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**Stanford Behavioral and Functional Neuroscience Laboratory (SBFNL)**

# MISSION STATEMENT

Our mission is to create a shared resource platform for academic labs, providing access to rodent testing in existing and novel paradigms for characterizing cognition and behavior.

We hope to accelerate progress in both fundamental and applied studies of nervous system function that will serve as a resource to further investigate normal and the diseased nervous system.

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Stanford Behavioral and Functional Neuroscience Laboratory has two locations on the Stanford campus:

SIM1 (Lokey Stem Cell Building) and Arastradero, providing over 5,000 sq. ft. of testing, housing, and laboratory space.

Both of our facilities are under a reverse light-dark cycle. This allows for testing during the rodents' natural active period.

The laboratory is operated by a team of experienced research scientists and research assistants.

SBFNL is supported by a NIH P30 Center Core Grant and the Stanford Neurosciences Institute (SNI).



SIM1 Building



Arastradero Building

## GOALS

Stanford Behavioral and Functional Neuroscience Laboratory aims to support and accelerate translational and preclinical neuroscience research through the following collaborations and services:

- Serve as an integrated infrastructure for experimental design oversight and robust data analysis
- Provide state-of-the-art biochemical and histological assays, behavioral testing paradigms, and surgical methods.
- Investigate novel therapies for a myriad of CNS disease models
- Conduct phenotyping assays for novel transgenic rodent lines



## Disease Model

- Alzheimer's Disease
  - Amyloid-Beta induced AD
- Down Syndrome
- Huntington's Disease
- Parkinson's Disease
- Autism
- Amyotrophic Lateral Sclerosis (ALS)
- Focal ischemia
- Global ischemia
- Post Traumatic Stress Disorder (PTSD)
- Addiction
- Experimental Autoimmune Encephalomyelitis (EAE)
- *In-vivo/vitro* inflammation model (LPS)

## *In-vivo/vitro* pharmacology

- Neuroinflammation
- Intracranial Volume Injections (ICV)
- Microdialysis
- Pharmacokinetics/dynamic
  - Blood pressure monitoring
- Blood Brain Barrier permeability studies
- Drug Efficacy studies

## Biochemical Tests

- Immunohistochemistry (IHC)
- Immunocytochemistry (ICC)
- Tetrazolium Chloride Staining
- Quantitative Polymerase Chain Reaction (qPCR)
- Enzyme-Linked Immunosorbent Assays (ELISA)
  - Pro-inflammatory cytokines
  - Amyloid Beta
- Western Blot
- Cell culture
- Cell-based Assays
  - Functional
  - Binding
- High-performance Liquid chromatography
- RNA scope

## 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
- Conditioned place preference
- Intellicage

### *Anxiety:*

- Elevated zero maze
- Elevated plus maze
- Light-Dark box
- Marble burying

### *Depression:*

- Forced Swim
- Tail Hang

## Social Tests:

- Tube dominance
- 3-Chamber test
- Interaction by pair test
- 5-trial social memory test
- 2-trial social test
- Automated social test
- Ultrasonic vocalization

## 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
- Pole Test
- Garcia neurological test
- Paw-whisker test
- 28 point neurological scoring
- Olfactory test
- Catwalk stride test
- Automated home cage activity
- Running wheel activity
- Pre-pulse inhibition

*...and many more!*

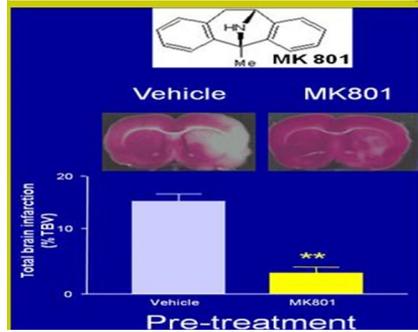
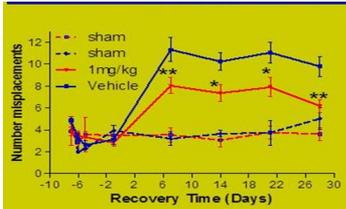
*Stanford Behavioral and Functional Neuroscience Laboratory can modify and expand upon existing behavior protocols or design new testing paradigms to best study the behavior you are interested in.*

SBFNL analyzes novel therapeutic efficacy through the generation of disease models and administration of experimental interventions. Downstream behavioral, biochemical, histological, and pathological evaluation of subjects and collected tissues further elucidate therapeutic effectiveness.

