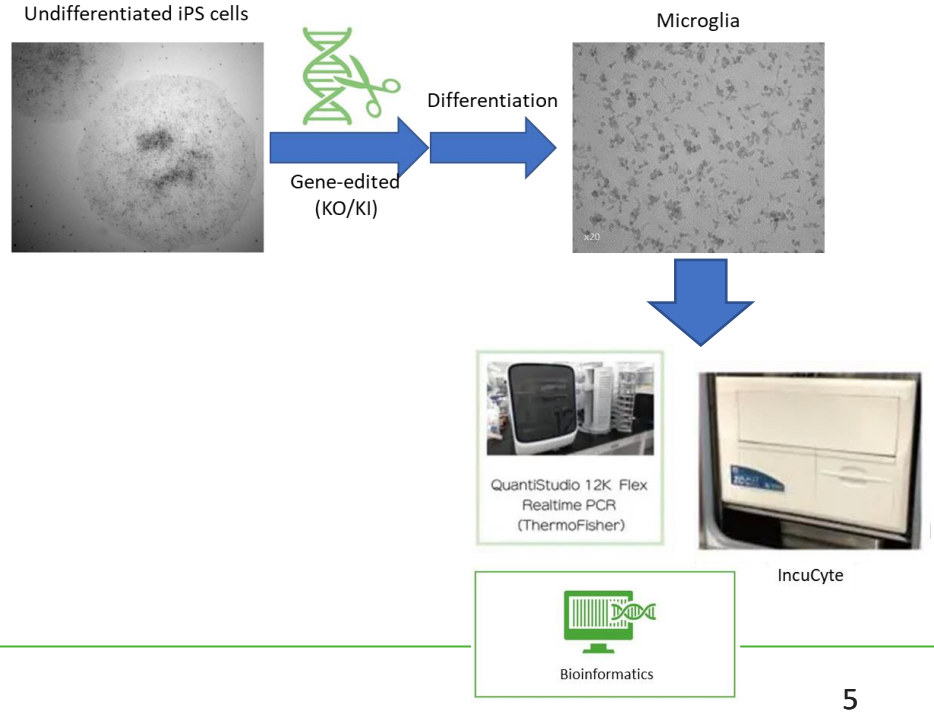


Gene-edited iPS cells

Can establish gene-edited iPS cells, differentiate them into various cells, and perform functional assay.

Examples

- Exploring correlation between phenotypes and change in genes of interest => target validation
- Established cells by introduction of gene mutation reported in patients => cell models



- Cell differentiation in house (microglia, neuron etc.)
- Collaboration with ORIZURU Therapeutic Inc.* (motor neuron, skeletal muscle etc.)

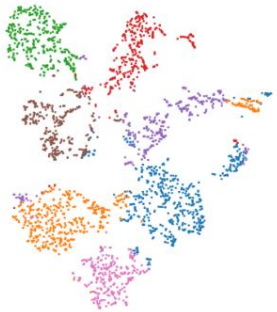
*This company is a spinout from Takeda Pharmaceuticals and Kyoto University, focusing on the development of iPS cells technology-based regenerative medicine.

Discovery of biomarkers

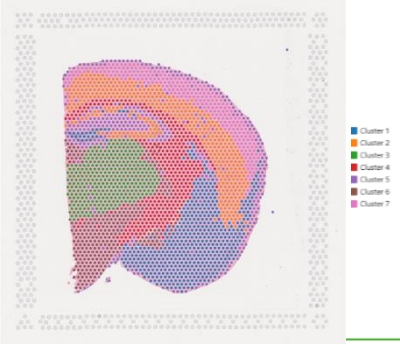
Spatial transcriptomics

Enables transcriptome analysis from a new perspective by obtaining comprehensive gene expression data associated with tissues/cells localized and structure information on tissue specimens.

