Stanford Behavioral and Functional Neuroscience Laboratory (SBFNL)

https://neuroscience.stanford.edu/shared-resources/behavioral-functional-lab

Email: neurobehavior@stanford.edu

BFNL Facilities

- **Two locations on the Stanford campus:**

  - **SIM1 (Lokey Stem Cell Building) and Arastradero, providing testing, housing and laboratory space.**

  - **The center is operated by a team of experienced full time Staff Scientists and Research Assistants.**

SIM1 Building

Arastradero Building

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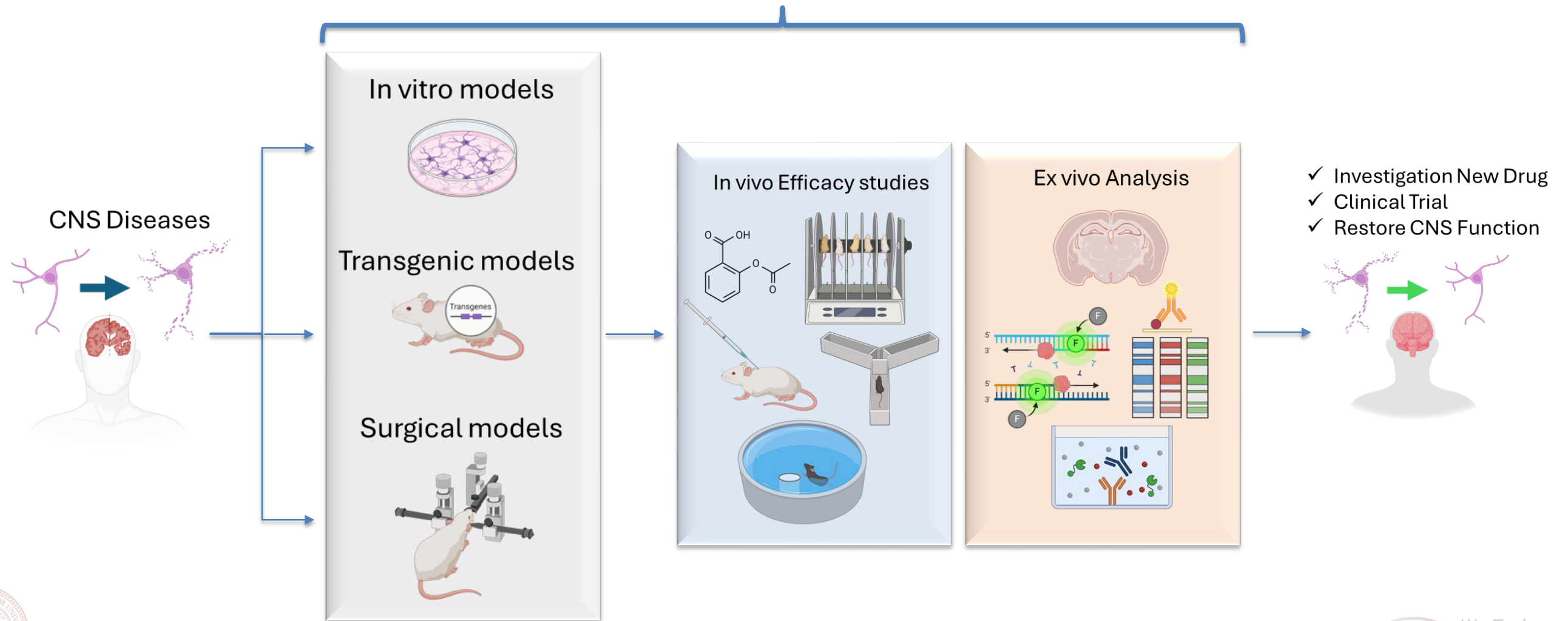
BFNL Facilities

- Over 20 procedure rooms

- 3 surgery rooms with 10 surgical stations and downdraft tables for tissue collection

- 3 dedicated colony rooms only for our users (Reversed/regular light cycle)

- Dedicated BSL2+ room for working with AAVs, Lenti, Alpha synuclein, Borrelia, etc

- Rodent behavioral testing equipment including Open Field Activity Chambers, Y-maze, Novel Place and Novel Object Recognition, Morris Water Maze, Fear Conditioning, Self-Administration Chambers, CatWalk Gait analysis, RotaRod and more...

- IncuCyte for live cell imaging, Cryostats, qPCR, WB, HPLC, Azure Biosystems for quantitative analysis of gels, blots, slides, tissue samples, Microplate Reader, Epifluorescence microscopy (EFM) and more....
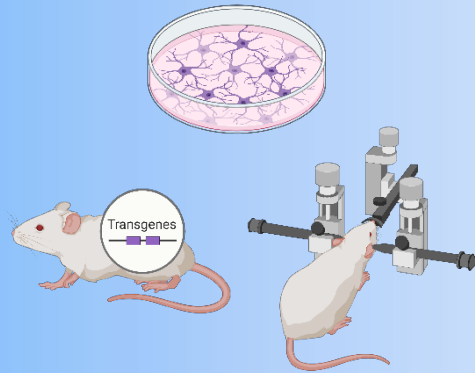
A community lab to serve the community- provide quality services and collaborations.

Behavioral & Functional Neuroscience Laboratory
(Translational studies for brain disorders and the target validation at cells and animal models)

CNS Diseases

In vitro models

Transgenic models

Transgenes

Surgical models

In vivo Efficacy studies

Ex vivo Analysis

✓ Investigation New Drug
✓ Clinical Trial
✓ Restore CNS Function

Stanford Behavioral and Functional Neuroscience Laboratory

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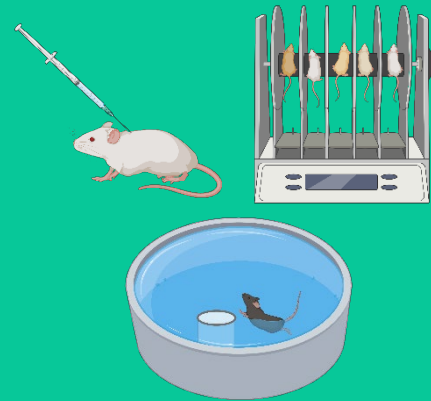Neurosciences Institute
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BFNL Services

Over 20 CNS disease models:

- Alzheimer's disease
- Huntington's disease
- Parkinson's disease
- Autism models
- Stroke models
- Down syndrome
- Pain
- Addiction
- Anxiety and depression
- Sleep disruption

**Over 100 *in vitro* and *in vivo* assays:**

- Synaptic degeneration
- IncuCyte- Live-cell imaging
- Sensorimotor Tests
- Learning and Memory Tests
- Social Interaction Tests
- Addiction Tests
- Anxiety and Depression Tests
- Nociception Test
- Toxicology, Pharmacokinetic & Pharmacodynamic
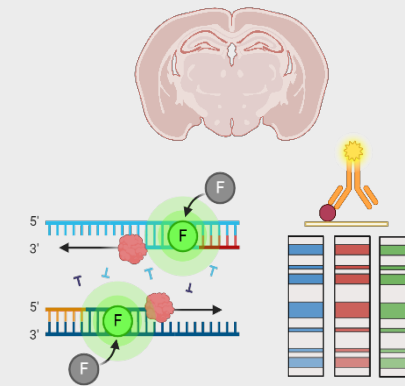
Ex vivo analysis:

- Histology
- IHC
- ELISA
- Western Blot
- iDISCO whole brain imaging
- HPLC
- PCR
- qPCR

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Morris Water Maze: Spatial Learning and Memory Test
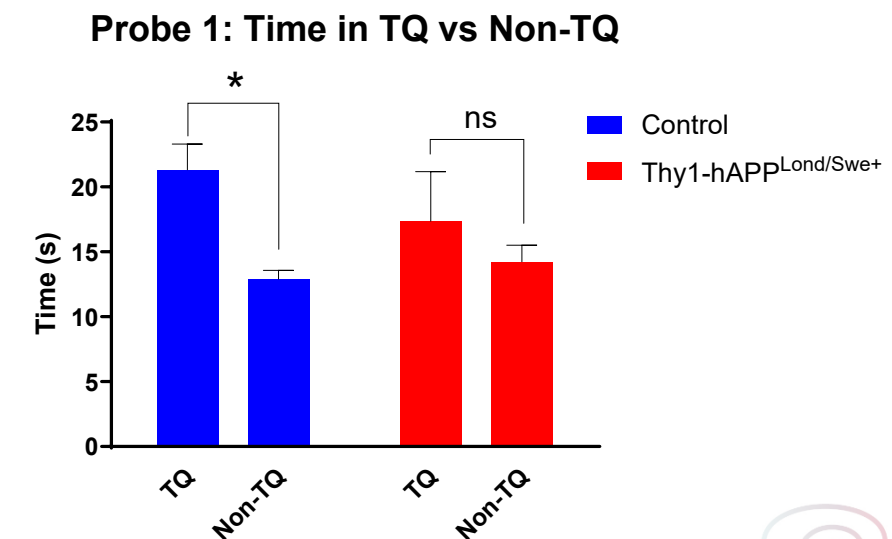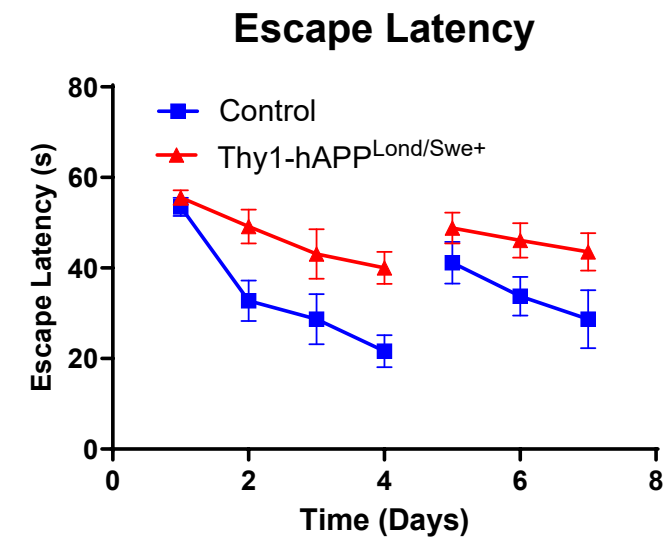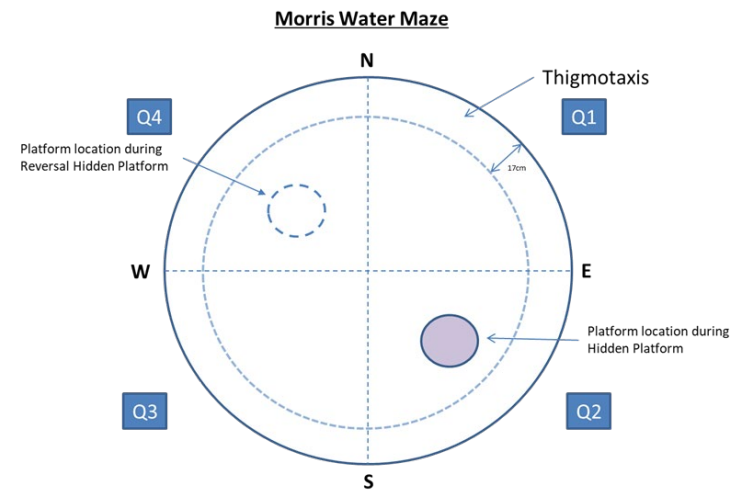
APP mice have amyloid pathology and reduced synaptic density in CA3 region of hippocampus