## DISEASE MODELS

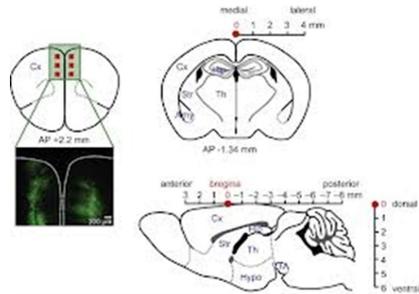
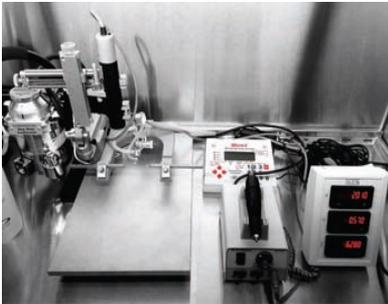
### Focal and Global Ischemia

- Therapeutic drug treatments
- Behavior Testing

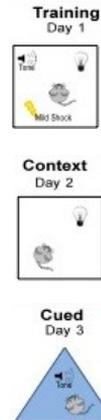
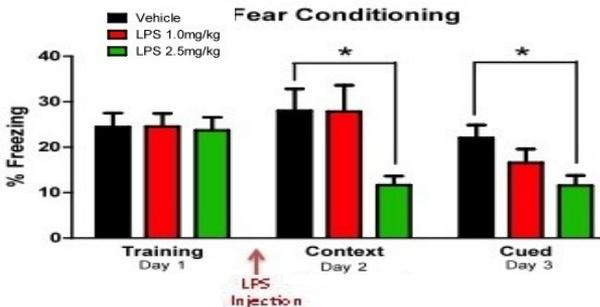


### Stereotaxic Injections

- Virus Injection
- Intracranial Injection

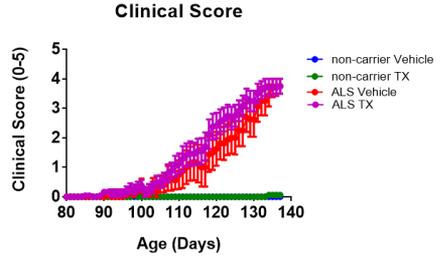


### LPS Induced Cognitive Deficits



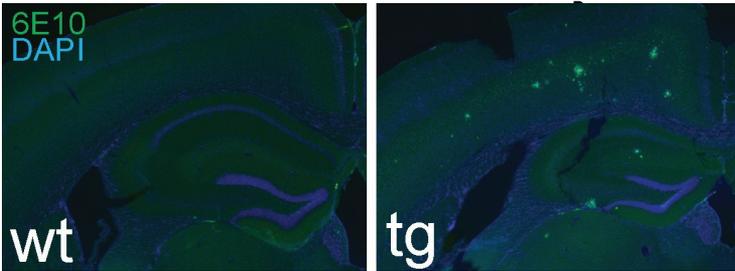
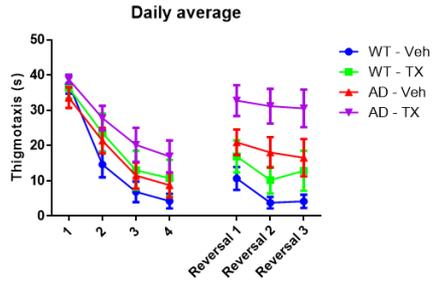
## Amyotrophic Lateral Sclerosis

- Therapeutic drug treatment
- Body weight monitoring
- Clinical score ranking
- Behavioral Testing



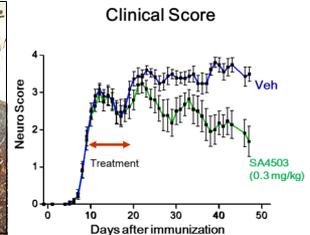
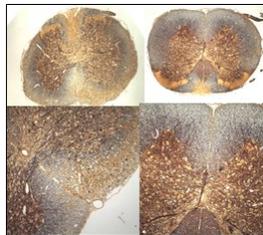
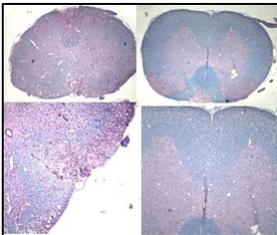
## Alzheimer's Disease

- Beta-adrenergic model
- Therapeutic drug treatment testing
- Behavior Testing



## Multiple Sclerosis

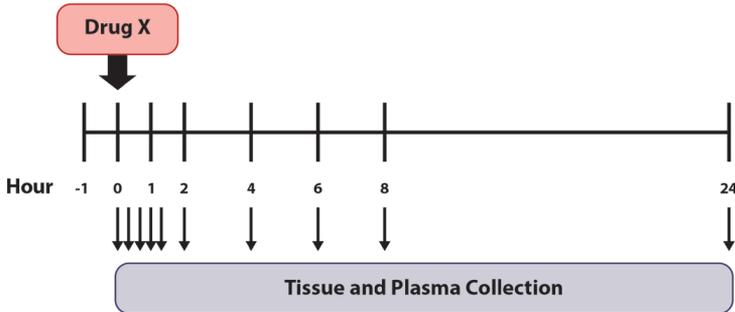
- Experimental Autoimmune Encephalomyelitis (EAE)