Morphological context

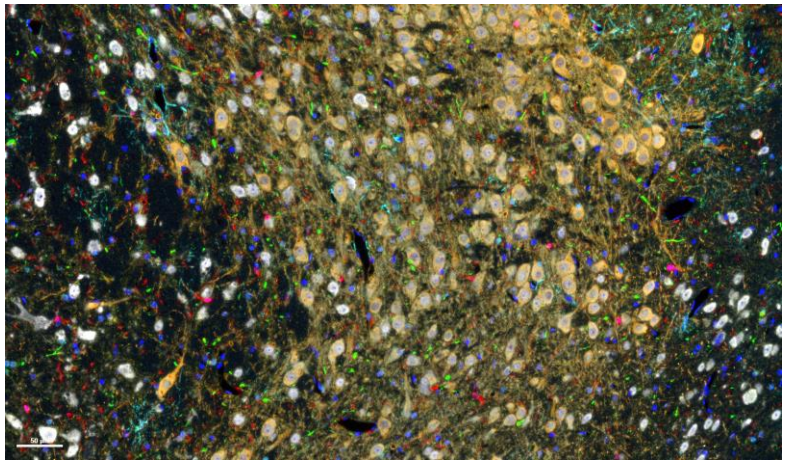


Cell subpopulation (NGS)



Multiplex-IHC

Enables simultaneous visualization of up to 6 targets such as various immune cells that exist in tissues. By performing image analysis on the entire tissue section, the microenvironment in the brain at the single-cell level can be elucidated.



1. GFAP
2. phospho-α-Synuclein
3. p62
4. TH
5. Iba-1
6. NeuN

Cell phenotyping and **Analysis of localization of phosphorylated α-synuclein** in mouse brain substantia nigra

Analysis of brain functions

Measuring brain and neural activity to assess functional recovery from deficits

1. Microdialysis (mice/rat)

- Over time collection of interstitial fluid to evaluate neurotransmitter release and soluble protein

2. Electroencephalogram (EEG) (mice/rat)

- Neural activity can be evaluated by measuring electrical signals from the brain. E.g., sleep EEG

3. Compound Muscle Action Potential (CMAP) (mice)

- CMAP is the evoked potential in muscle by electrical stimulation to multiple motor nerves.

1. Microdialysis

We support your research by analyzing dynamic change of analyte concentrations at a specific site in brain that cannot be captured by brain tissue or CSF sampling,

Stereotaxic frame

Axcelead's Microdialysis allows to

- ✓ Collect samples in interstitial fluid over time from free moving animals (mouse, rat)

  →Enable evaluation of analyte concentrations without influence of anesthesia
- ✓ Monitor various molecules such as monoamine, amino acid, peptide, proteins etc.
- ✓ Monitor molecules of interest at a specific site in brain by precisely fixing probes using a stereotaxic frame.
- ✓ Simultaneously measure multiple molecules by LC/MS

Microdialysis system

2. Electroencephalogram(EEG)

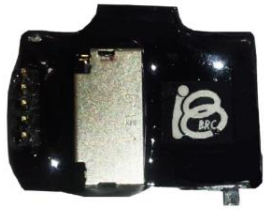
We strongly support your research by monitoring neural activity in real time by EEG.

Axcelead's EEG allows to

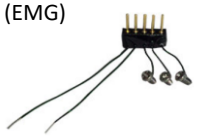
- ✓ Monitor the neural activity over time (~48 h)
- ✓ Record not only on the brain surface but also in targeted brain site such as the hippocampus.
- ✓ Analyze the sleep stage and circadian rhythms using recorded EEG.

ELG-2 EEG LOGGRER-2 **EEG LOGGER**

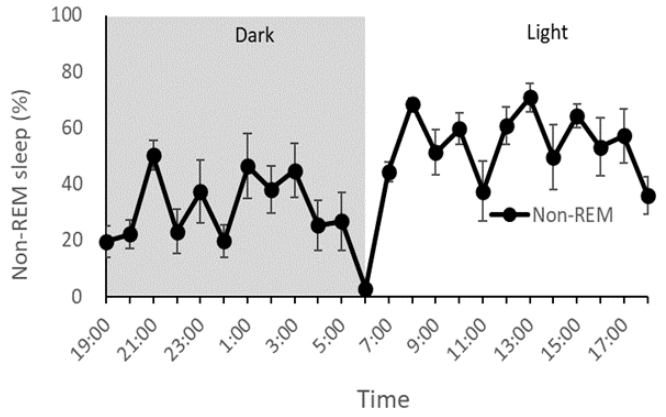
Body



Electrode： Electroencephalogram (EEG)、electromyogram (EMG)