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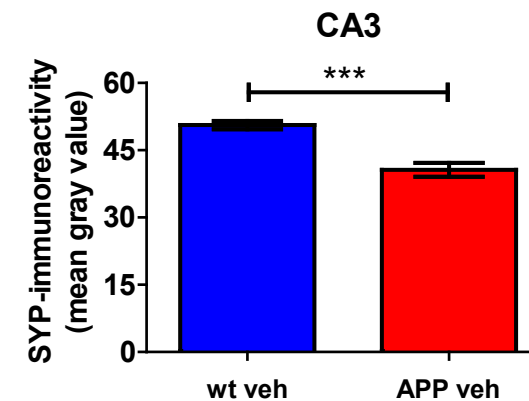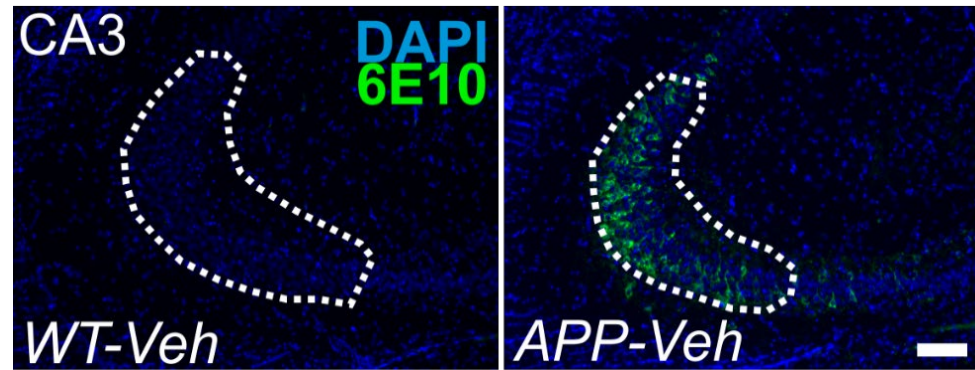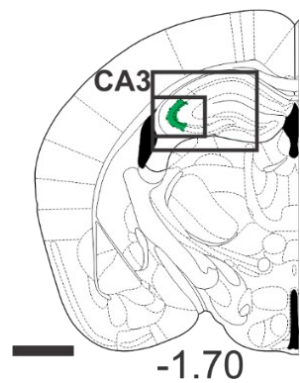
Amyloid Beta infusion for 4 weeks causes synaptic degeneration

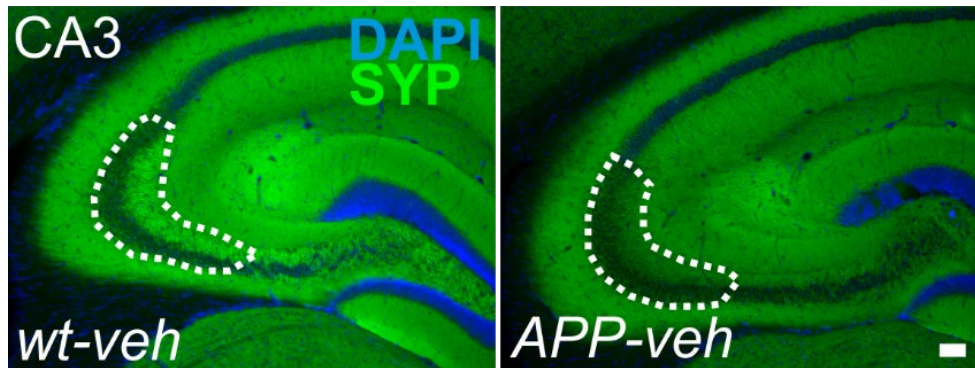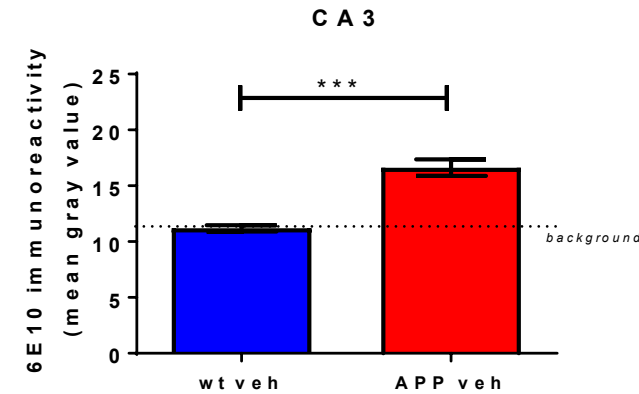

Alzet pump and Alzet Brain Infusion Kit
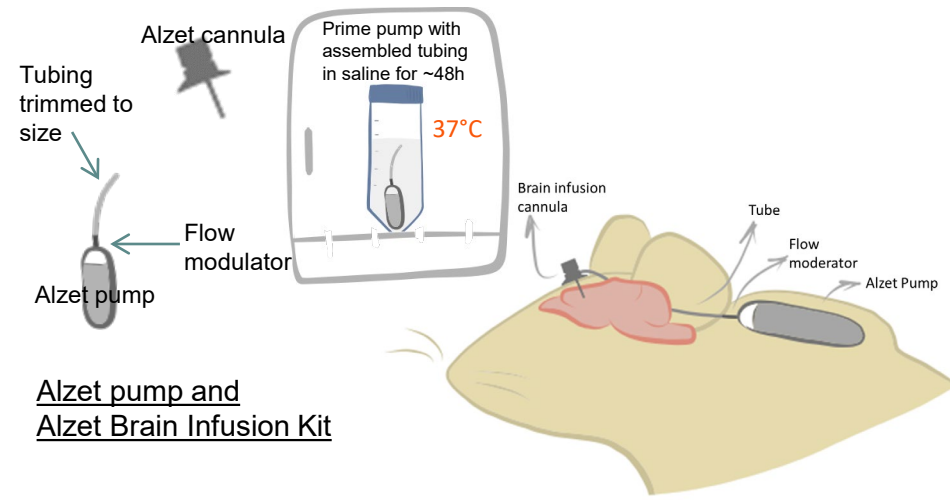
Mice with Aβ infusion have impaired Novel Object Recognition
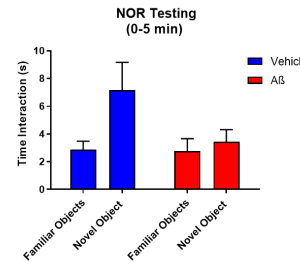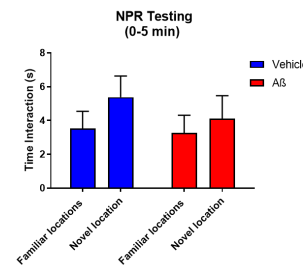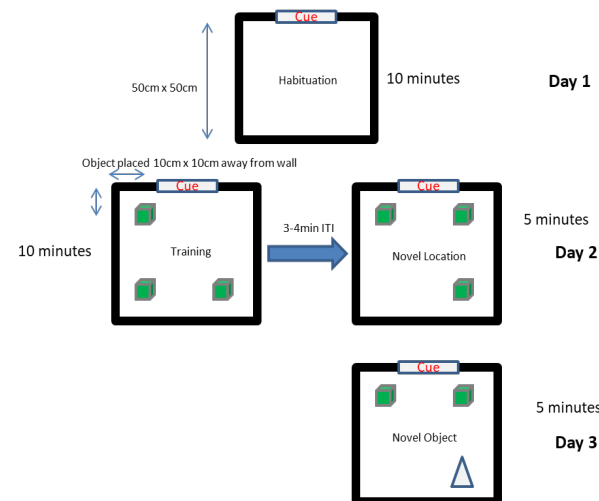
Quantitative Western Blot of Contralateral hippocampal lysate samples (9 ug/lane)

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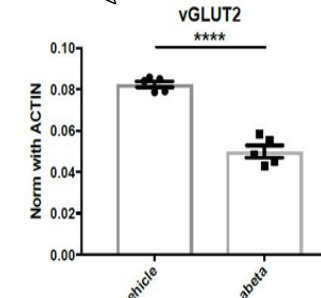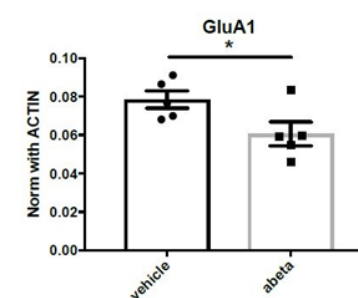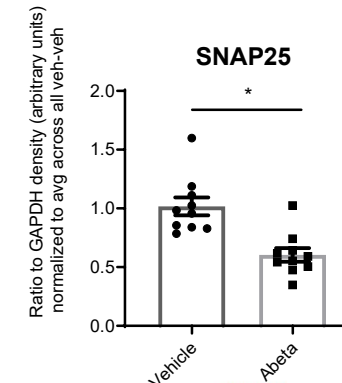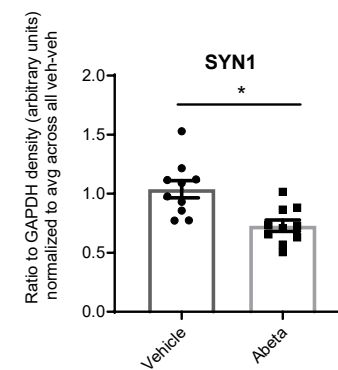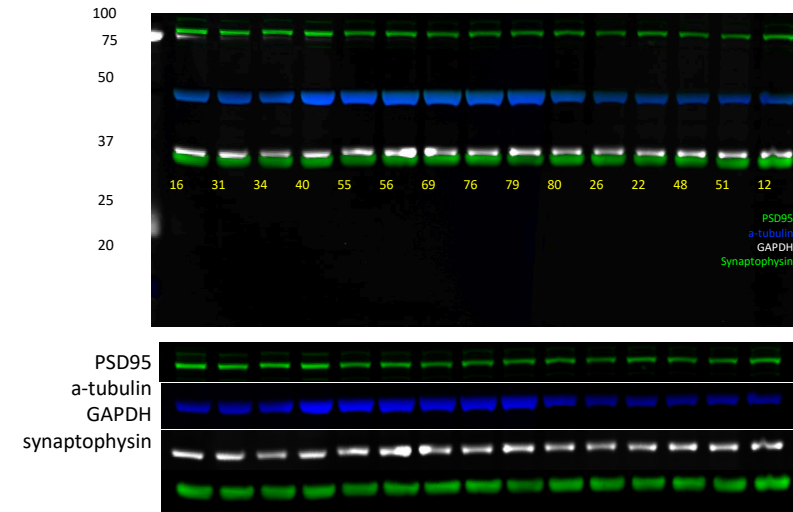
In-vitro model of synaptic degeneration driven by activated microglia and Dose-dependent prevention of synaptic degeneration

Phagocytosis assay with microglia

Mouse/Rat/Human Microglia are cultured in vitro to study the effect of the drug/treatment on basic microglia functionality. The cultured microglia are useful in studying drug effects of synaptic pruning and phagocytosis of cell debris. Cell phagocytosis will be evaluated using automated imaging from the IncuCyte live cell imaging system.