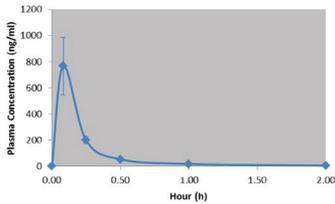
# IN-VIVO PHARMACOLOGY

## Pharmacokinetic & Pharmacodynamics (PK/PD)

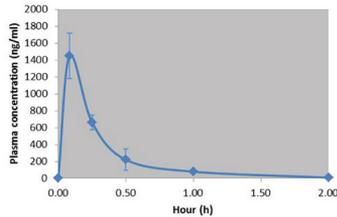
- Bioavailability, biological half-life, elimination rate
- Blood Brain Barrier Permeability
- ICV and other modes of drug delivery



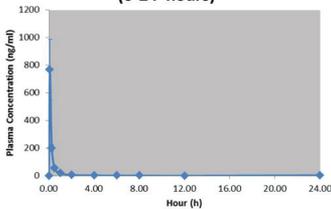
PK Analysis of Plasma IV dosing (0-2 hours)



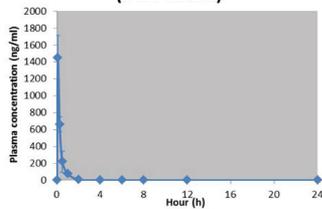
PK Analysis of Plasma SC dosing (0-2 hours)



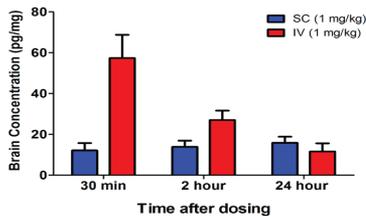
PK Analysis of Plasma IV dosing (0-24 hours)



PK Analysis of Plasma SC dosing (0-24 hours)

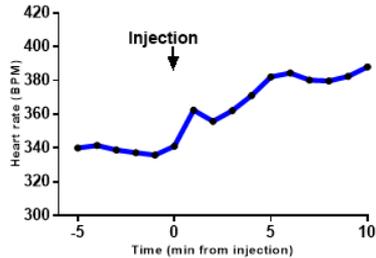
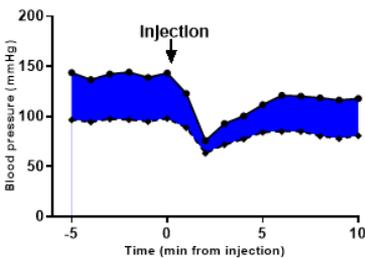
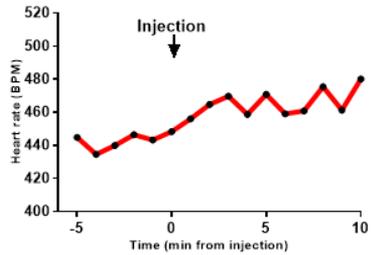
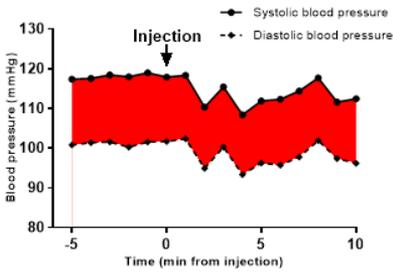
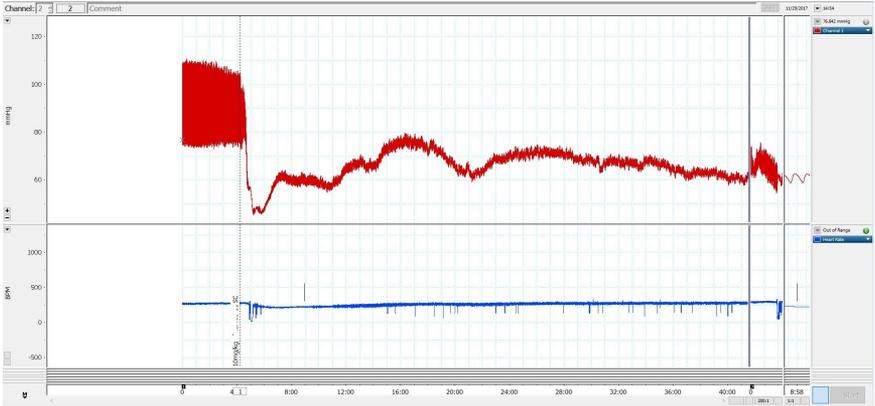


PK Analysis in Brain (0-24 hours)