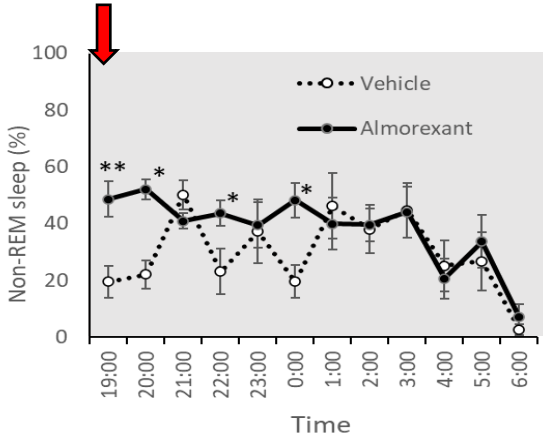


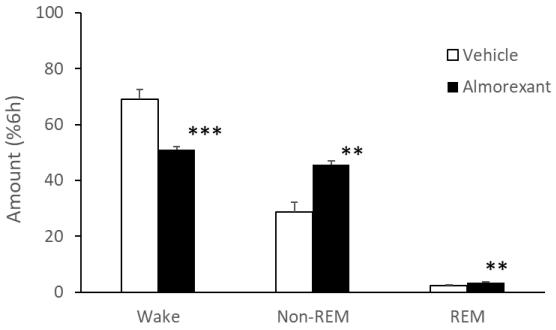
Non-REM sleep (Circadian rhythm)



Almorexant

Non-REM sleep (almorexant 100 mg/kg)

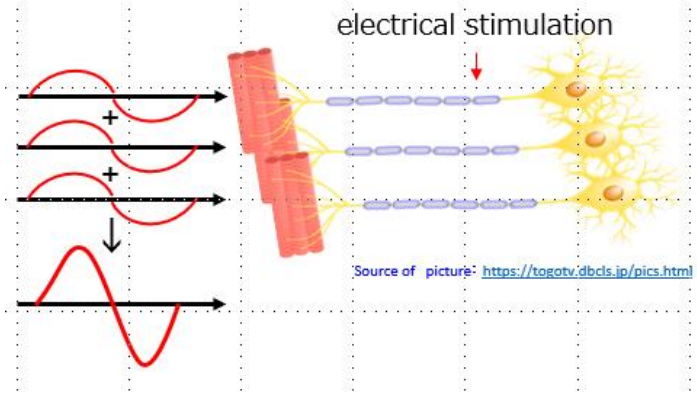




Mean ±S.E.M. (n=6). **p< 0.01, ***p< 0.001, vs Vehicle group by paired t-test.

3. Compound Muscle Action Potential (CMAP)

Time dependent neurotransmission, functional and morphological changes can be evaluated in a single study protocol.(mice)



Axcelead's CMAP allows to

- ✓ Evaluate the motor neurophathy in the lower motor neurons over time.
- ✓ Detect the functional changes before morphological changes observed such as muscle weakness and muscle atrophy.
- ✓ Improve the clinical translatability, because CMAP is used for diagnosis and prognosis in neuromuscular disease such as amyotrophic lateral sclerosis (ALS) in the clinic.