Automated Phagocytosis Assay

- pHrodo-labelled α-synuclein fibrils
- pHrodo-labelled amyloid beta fibrils
- pHrodo-labelled synaptosome

neuron

synaptosomal preparation

rat brain

BV2 mouse or human microglia

pHrodo

pH - 6

pH - 5

pHrodo

pHrodo-labelled synaptosomes change color due to pH differences. Phagocytosis can be measured by the color intensity of engulfed synaptosomes

Synaptic phagocytosis

Phagocytosis Inhibitor

0d 0h 0m

0d 0h 0m

Synaptic phagocytosis

Phagocytosis Inhibitor

DAPI/pHrodo
Nucleus of the cell
pHrodo-labeled synaptosome

DAPI/pHrodo/iba1

Phagocytosis Inhibitor

Total Integrated Intensity (OCU × µm²/Image) vs Time (hours)

- untouched
- vehicle
- 0.1 nM
- 1 nM
- 10 nM
- 100 nM
- 1000 nM
- UNC

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Self-Administration for addiction studies
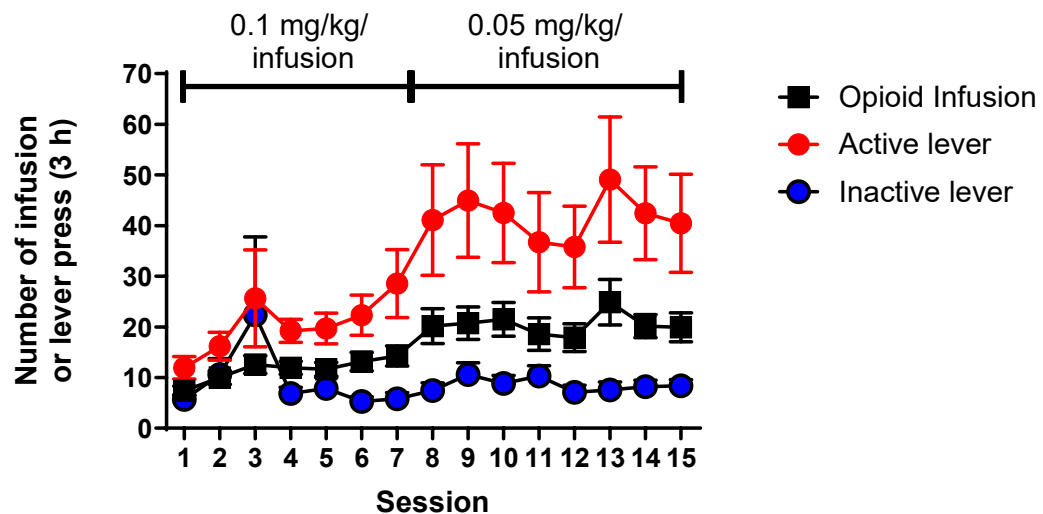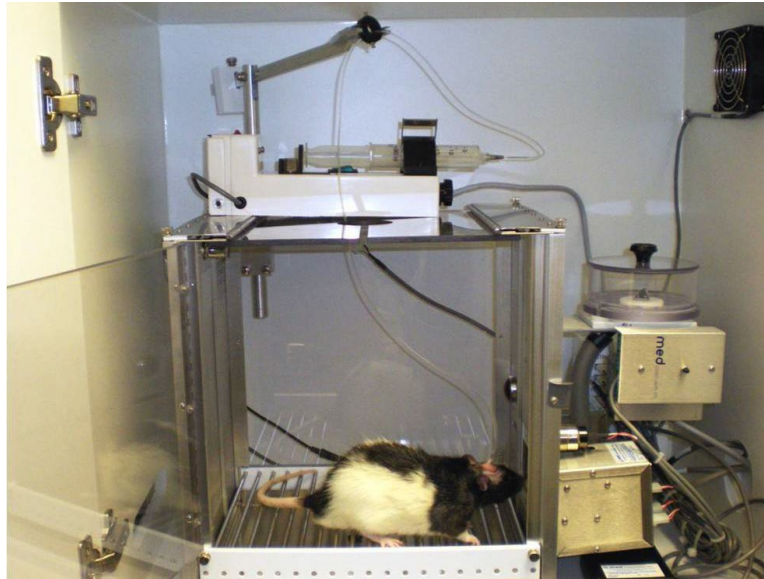
✓ Useful for studying animal model of addiction

✓ Rats become addicted to heroin that they self-administer.

✓ Over several sessions in these special cages, they increase active lever presses that deliver heroin

✓ 16 cages for rats

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Microdialysis and HPLC-MS systems (DA, NE, Serotonin,..)
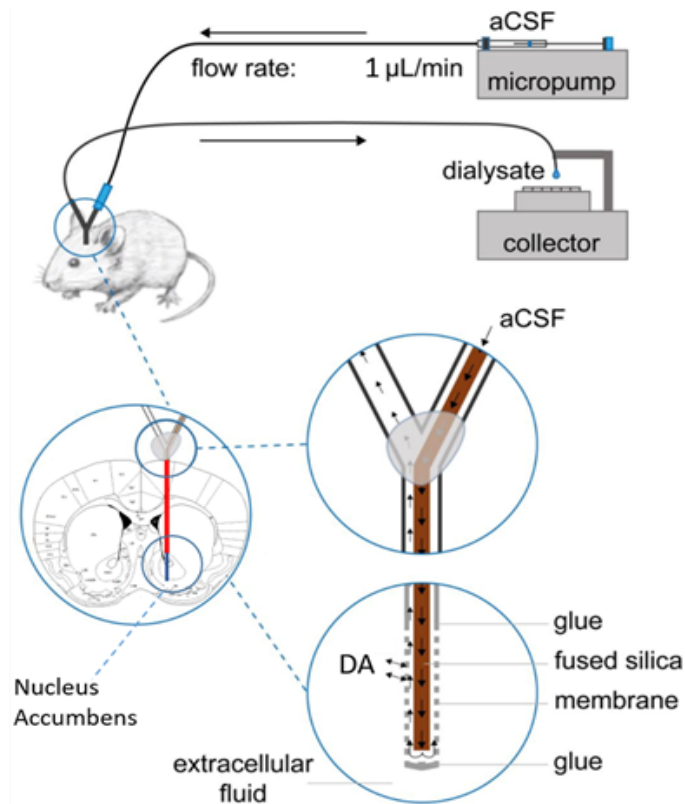
Preparative and Analytical HPLC-MS machine
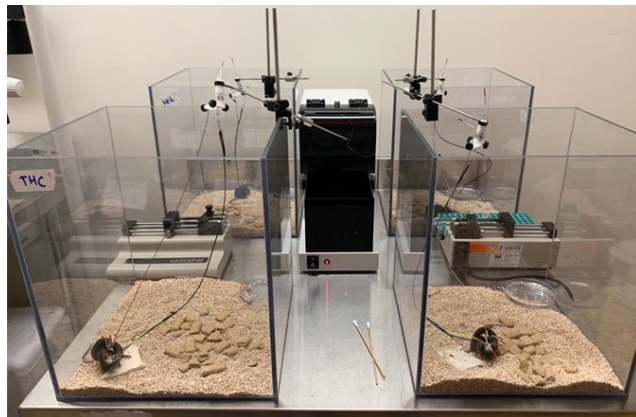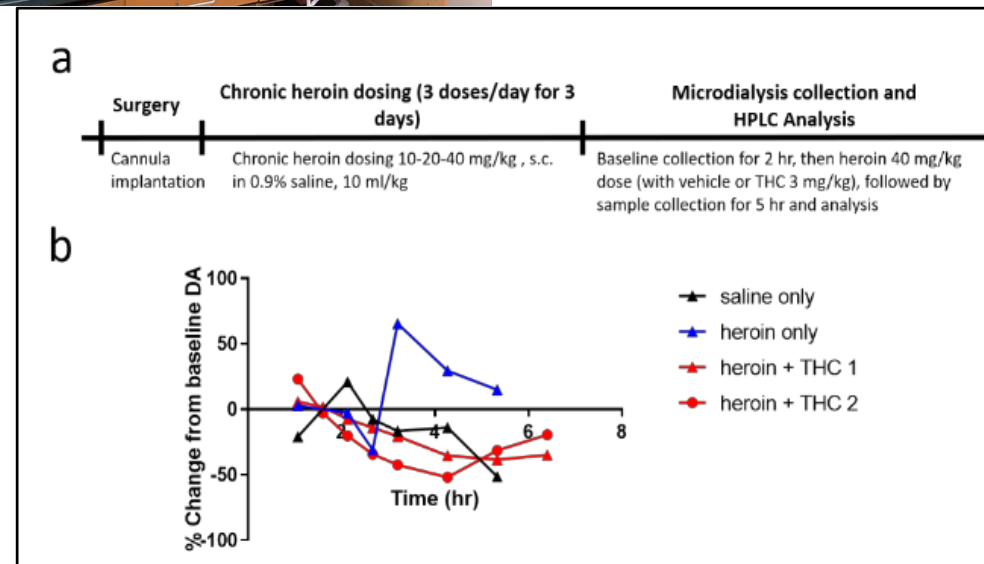
6-OHDA Convention Enhanced Delivery model of Parkinson's Disease
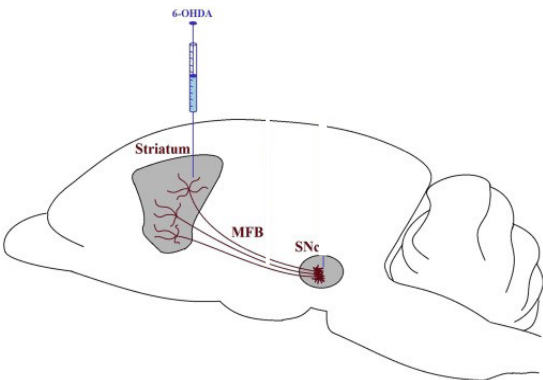
- Striatal lesion produces a more desirable retrograde and <u>progressive damage</u> of the nigrostriatal system
- Wider Therapeutic window compare to classical 6-OHDA model
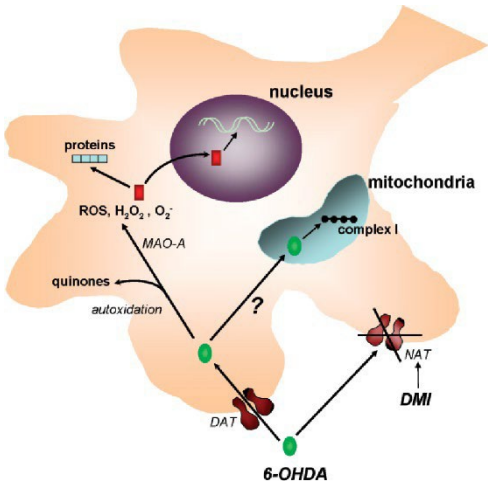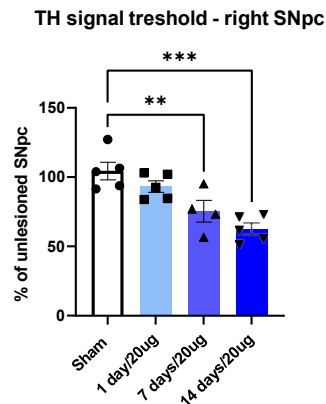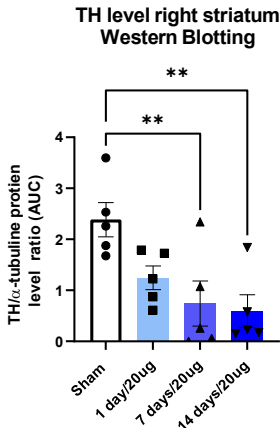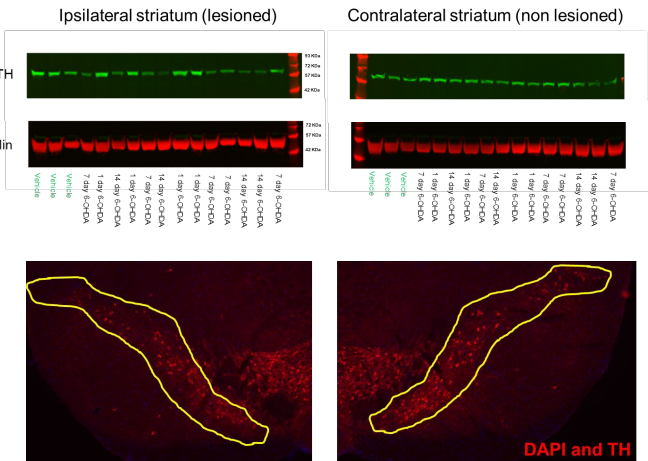
✓ Autoxidation to quinone and radical semi-quinone