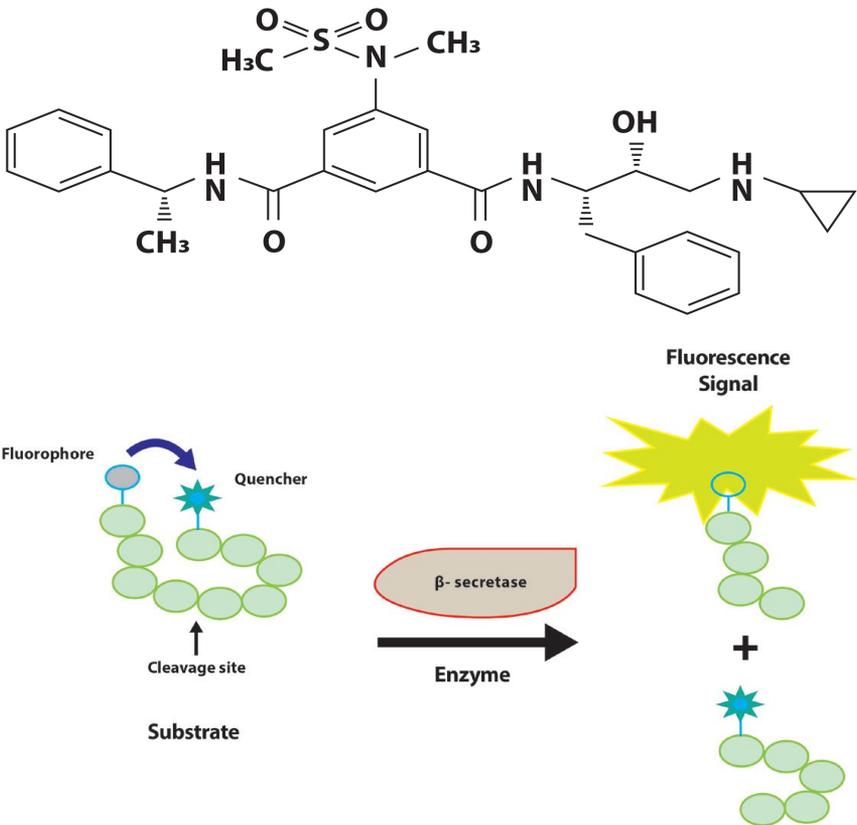
# Blood Pressure Monitoring

- Drug effect on blood pressure and HR
- Data analysis

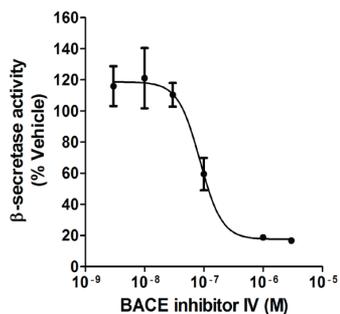


# IN-VITRO PHARMACOLOGY

## $\beta$ -secretase assay



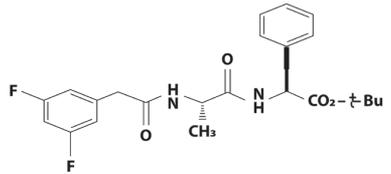
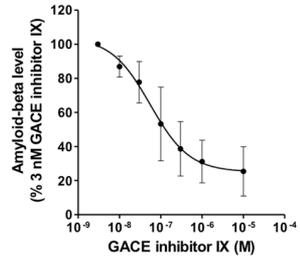
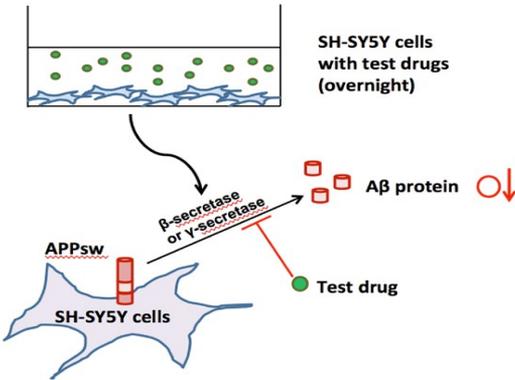
$\beta$ -secretase is strongly implicated in the pathology of Alzheimer's disease. Thus, modulation of the  $\beta$ -secretase may lead to therapeutic benefits for the treatment of the disease. In this assay, compounds' effects on the activity of  $\beta$ -secretase are tested using the enzyme substrate,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



# Cell based Assays

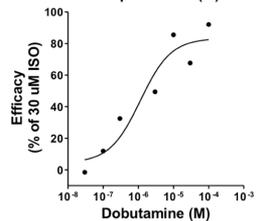
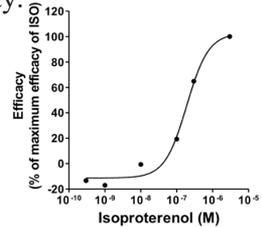
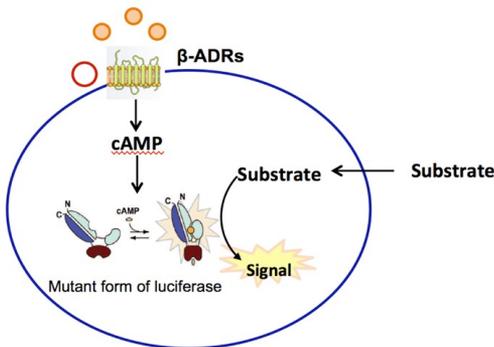
## Amyloid beta toxicity assays