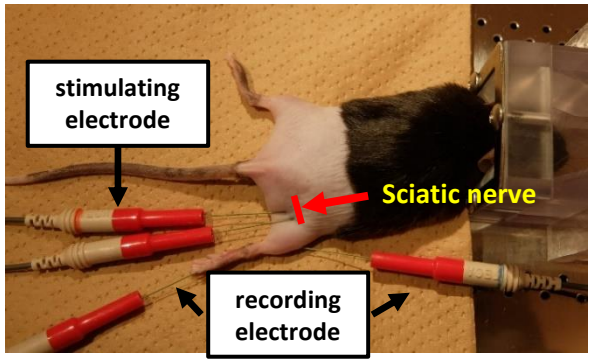
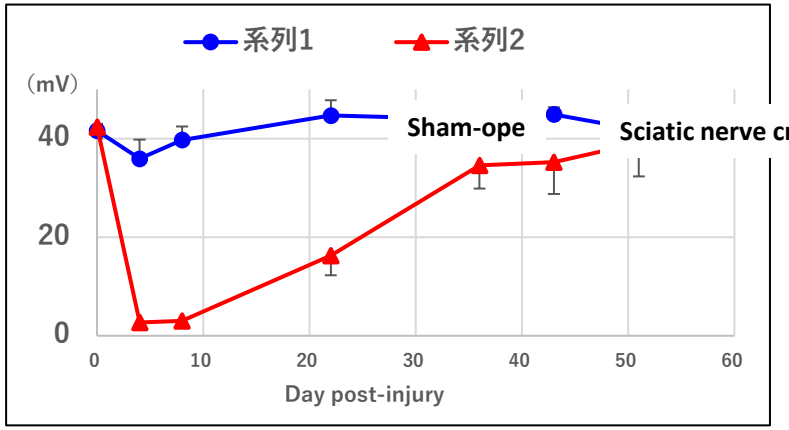


Figure Positioning of the electrodes for CMAP measurements.



Data is Time course of Compound Muscle Action Potential (CMAP) following sciatic nerve crush.(n=5)

Animal models/behavioral test

Animal model

Schizophrenia, Parkinson, autism, MS, cognitive dysfunction model etc.

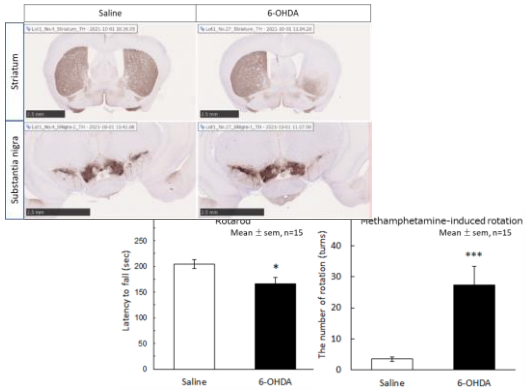
Modality

Small molecule, Antibody, peptide, ASO

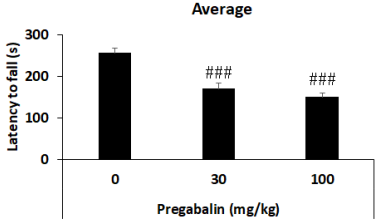
Behavioral test

Motor function, cognitive function, sociability etc.

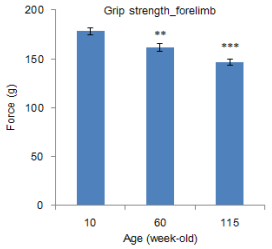
6-OHDA model (mice)



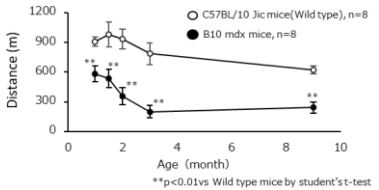
Rotarod (Motor coordination) (mice/rat)



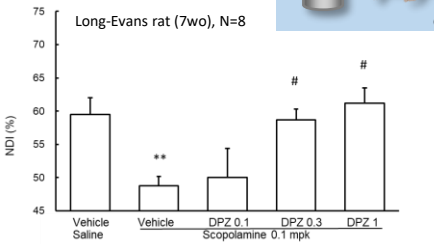
Grip strength test (mice/rat)