✓ Oxidation by the MAOB and production of hydrogen peroxide and other ROS

✓ Putative inhibition of mitochondrial complex I

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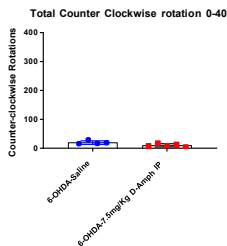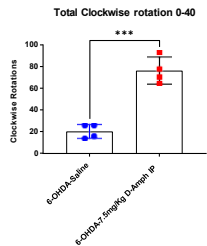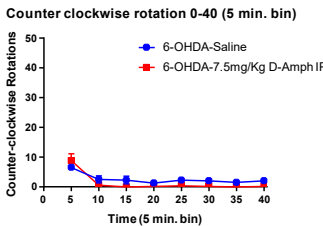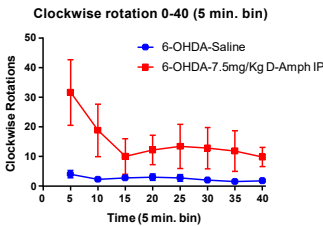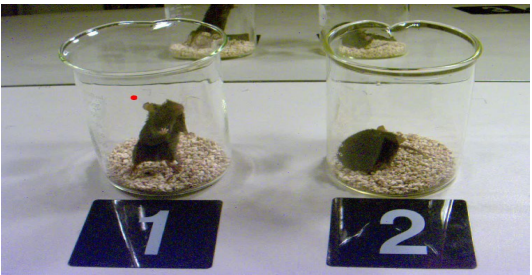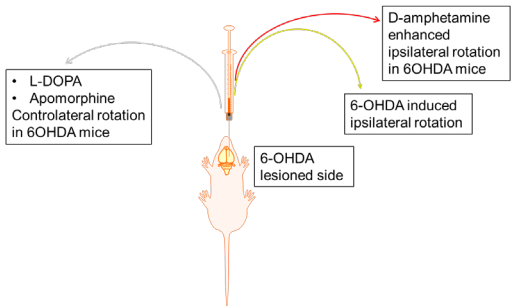
α-synuclein mouse model of Parkinson's Disease

Mouse fibrillary form of A-synuclein

Injection schematic

Ctx

Str

-2.6 mm

Bregma +0.20 mm
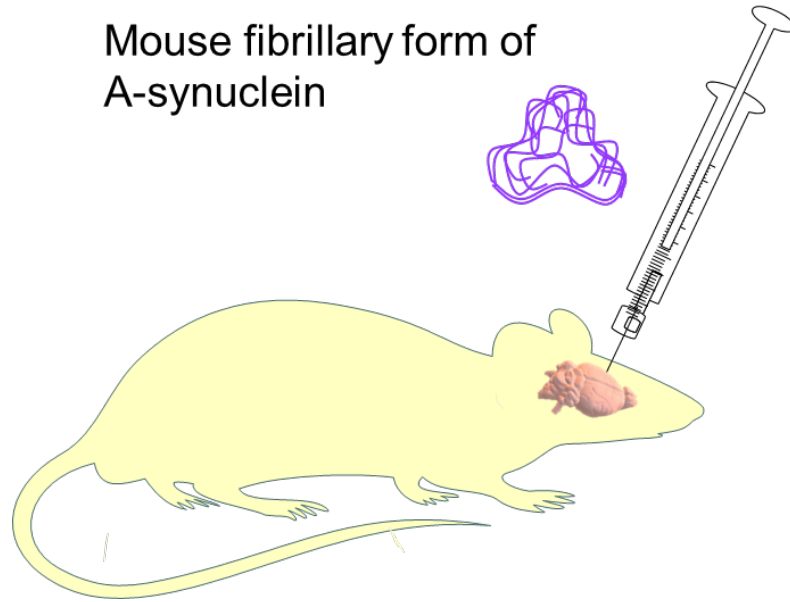
Pathological accumulation of α-synuclein in the striatum and the corpus callosum 4 weeks post surgery

COTROL

α-Syn
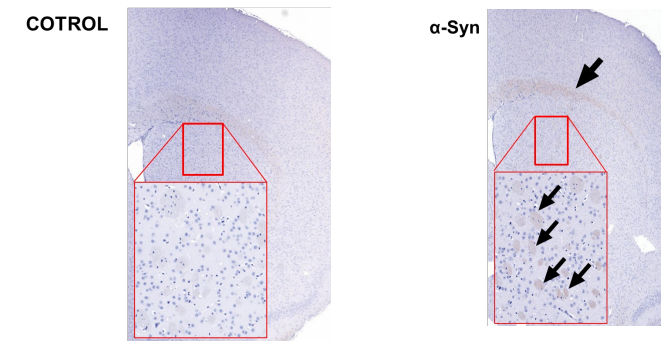
mice injected with α-Syn have lower latency to fall in wire hang test
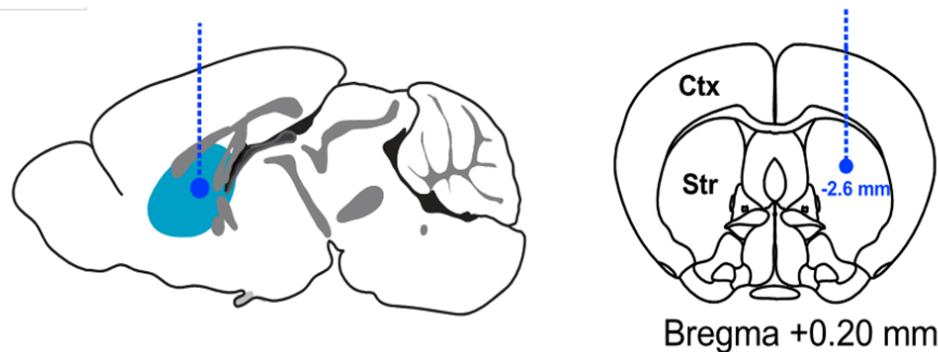
Wire Hang: Average Latency to fall
Baseline-Post Surgery

Latency to fall (s)

150

100

50

0

Baseline   PS week 2   PS week 3   PS week 4

■ Vehicle
■ α-Syn (5µg/2.5µl)

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Ischemic Stroke Models

Ischemic penumbra
Core of infarct
MCA
Cerebral embolism
Blood clot blocks MCA
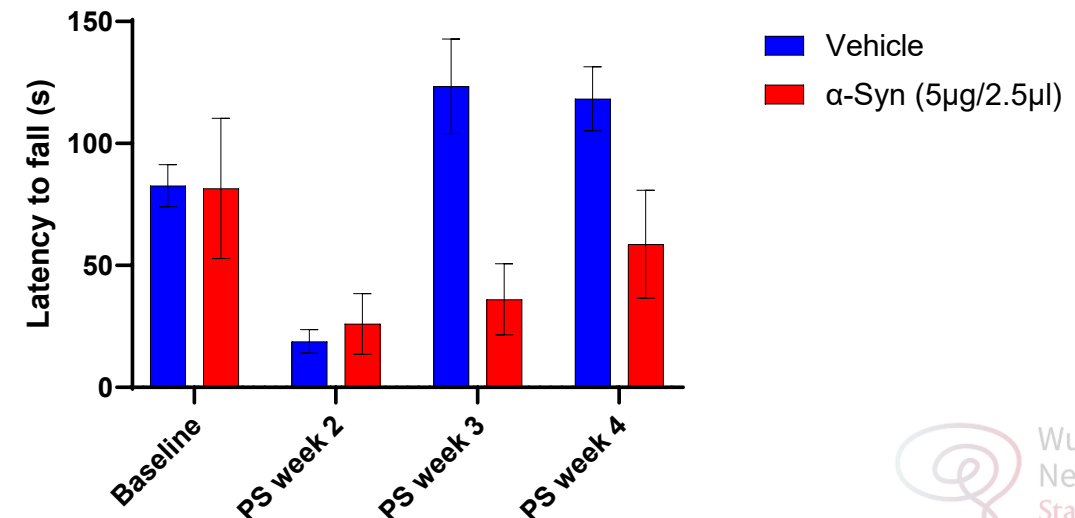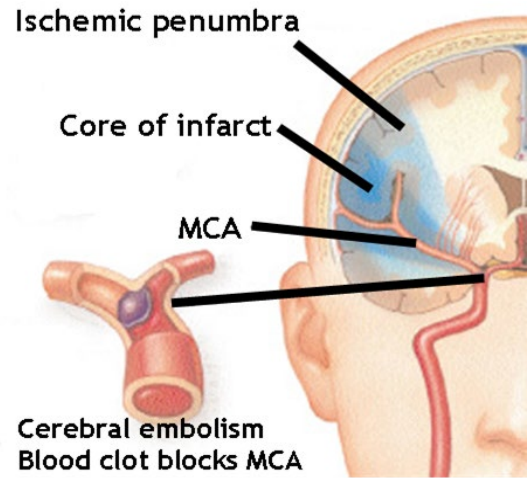
MCAO model of Ischemic stroke

Infarction area
Middle cerebral artery
Surgical clamp
Carotid artery
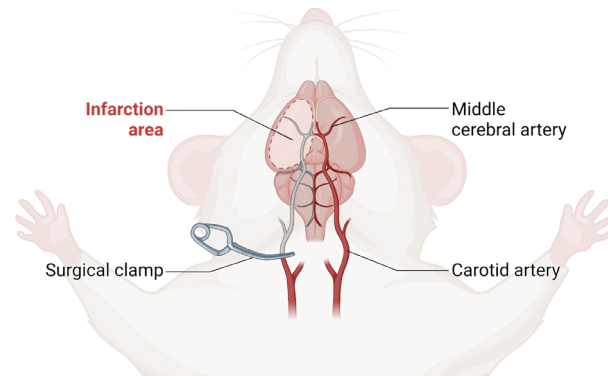
Ischemic Cell Death prevented by treatment with CBX
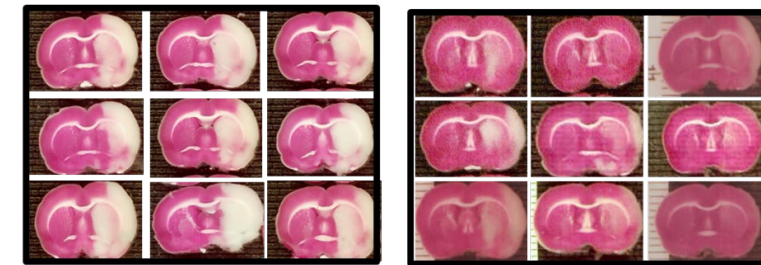
Vehicle

CBX 3 and 7 h

Triphenyltetrazolium chloride (TTC) stained brain sections

Sham   tMCAo
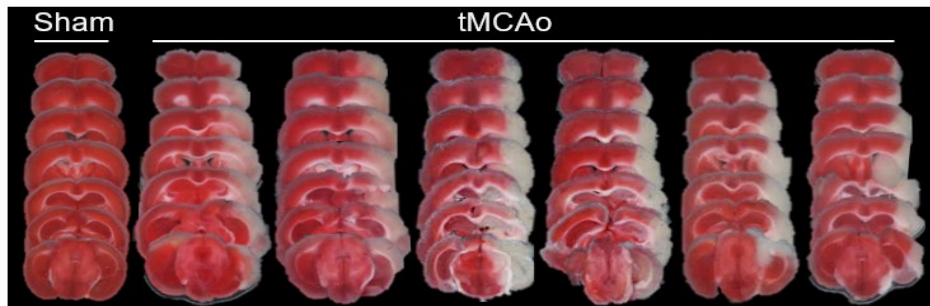
Behavioral Outcome after Experimental Stroke

Cylinder

Paw-Whisker Cross Left

P<0.01   P<0.01   P<0.01
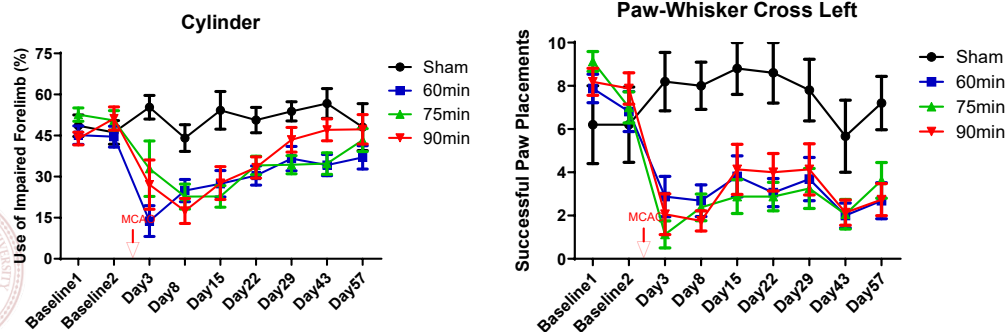
Total brain infarction (mm^3)