Amyloid peptide ( $A\beta$ ) has a central role in the pathogenesis of Alzheimer's disease. In this assay, test compounds' effect on the  $A\beta$  level are studied using the human neuroblastoma cell line stably expressing Swedish mutant amyloid precursor protein. The levels of  $A\beta$  produced with or without test compounds are measured with sandwich ELISA.



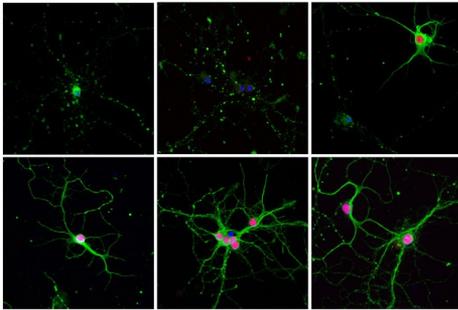
## GPCR (G-protein coupled receptors)

G-protein coupled receptors including adrenergic receptors are one of the most important drug targets. In this assay, effects of test compounds on  $\beta$ -adrenergic receptors are studied using the mutant inactive luciferase, which becomes active in response to cyclic AMP. Activation of the receptor with  $\beta$ -adrenergic receptor agonists lead to the production of cyclic AMP, which in turn increase the level of luminescence signal produced by the luciferase activity.

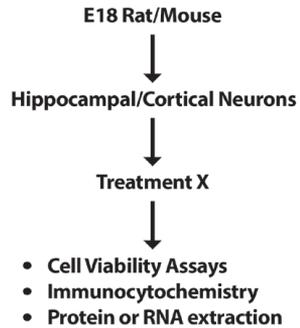


# Primary Cortical Neurons (PCN) and Primary Hippocampal Neurons (PHN) Cell Culture

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

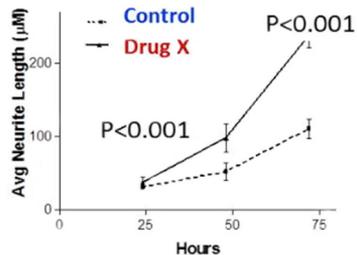
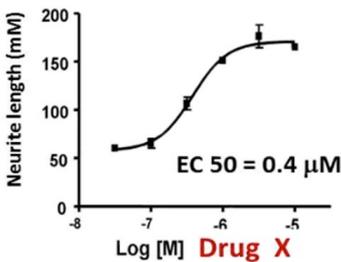
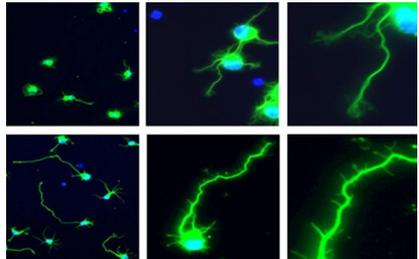


APP Toxicity in PCN



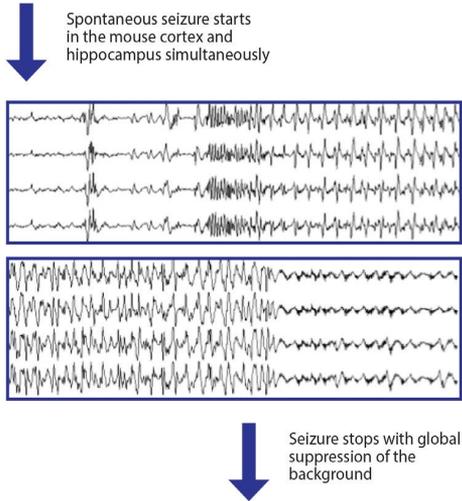
## Neurite Outgrowth Assay in PCN and PHN