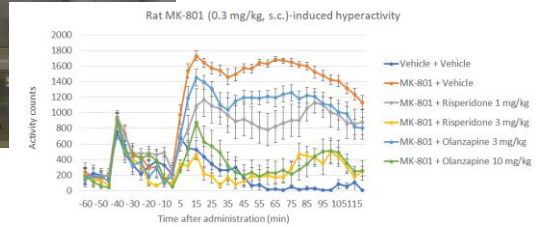
Treadmill test (mice)



Novel object recognition test (NORT) (mice/rat)

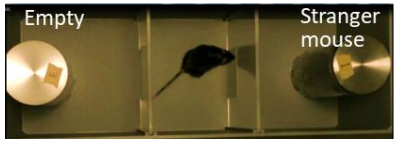


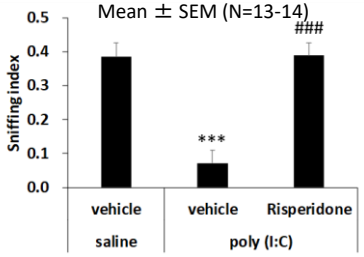
Locomo.tor Activity (mice/rat)



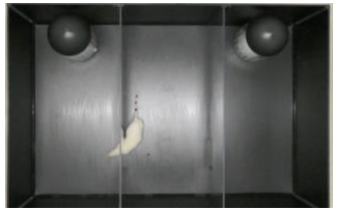
3-chamber Social Interaction test

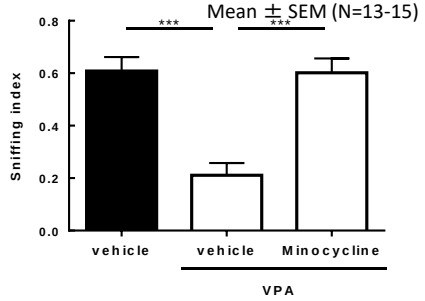
Mice (poly IC model)





Rat (Autism spectrum disorder model)



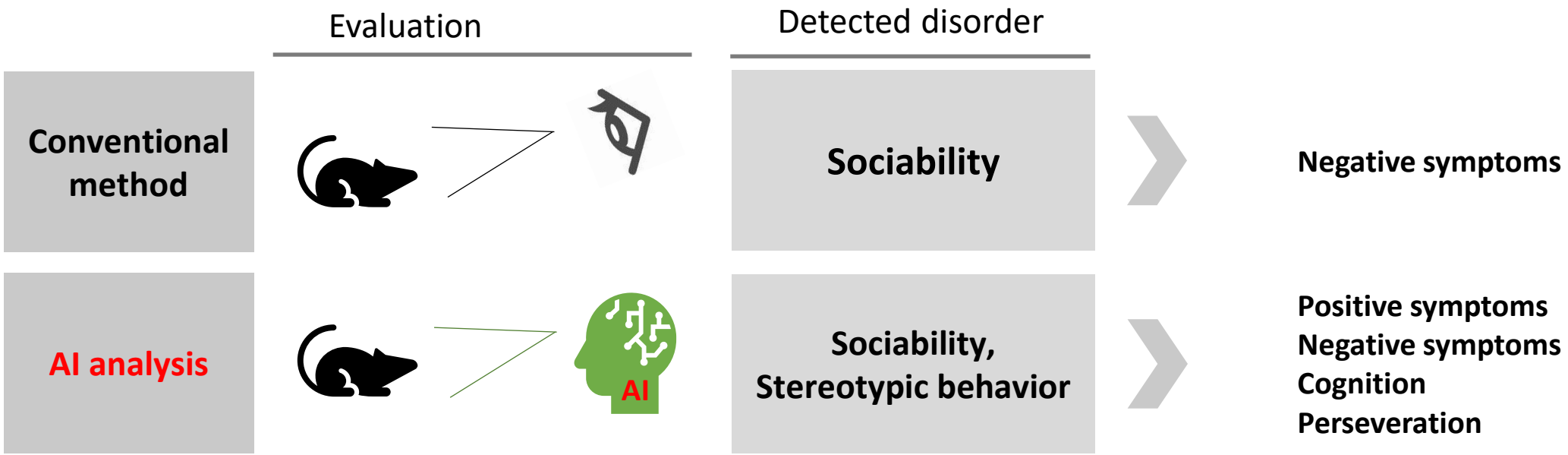


AI analysis of Animal Behavior

AI behavioral analysis makes it possible to evaluate behavioral changes that can not be visually undetected by human eyes. It could unveil novel behavioral abnormalities and effects of drugs (efficacy and safety signals).