Vehicle   1.5h   3h   6h

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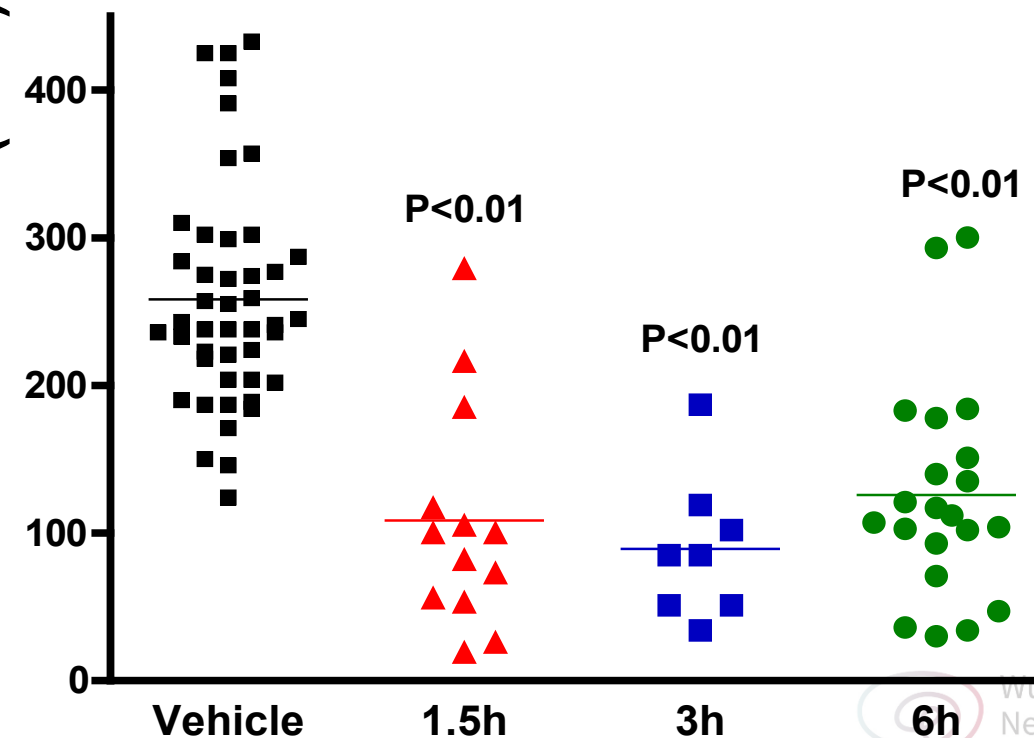
Digilab Home cage monitoring system

Benefits at a Glance!

AVOID ANY ROOM LIGHT MANAGEMENT
Each cage is autonomously providing proper darkness levels to the animals thanks to specific plastic materials shielding the room lights. Simply using the Red or Black IVC cages in the standard IVC Rack, you don't need to work at different room light levels.

MULTI-EXPERIMENT FLEXIBILITY
Every single cage can be programmed with a specific illumination cycle making it independent from the other cages, and allowing to run multiple experiments in the same room and on the same rack.

5xFAD Alzheimer's Disease model mice are hyperactive. Average data during light and dark cycle over 30 days of recording. Blue = WT, Red = 5xFAD..

9pm-9am (Light ON)   9am-9pm (Light OFF)

- A home cage monitoring system showing activity level of mice from different vendors.

- Phenotype transgenic mice, post surgery monitoring, drug screening

- Continuous live recording of animal for months with cloud-computing

Digilab: Distance Moved 1hr Post-Meth

- Veh - Veh
- Veh - Meth (1mg/kg, IP)
- Treatment + Meth (1mg/kg, IP)

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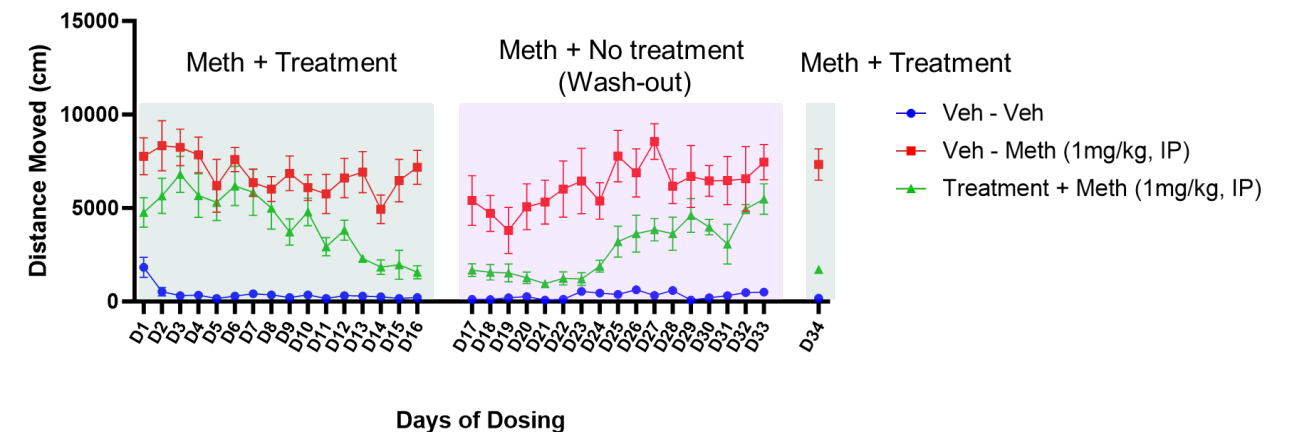
Metabolic cages
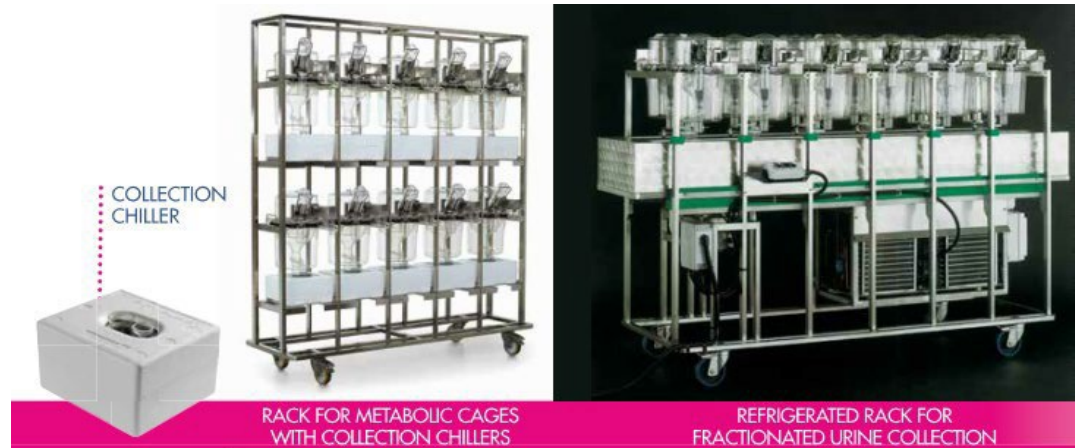
- ✓ Urine and feces collection from animal model of addiction or treated with novel treatment

- ✓ E-chiller for storing samples at low temperatures without compromising sample integrity

- ✓ Cages for both rats and mice
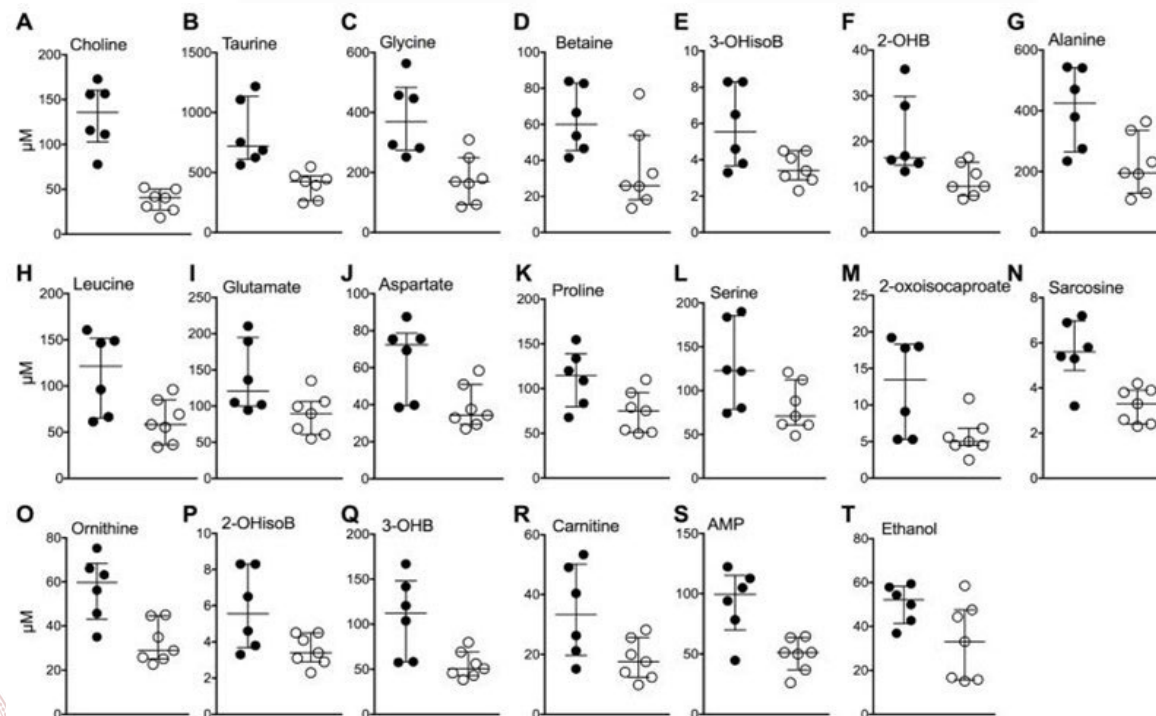
Cognitive Tests:

Learning and Memory:
- Morris water maze
- Delayed-Matching-to-Place water maze
- Barnes maze
- T maze
- Y maze
- Fear conditioning
- Novel object recognition
- Novel location recognition
- Passive avoidance test
- Conditioned place preference

Anxiety:
- Elevated zero maze
- Elevated plus maze
- Light-Dark box

Social Tests:
- Tube dominance test
- 3 Chamber test
- Interaction by pair test
- 5-Trial social memory test
- 2-Trial social memory test
- Automated social test
- Ultrasonic vocalization test

Stanford Behavioral and Functional Neuroscience Laboratory can modify and expand upon existing behavior protocols or design new testing paradigms to best study the behavior you are interested in.

Sensorimotor Tests:
- Open field activity test
- Activity chamber test
- Basket test
- Cylinder test
- Hot plate test
- Grip strength test
- Rota-rod performance test
- Horizontal ladder test
- Paw misplacement test
- Inclined platform test
- Beam walk test
- Garcia neurological test
- Twenty-eight point neurological scoring
- Olfactory test
- Catwalk Gait Analysis
- Automated Home Cage activity
- Running Wheel activity
- Pre-pulse inhibition
- IntelliCage

Disease Models:
- Alzheimer's disease
- Down syndrome
- Huntington's disease
- Parkinson's disease
- Autism
- ALS
- Addiction
- Focal ischemia
- Global ischemia
- EAE
- ICV, and other modes of Delivery
- PK
- PD
- BBB permeability studies
- Non-GLP safety studies

...and many more!