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

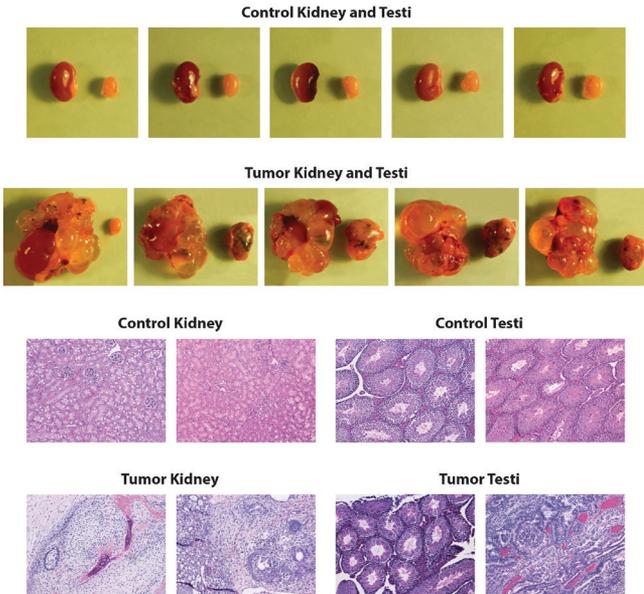


# Neurophysiology (Dr. Brenda Porter)

## Continuous EEG to assess rodents for seizure activity

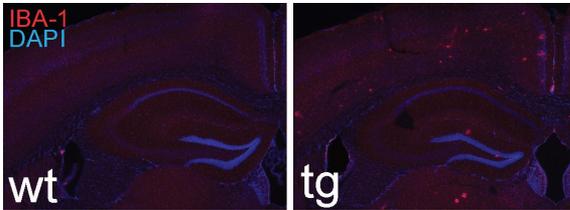


## Non-GLP Safety and Pathological Evaluation



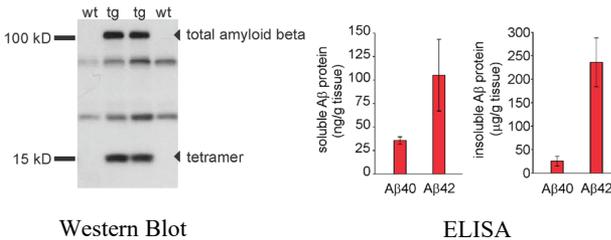
# BIOCHEMICAL AND HISTOCHEMICAL EVALUATION

- General histological stains
- Immunohistochemistry
- Immunofluorescence for various cellular markers
- Biochemical evaluation of samples



Microglia Staining (IBA-1-immunoreactivity)

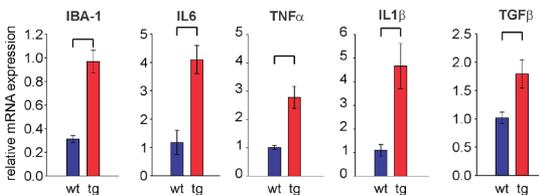
- Amyloid Beta Plaque Load



Western Blot

ELISA

- Quantitative rtPCR



Relative Gene Expression

# BEHAVIORAL ASSAYS

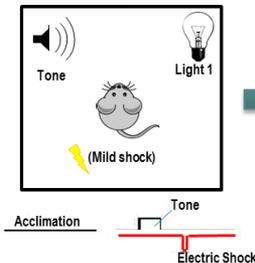
Stanford Behavioral and Functional Neuroscience Laboratory can provide expertise in all aspects related to the design and implementation of behavioral experiments as well as data analysis and interpretation. The core is equipped to accommodate a full battery of behavioral tests relevant to learning and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

## FEAR CONDITIONING

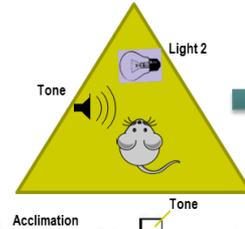
Trace Fear Conditioning (FC) is used to assess Pavlovian learning and memory in rodent models of CNS disorders. Subjects learn to associate a neutral Conditional Stimulus (CS; a tone) with an aversive Unconditional Stimulus (US; a mild electrical foot shock) and exhibit a Conditional Response (CR; freezing). 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.



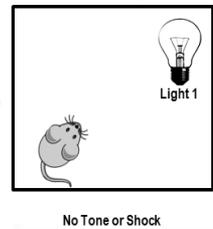
Day 1: Training Context  
(Tone, Odor 1, Light 1, Mild shock)



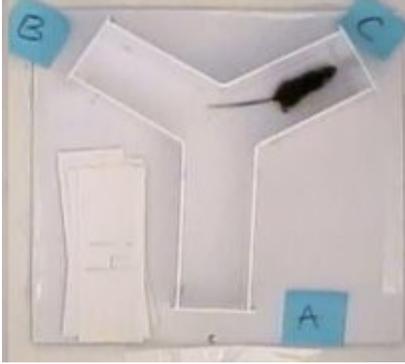
Day 2: Novel Context  
(Tone, Odor 2, Light 2)



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,

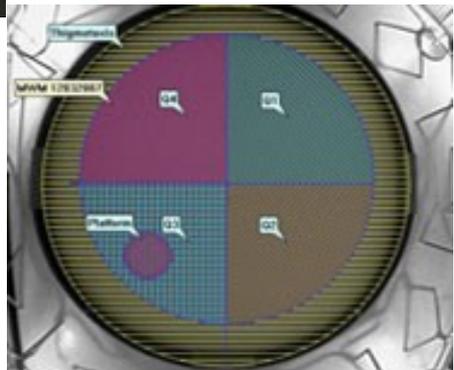
septum, basal forebrain, and prefrontal cortex--are involved in this task. This test is used to quantify cognitive deficits in transgenic strains of mice and evaluate novel chemical entities for their effects on cognition.