- Improvement of clinical translatability
- Discovery of new therapeutic hypotheses and novel targets
- Discovery of potential actions of drug candidates

e.g.) Detecting multiple parameters and then evaluating effects on multiple disorders in a schizophrenia model (collaborative research underway)



Summary

1. Validation of target/hypothesis

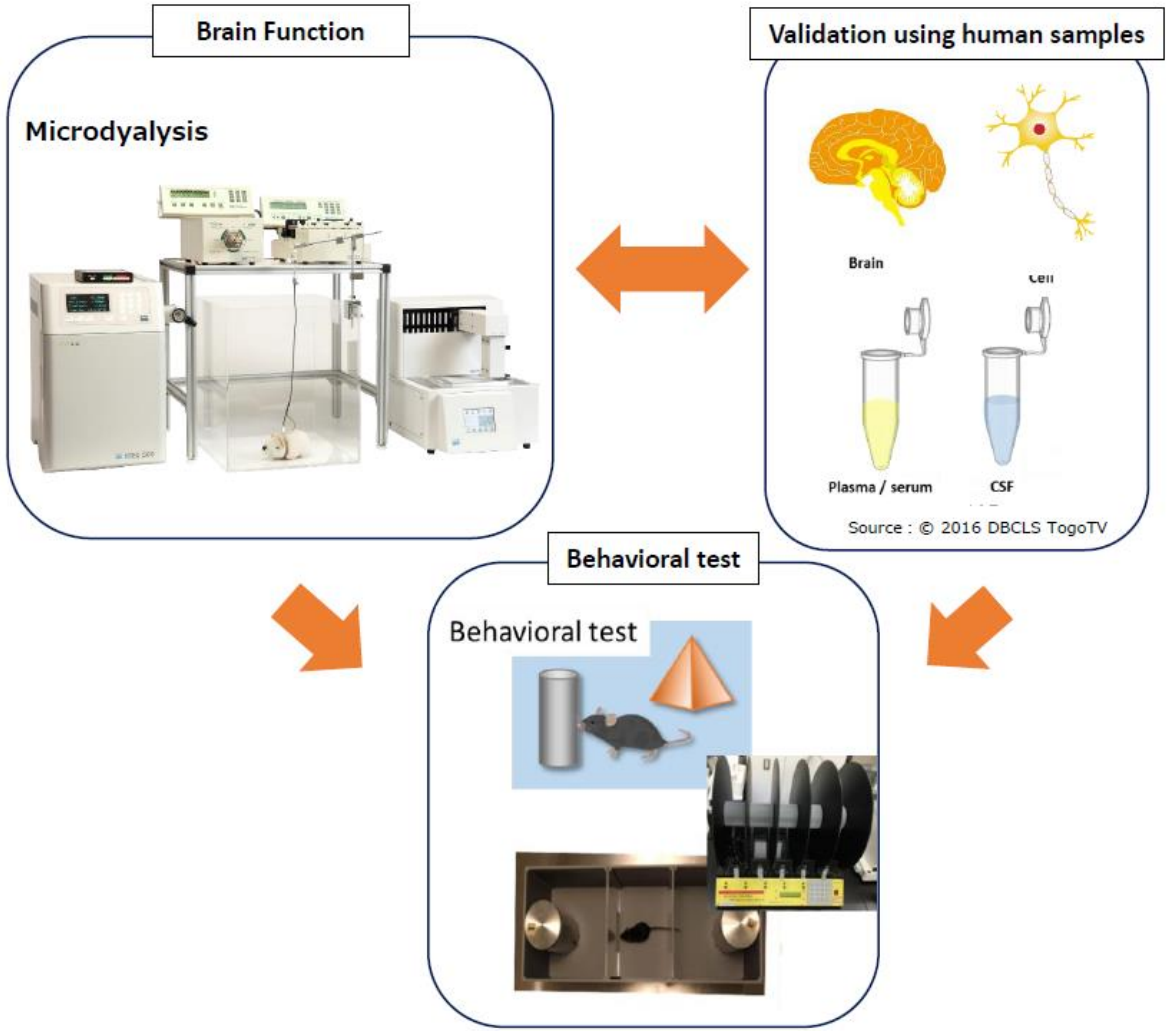
- Validation of hypothesis based on the change of gene and protein expression using iPS cells, patient-derived CSF, plasma, brain etc.
- Sampling of CSF, plasma, brain and cells from animal model and comparison the change of patients with that of animal model (Back translation)
- Biomarker identification by comprehensive analysis using multiomics analysis and bioinformatics