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Stanford Behavioral and Functional Neuroscience Laboratory
can provide expertise in all aspects related to the design and implementation of behavioral experiments as well as data ana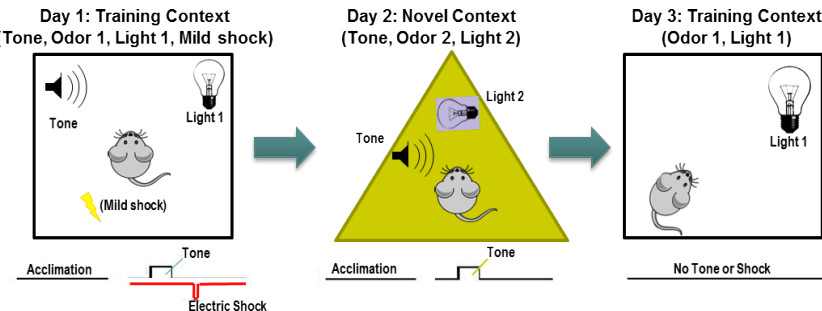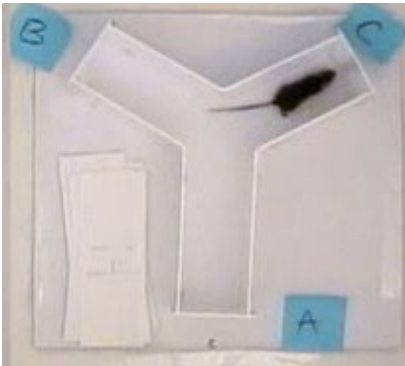lysis and interpretation. The core is equipped to accommodate a full battery of behavioral tests relevant to learning and memory, sensory gating, motor function, nociception, and anxiety-related behaviors. Here are some examples of the services we can provide:

Fear Conditioning

Trace Fear Conditioning (FC) is used to assess Pavlovian learning and memory in rodent models of CNS disorders. Subjects learn to associate a neutral Conditional Stimulus (CS; a tone) with an aversive Unconditional Stimulus (US; a mild electrical foot shock) and exhibit a Conditional Response (CR; freezing). After repeated pairings of CS and US, the subjects learn to freeze in response to both the tone and training context. The percentage of freezing during a specified time is reported. FC is a useful test for neurobehavioral and genetic studies on transgenic strains of mice, and for pharmacological studies evaluating the effect of novel chemical entities on cognition.

Day 1: Training Context (Tone, Odor 1, Light 1, Mild shock) — Day 2: Novel Context (Tone, Odor 2, Light 2) — Day 3: Training Context (Odor 1, Light 1)

Y Maze

Y Maze Spontaneous Alternation is a behavioral test for measuring spatial working memory based on the willingness of rodents to explore new environments. Rodents typically prefer to investigate a new arm of the maze rather than returning to one that was previously visited. Many parts of the brain--including the hippocampus, septum, basal forebrain, and prefrontal cortex--are involved in this task. This test is used to quantify cognitive deficits in transgenic strains of mice and evaluate novel chemical entities for their effects on cognition.

Morris Water Maze

The Morris Water Maze is designed to test spatial memory by observing the subject's ability to find a hidden platform in an opaque water tank.
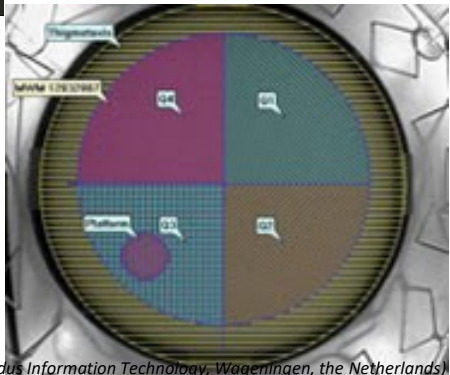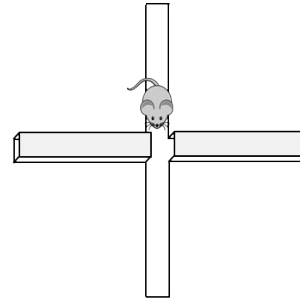
The subject is trained to use visual cues surrounding the tank to locate the platform. Successful learning of the Morris Water Maze is determined by the gradual decrease in the time it takes to find the platform.

*Ethovision Pro(Noldus Information Technology, Wageningen, the Netherlands)*

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Elevated Plus Maze

The Elevated Plus Maze test is used to assess anxiety-related behavior in rodent models. The apparatus consists of a "+"-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center area. The subjects freely explore the maze as their behavior is recorded. The preference for being in open arms over closed arms is calculated to measure anxiety-like behavior. This test can be used to phenotype strains of transgenic mice and to screen for putative anxiolytic compounds.
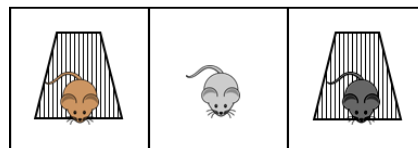
Three Chamber Social Test
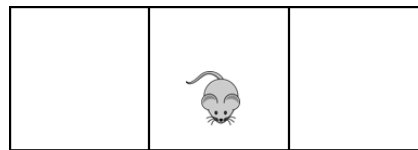

Habituation: Empty Apparatus
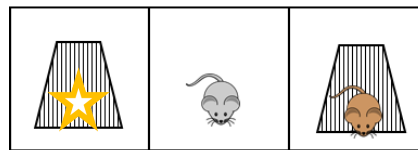
Sociability: Novel Object; Mouse 1

Social Novelty: Mouse 1; Mouse 2

The Three-Chamber test assesses general sociability and interest in social novelty in rodent models of CNS disorders. Rodents normally prefer to spend more time with another rodent (sociability) and will investigate a novel intruder more so than a familiar one (social novelty). Based on these inclinations, the Three Chamber Test can help identify rodents with deficits in sociability and/or social novelty.

Automated Social Test

nose poke
guillotine door
shelter
IR Beams
food and water
interaction wall
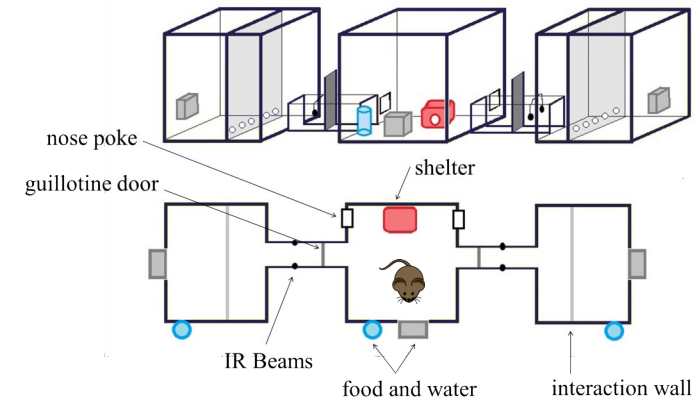
Sociability: Novel Object; Mouse 1

Social Novelty: Mouse 1; Mouse 2
Ethovision XT (Noldus Information Technology, Wageningen, the Netherlands)

The Automated Social Test is a perfect representation of SBFNL's commitment to continually expand and improve upon existing behavioral paradigms. The Automated Social Test takes the ideas behind the Three-Chamber Social Test and allows scientists to expand the test over several days in a home-cage environment with no disruptions from the experimenter. Furthermore, the design of the chambers enables scientists to modify the difficulty of access to interactions by requiring a certain number of nosepokes to open doors to the interaction chambers. This allows us to distinguish between the desire to socialize and mere exploration. No such test of social motivation currently exists, bringing SBFNL to the forefront.

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Automated Home Cage Testing

Automated Home Cage testing allows scientists to monitor home cage activity levels and behavior of subjects over several days. The addition of running wheels gives an additional way to assess differences in activity. The Automated Home Cage setup is a high-throughput method for screening subjects after drug treatment or genetic manipulation. Up to 24 cages can be processed at one time.
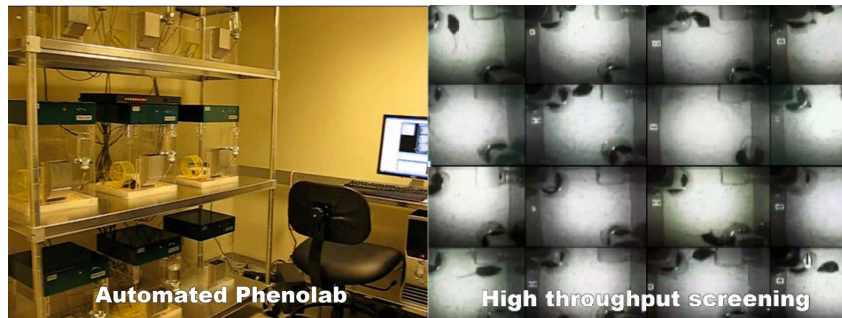
Ethovision XT (Noldus Information Technology, Wageningen, the Netherlands)

Catwalk Gait Analysis
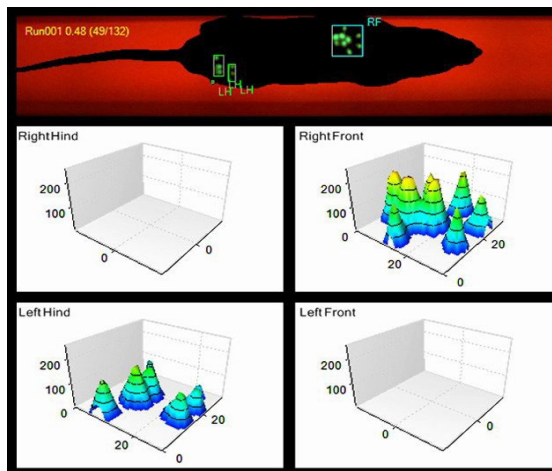
The Catwalk is an automated gait analysis system used to assess motor function and coordination in rodent models of CNS disorders. Subjects walk across an illuminated glass platform while a video camera records from below. Gait related parameters—such as stride pattern, individual paw swing speed, stance duration, and pressure—are reported for each animal. This test is used to phenotype transgenic strains of mice and evaluate novel chemical entities for their effect on motor performance.

Open Field

The Open Field task is a sensorimotor test used to determine general activity levels, gross locomotor activity, and exploration habits in rodents. The animal is allowed to freely move about a large, square arena for 10 minutes while activity is recorded. The Open Field test is useful for phenotyping transgenic strains of mice and evaluating the effect of novel chemical entities on activity, anxiety, and exploration.
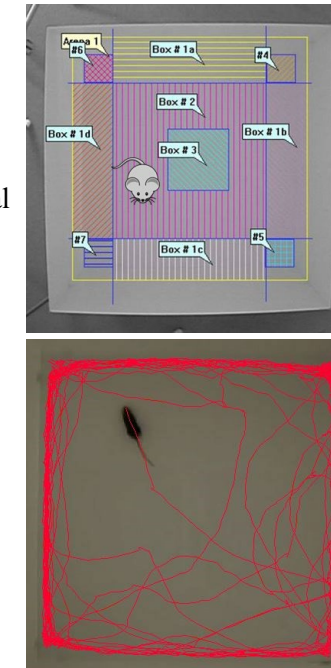
Ethovision XT (Noldus Information Technology, Wageningen, the Netherlands)

Olfactory Test
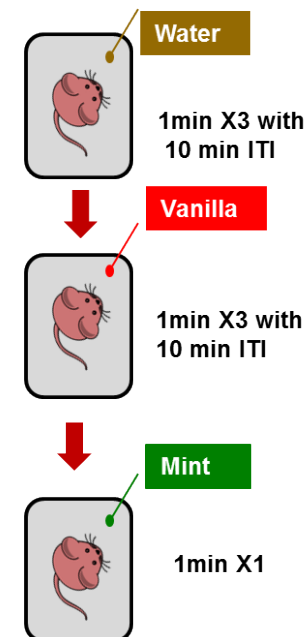
The Olfactory Test is a sensory assay used to measure the olfactory ability, degree of social interest, and perception of social novelty in rodent models of CNS disorders. The nonsocial olfactory test employs water and synthetic odorants while the social olfactory test uses water and urine samples from other animals. Subjects are exposed multiple times to three different odorants and time sniffing the odor is recorded. The olfactory test is useful for evaluating the effect of novel chemical entities and transgenic modifications on olfactory sensation, discrimination, and sociability.