## MORRIS WATER MAZE



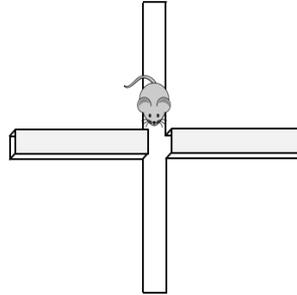
The Morris Water Maze is designed to test spatial memory by observing the subject's ability to find a hidden platform in an opaque water tank.

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.



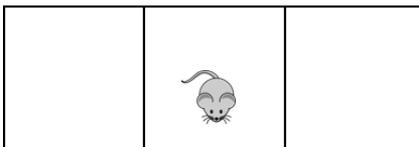
*Ethovision Pro(Noldus Information Technology, Wageningen, the Netherlands)*

## ELEVATED PLUS MAZE

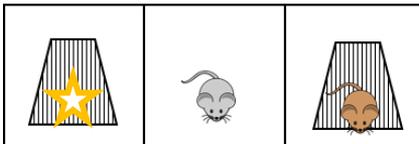


The Elevated Plus Maze test is used to assess anxiety-related behavior in rodent models. The apparatus consists of a “+”-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center area. The subjects freely explore the maze as their 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.

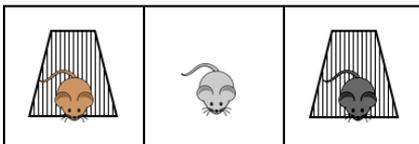
## THREE CHAMBER SOCIAL TEST



Habituation: Empty Apparatus



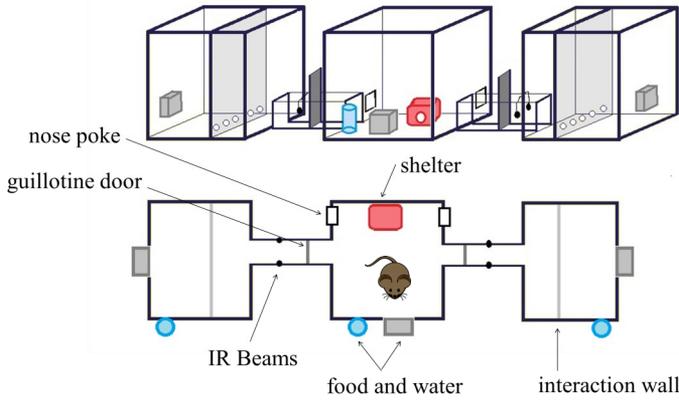
Sociability: Novel Object; Mouse 1



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



Sociability: Novel Object; Mouse 1



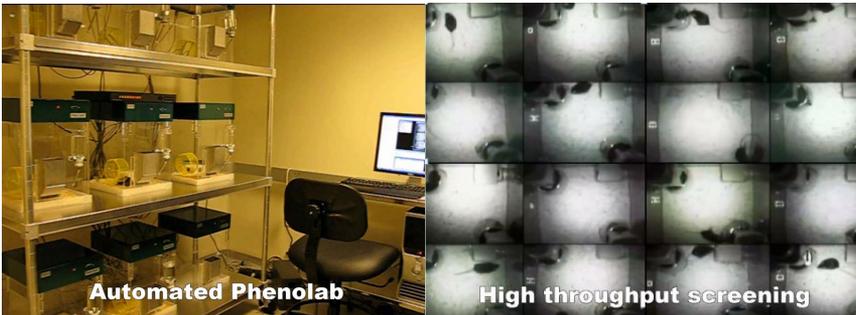
Social Novelty: Mouse 1; Mouse 2

*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.