2. Brain Function

- Evaluation the potential of compound by neurotransmitter release linked to the brain function using microdialysis.
- Improve predictability in clinical trials by biomarkers etc.

3. Efficacy: Behavioral test

- Various behavioral tests that are essential to evaluate the efficacy of compound in CNS area
- Experience and knowledge that have been involved in the establishing various assay systems

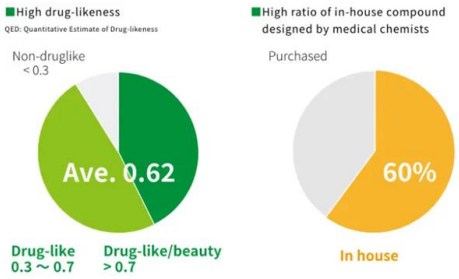


❖ **Our integrated service provides appropriate solutions to various issues in drug discovery research in CNS area.**

CNS Services Supported by Strong Screening and Chemistry Expertise

Target selection → Hit compound ID → Lead Gen. → Lead Opt. → Pre-clinical → IND enabling

1.5 million compounds



Pharma origin, huge, high-quality and diverse library

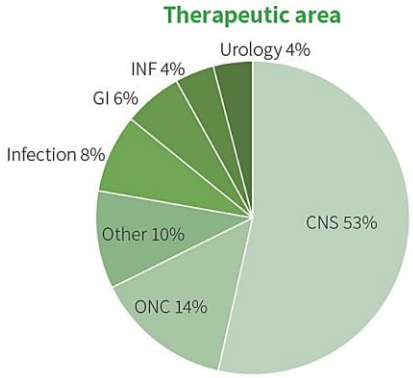
>700
Screening Experience

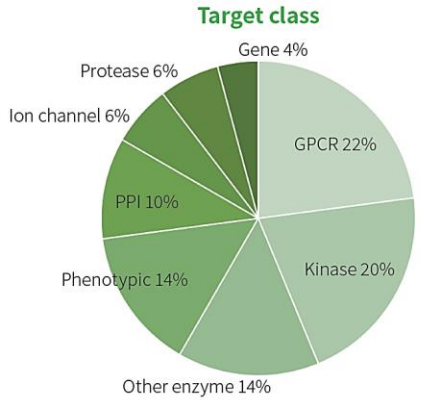
>90%
Hit Ratio of Screening

Proven track record

Superior medicinal chemist's expertise

Axcelead has been involved in many drug discovery PJs (LG/LO), covering major therapeutic areas and target classes.





>100
Successful IND complete

>20
New Drug Applications

- Full capability of Drug Discovery
- Pharma Scientists
- Knowledge Experience
- Legacy Data
- Brand-new Infrastructure

- ***Provide candidate compounds within 24 months***
- ***One-stop and all-in-one support***
- ***Avoid complicated coordination of CROs***

- ***Provide Eligible hit compounds for Lead Generation***
- ***Increase success possibility of drug discovery project***
- ***Minimize timeline for candidate identification***

- ***Achieve optimal IND with the fastest and minimum way***
- ***Address safety issue appropriately***

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AXCELEAD Drug Discovery Partners

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We value your concerns and questions!