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Stanford Behavioral and Functional Neuroscience Laboratory is interested in studying the complex, whole-system effects of disease models and potential treatments. We utilize our expertise in not only BEHAVIORAL testing, but in PHARMACOLOGICAL and DISEASE models to better study neurological disorders and treatments.

Behavioral testing in tandem with molecular and cellular biology techniques allows us to provide a complete picture of the systems we study. Here is an example of how this merger of information can provide a full story, leading us to significant and exciting discoveries.

## Post-treatment and Neuroprotection in the MCAO Rodent Model of Stroke
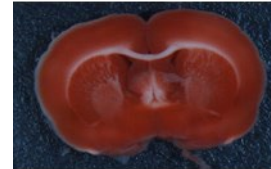

sham   2h MCAO

Middle cerebral artery occlusion (MCAO) is a surgically induced cerebral ischemia in rats. In this experiment, we test the efficacy of a drug (CBX) on inducing neuroprotection when given as a post-stroke treatment.

Two main neurological tests are used to assess motor and behavioral deficits in rat models of CNS disorders. Both the Garcia test and the 28-point Neuroscore test involve a battery of motor tests, ranging from simple observation to traversing a horizontal bar. These tests are well-suited for assessing neurological damage due to ischemia and recovery.
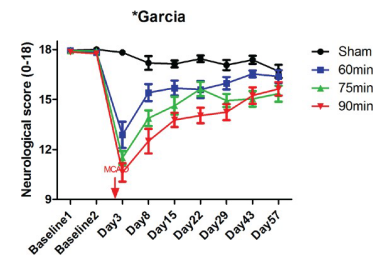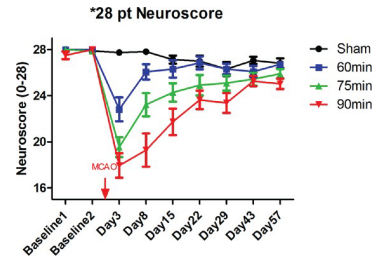
The Cylinder test is designed to evaluate locomotor asymmetry. As the animal moves within an open-top, clear plastic cylinder, its forelimb activity while rearing is recorded. In MCAO rats, the left forelimb is impaired after stroke. Recovery from stroke can be shown by increased use of the impaired forelimb during the cylinder test.
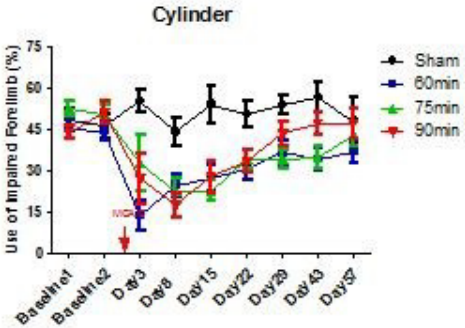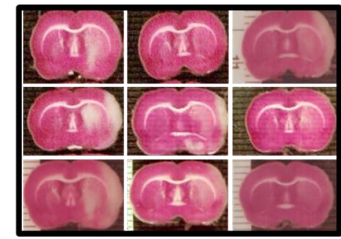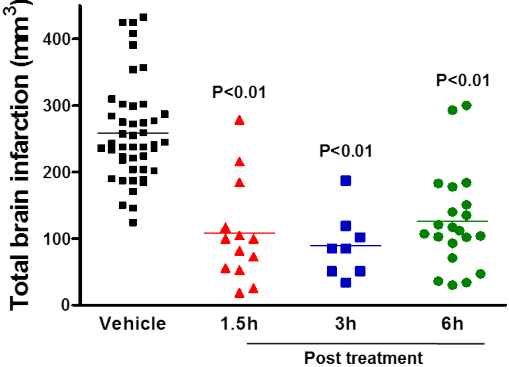

Neuroprotection Achieved by CBX

CBX treatment post-stroke reduces immediate damage to cerebral tissue caused by ischemia.

Vehicle

CBX 3 and 7 h

Stanford Behavioral and Functional Neuroscience Laboratory

Wu Tsai Neurosciences Institute
Stanford University

In addition to behavior testing, Stanford Behavioral and Functional Neuroscience Laboratory creates disease models and performs further analysis via biochemical, histochemical, and pathological evaluation of tissues and samples generated from studies.
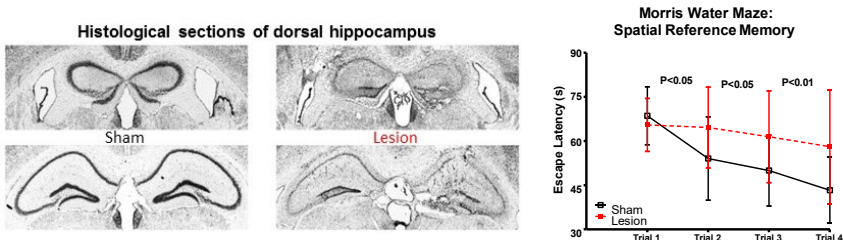
Experimental Stroke Models
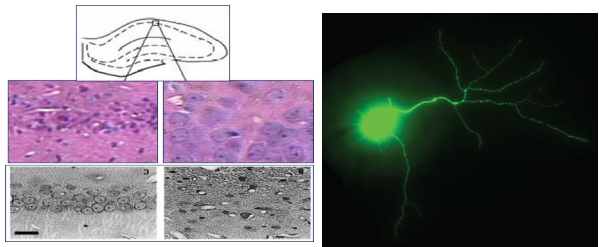
Multiple Sclerosis

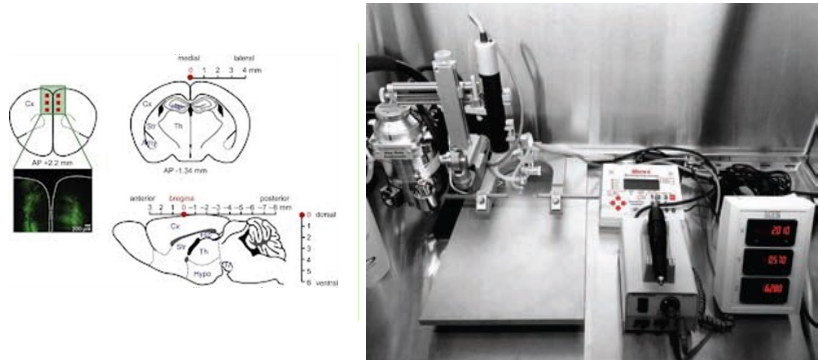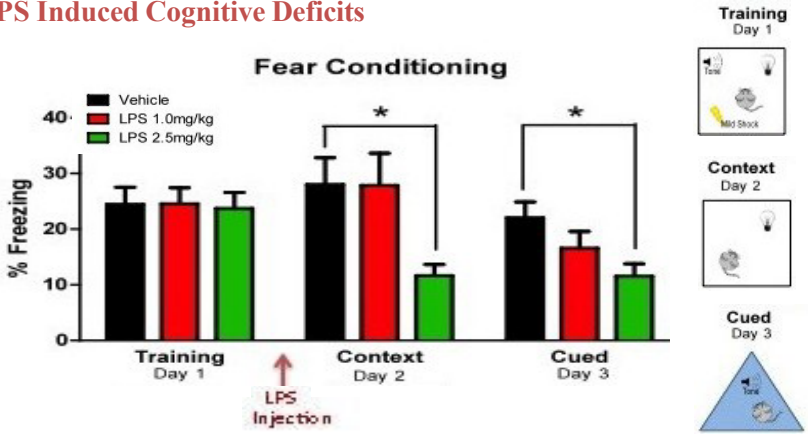- Experimental Autoimmune Encephalomyelitis (EAE)
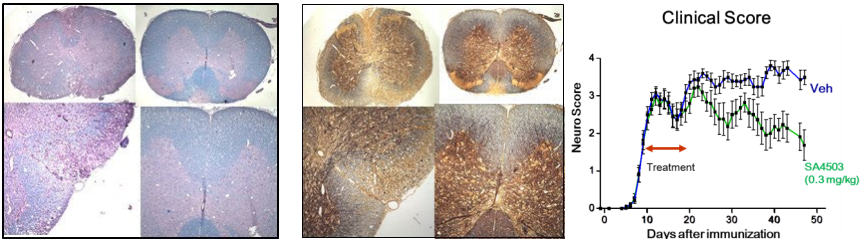
Hippocampal Lesions

Stereotactic Neurosurgical Procedures

¤Virus and Aβ Injection ¤ Intracranial Injection ¤ Optogenetics ¤Microdialysis ¤ 6-OHDA model of Parkinson's disease

LPS Induced Cognitive Deficits

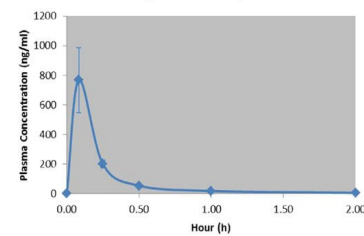Wu Tsai Neurosciences Institute Stanford University
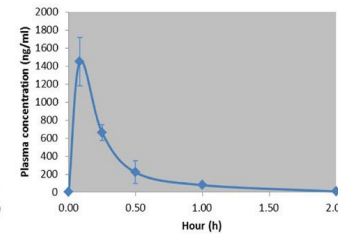
Pharmacokinetic & Pharmacodynamic (PK/PD)