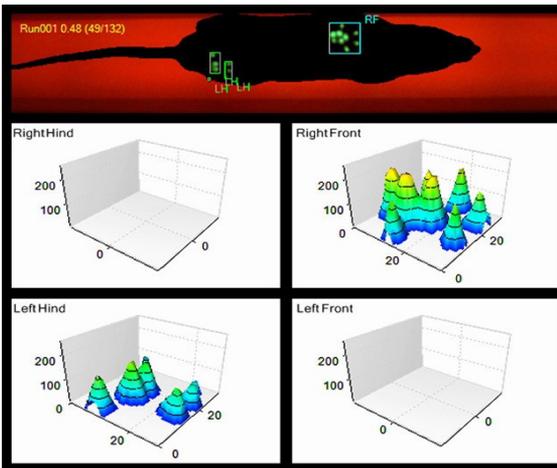
## AUTOMATED HOME CAGE TESTING

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

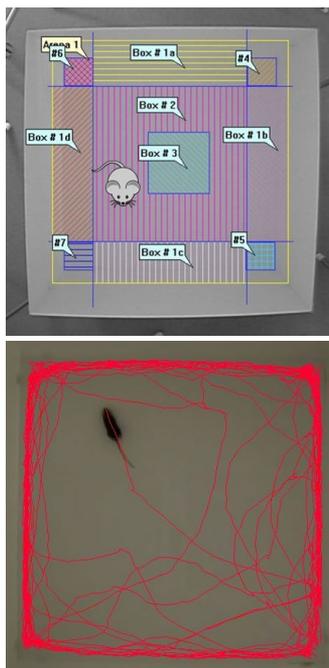


The Catwalk is an automated gait analysis system used to assess motor function and coordination in rodent models of CNS disorders. Subjects walk across an illuminated glass platform while a video camera records from below. Gait related parameters—such as stride pattern, individual paw swing speed, stance

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.

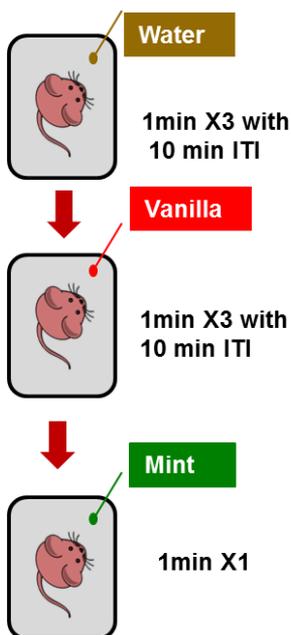
## OPEN FIELD

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.

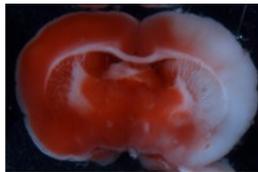
Stanford Behavioral and Functional Neuroscience Laboratory is interested in studying the complex, whole-system effects of disease models and potential treatments. We utilize our expertise in pharmacological and disease models to better study neurological disorders and treatments.

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

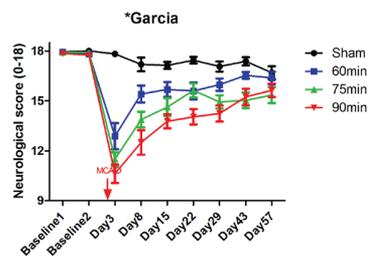
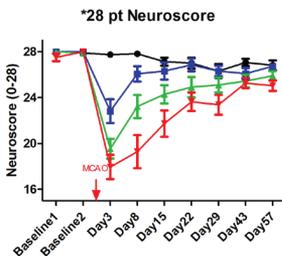


2h MCAO

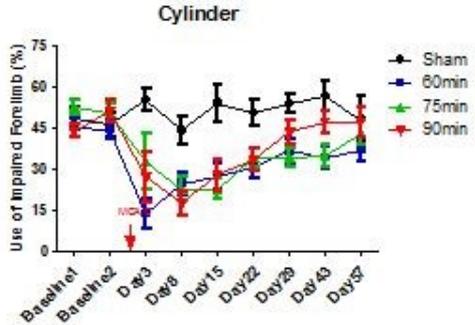
Middle cerebral artery occlusion (MCAO) is a surgically induced cerebral ischemia in rats. In this experiment, we test

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.

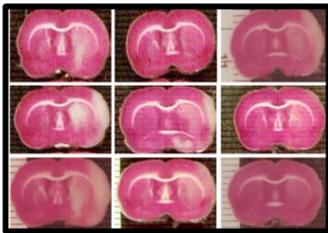


### NEUROPROTECTION ACHIEVED BY CBX

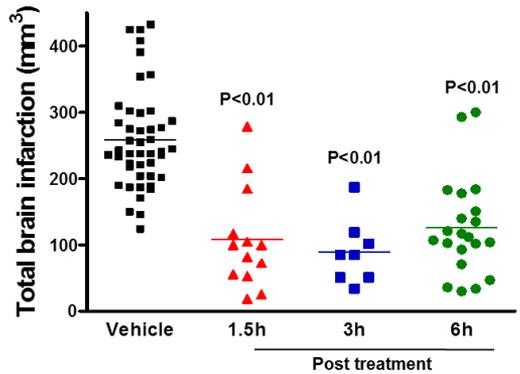
CBX treatment post-stroke reduces immediate damage to cerebral tissue caused by ischemia.



Vehicle



CBX 3 and 7 h



## CONTACT US

For more information about Stanford Behavioral and Functional Neuroscience Laboratory, please visit our website at: <http://sbfnl.stanford.edu>, or contact :

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