- Bioavailability, biological half-life, elimination rate
- Blood Brain Barrier Permeability
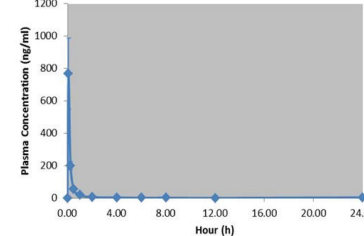- ICV and other modes of drug delivery
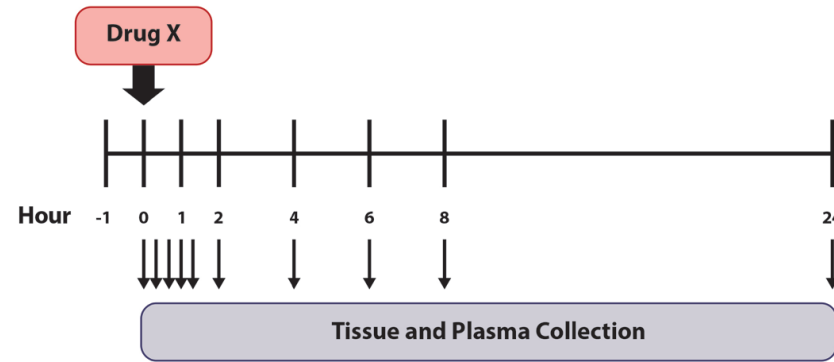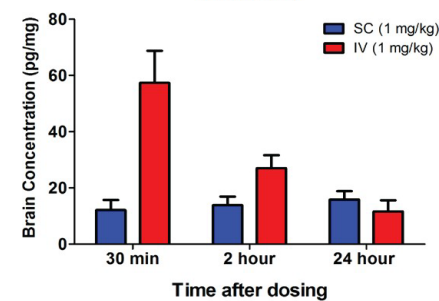
In Vitro Pharmacology
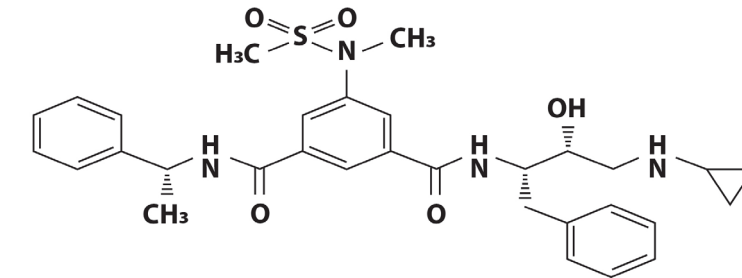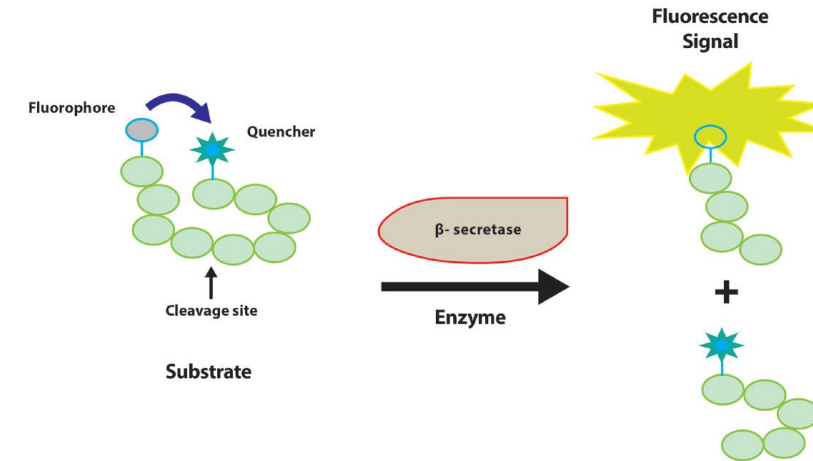
- β-secretase assay
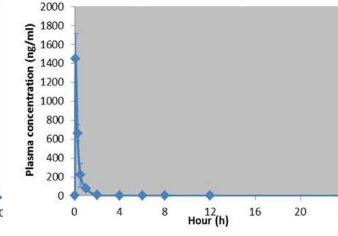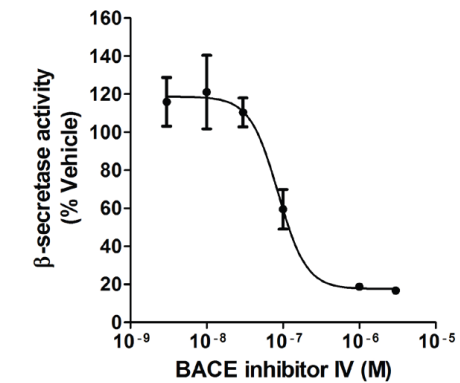

β-secretase is strongly implicated in the pathology of Alzheimer's disease. Thus, modulation of the β-secretase may lead to therapeutic benefits for the treatment of the disease. In this assay, compounds' effects on the activity of β-secretase are tested using the enzyme substrate, which is linked to a fluorophore at one end and to a quenching agent at the other.

...ord Behavioral and Functional Neuroscience Laboratory

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Cell based Assays

- Amyloid beta toxicity assays

Amyloid peptide (Aβ) has a central role in the pathogenesis of Alzheimer's disease. In this assay, test compounds' effect on the Aβ level are studied using the human neuroblastoma cell line stably expressing Swedish mutant amyloid precursor protein. The levels of Aβ produced with or without test compounds are measured with sandwich ELISA.
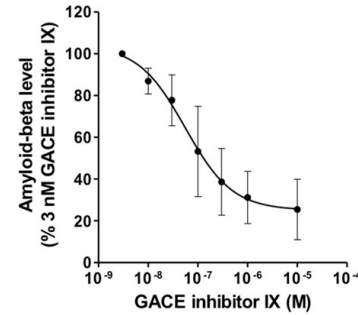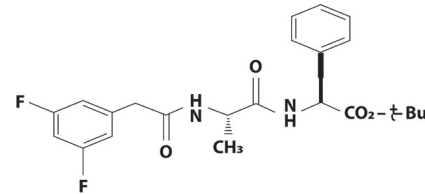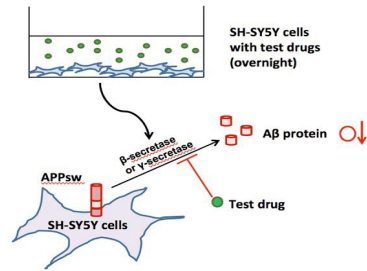
- GPCR (G-protein coupled receptors)
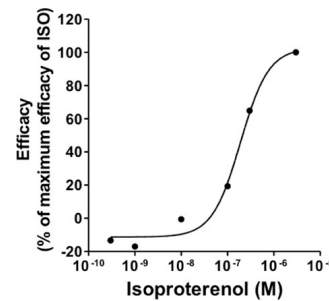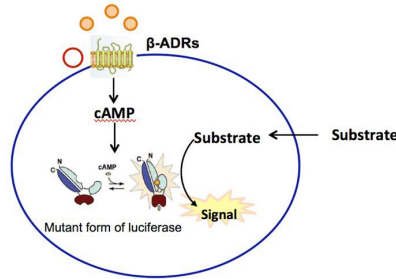
G-protein coupled receptors including adrenergic receptors are one of the most important drug targets. In this assay, effects of test compounds on β-adrenergic receptors are studied using the mu- tant inactive luciferase, which becomes active in response to cyclic AMP. Activation of the receptor with β-adrenergic receptor agonists lead to the production of cyclic AMP, which in turn increase the level of luminescence signal produced by the luciferase activity.
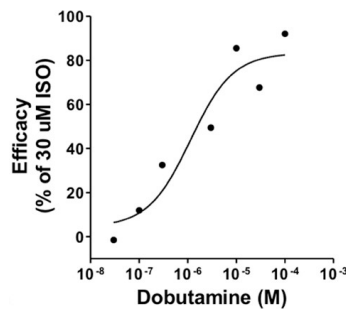
- Primary Cortical Neurons (PCN) and Primary Hippocampal Neurons (PHN) Cell Culture

Embryonic hippocampal/cortical neurons are cultured in vitro to study the effect of the drug/treatment on gene or protein ex- pression. The cultured neurons are also useful in studying the morphology of neurons and synapse formation/density. Cell sur- vival and toxicity will be evaluated using different techniques such as western blotting, qPCR, and immunocytochemistry.
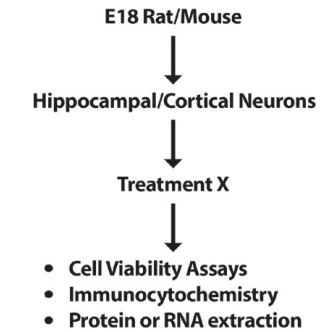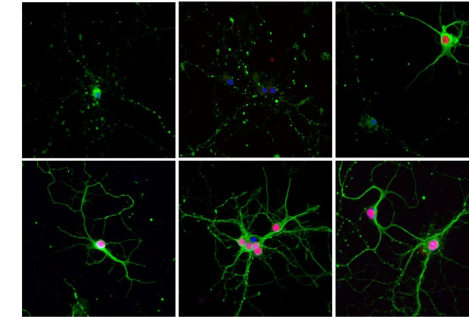
APP Toxicity in PCN

- Neurite Outgrowth Assay in PCN and PHN

Neurite outgrowth is an important morphological pheno- type of neuronal cells. As the cellular processes are essential for cell health and function, test compounds that can affect the growth of neu- rites may have potential as drugs for neuropatho- logical disorders. In this assay, effects of test compounds on neurite outgrowth are tested with fluorescent staining.

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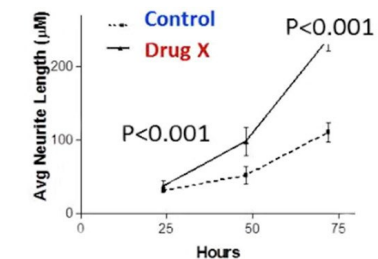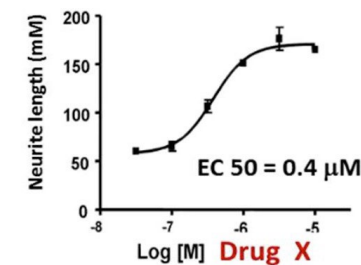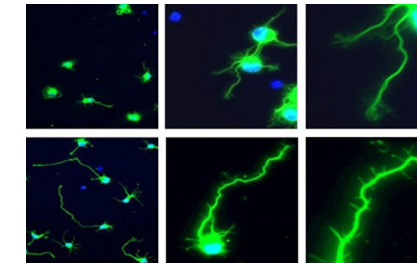
Biochemical and Histochemical Evaluation

- General histological stains
- Immunohistochemistry using the ABC/DAB method
- Immunofluorescence for various cellular markers
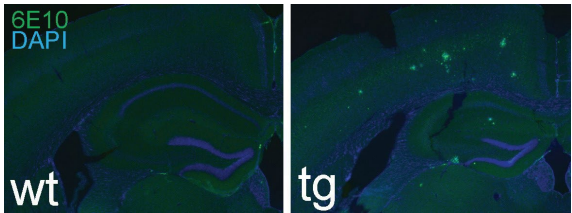- Biochemical evaluation of samples

Neuropathological assessment in models of Alzheimer's Disease
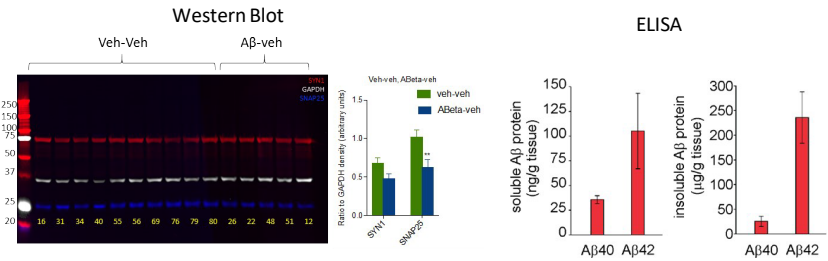
- Immunohistochemistry
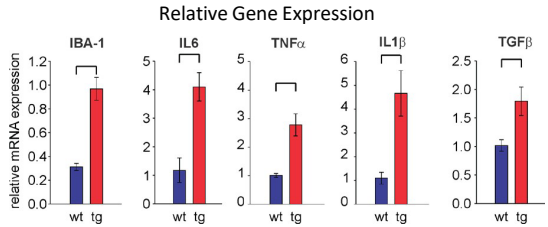
Microglia Staining (IBA-1-immunoreactivity)
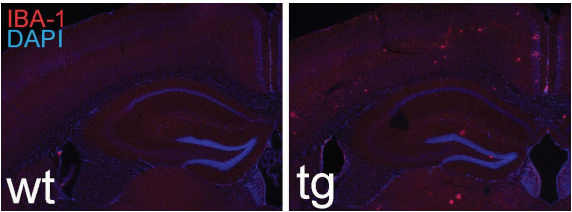
Amyloid Beta Plaque Staining (6E10-immunoreactivity)

- Amyloid Beta Plaque Load

- Quantitative rtPCR

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Rates are updated every fiscal year
Example rate provided below. Refer to BFNL website for the current rate.

Service	Rate/Hr
Experiment performed by BFNL Staff	$200
Facility/BFNL Equipment Use	$80
Technical Procedure Room Use	$25-70

* Indirect cost (IDC) 54% for external users

Please visit our website to learn more about Stanford Behavioral and Functional Neuroscience Laboratory.
https://neuroscience.stanford.edu/shared-resources/behavioral-functional-lab
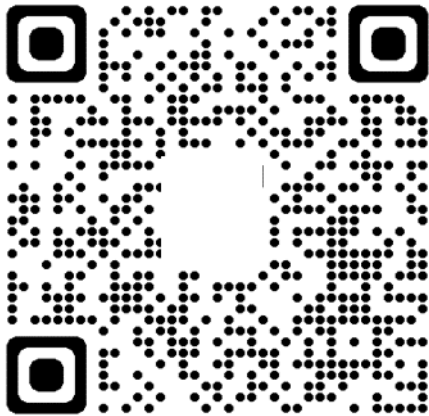
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