



- Neurology and Psychiatry Drug Profiling
- *In Vivo* and *In Vitro* Assays
- Histological Analysis
- Behavioural Tests
- Transgenic/Knockout Mouse Phenotyping

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## 1. *IN VITRO* ASSAYS

NEUROFIT offers a large range of models and assays for evaluating neurotoxicity, neuroprotection or neurotrophic activity of new test compounds.

NEUROFIT can also develop custom protocols to determine mechanism of action, or define therapeutic indication of new drug candidates.

### **RODENT PRIMARY CULTURE**

- **Cortical, Hippocampal, Mesencephalon neurons**
- **Spinal cord motoneurons**
- **Sensory neurons**
- **Schwann cells – sensory neurons co-culture**
- **Rat nerve - human muscle co-culture**
- **Glial cells: astrocyte or oligodendrocyte precursor**

### **MODELS**

- **Neurotrophic effect:** effects of test compounds on survival rate or neurite outgrowth
- **Exitotoxicity:** neurons intoxicated with glutamate or NMDA
- **Anti-mitotic intoxication:** DRG intoxicated with cisplatin, vincristine or taxol; Spinal motor neurons intoxicated with vincristine
- **Parkinson's models:** Mesencephalon neurons intoxicated with MPP<sup>+</sup>
- **Alzheimer's model:** Hippocampal neurons intoxicated by A $\beta$ 1-40
- **Co-culture nerve/muscle:** effects of test compounds on co-culture innervation
- **Multiple sclerosis:** Oligodendrocyte precursor proliferation

### **CANCER CELL LINES**

- **Growth inhibition of MCF7, SK-MES1, ... cell lines by chemotherapeutic agent**

### **ASSAYS**

- **Viability and survival tests:** LDH, AP, MTT assays
- **Neurotrophic effect:** neurite length, percentage of cells displaying neuritis,
- **Western blotting of proteins**
- **Co-culture:** length of neuritis, surface of innervated muscle fiber area, video samples

## 2. NEUROLOGY PROGRAM

NEUROFIT has developed powerful testing *in vivo* methods in rodents for assessing the neurotoxicity, neuroprotective or neurotrophic effects of drug candidates. Studies may include quantitative behavioural measurements, or analyses of effects upon neurodegenerating nerve fibers (axonopathy) or nerve cells (neuronopathy).

### NEUROLOGICAL AND NEURODEGENERATIVE MODELS

- **ALZHEIMER'S DISEASE:** Scopolamine, MK-801 or PCP-induced cognitive deficits
- **EPILEPSY:** PTZ-induced convulsion
- **MULTIPLE SCLEROSIS:** DTH, EAE, RR-EAE rats
- **ARTHRITIS:** Collagen induced arthritis
- **PARKINSON'S DISEASE:** Fri/Fri mice
- **PERIPHERAL NEUROPATHIES**
  - **Traumatic neuropathies:** sciatic nerve crush
  - **Diabetic neuropathy:** STZ rats
- **PAIN AND NEUROPATHIC PAIN**
  - Diabetes-induced neuropathic pain: STZ rats
  - Formalin-induced pain
  - Acute pain

### TESTS

- **MOTOR COORDINATION**
  - Rotarod
  - Bar test
  - Gait analysis
  - Grid test
  - Hanging test
  - String test
- **MUSCULAR STRENGTH, MUSCLE ENDURANCE**
- **SPONTANEOUS ACTIVITY** (test for sedation)
- **SENSITIVITY TESTS:** Tail flick, hot plate test
  - Allodynia evaluation: Von Frey test
  - Hyperalgesia test: Hot plate test (52°C), Paw pressure test (Randall-Sellito)
- **EMG TEST**
  - Sensory and motor nerve conduction velocity (SNCV and MNCV)
  - CMAP
  - H reflex

### **3. HISTOLOGICAL ANALYSIS**

**NEURODEGENERATION INVESTIGATION ON PERIPHERAL NERVES** (computer-assisted analysis of nerve fiber morphometry)

- axonal area
- g-ratio (relative myelin thickness)
- fiber size distribution
- fiber density

**STANDARD COLORATION ON PARAFFIN & CRYOSECTIONS**

- HE staining
- Toluidin blue

## 4. PSYCHIATRIC & COGNITIVE DISORDERS

NEUROFIT offers a variety of well-accepted behavioural tests to assess the potential anxiolytic, antidepressant, antipsychotic or cognitive effect of test compounds.

### **ANXIETY**

- Light-dark test
- Marble burying
- Elevated plus maze
- CCK-4 –induced panic anxiety

### **DEPRESSION**

- Tail suspension test
- Forced swimming test

### **SCHIZOPHRENIA**

- MK801-induced hyperactivity test
- D-amphetamine-induced hyperactivity test

### **CATALEPSY**

### **COGNITIVE FUNCTION**

- Object recognition test
- T-maze
- Conditioned place preference test
- Passive avoidance test

### **SIDE EFFECTS**

- Open field
- Rotarod test
- Object recognition test
- T-maze
- Passive avoidance test
- String test

## **5. PHENOMOUSE™ - TRANSGENIC AND KNOCKOUT MOUSE PHENOTYPING**

Neurofit offers phenotype screening services for transgenic or knockout mice. Typically this is carried out in a series of stratified tests beginning with behavioural observations and quantitative behavioural measurements. Based upon these initial results, secondary and tertiary level tests may be indicated for a more exhaustive assessment of the mouse phenotype. For the primary test battery (behavioural observation) 8-10 animals of each phenotype are recommended, i.e. 8-10 homozygous mutant (-/-) and 8-10 wild-type (+/+).

For the secondary and tertiary test batteries, a minimum of 10 animals of each phenotype are preferable.

### **STEP 1: PRIMARY TEST BATTERY**

- Bizarre and stereotyped behaviour
  - Urination/defecation
  - Stereotypies
  - Body position
  - Spontaneous activity
  - Respiration rate
  - Tremor
  
- Behavior in a new environment
  - Palpebral closure
  - Locomotor activity
  - Tail elevation
  - Touch escape
  - Startle response
  - Positional passivity
  
- General behavior / reflexes
  - Visual placing
  - Grip strength
  - Body tone
  - Pinna reflex
  - Corneal reflex
  - Aggressively
  - Convulsion
  - Righting reflex
  - Skin color

Evolution of body weight is recorded over one week.

## **STEP 2: SECONDARY TEST BATTERY**

- Locomotor activity (in open-field twice a day at the beginning and the end of the animal cycle)
- Anxiety (Light/dark boxes test)
- Depression (Forced swimming test)
- Memory (Object recognition test)
- Analgesia (Hot plate)

## **STEP 3: TERTIARY TEST BATTERY**

According to the deficits observed in the first batteries, an acute observation in one or several pathologies:

- Anxiety
  - Light/dark boxes
  - Marble burying
  - Elevated plus-maze
- Memory and learning
  - Object recognition test
  - T-maze
- Schizophrenia
  - Hyperactivity test
- Peripheral disorders
  - Electromyographical testing (EMG testing)
  - Motor-coordination tests
  - Sensorimotor analysis
- Biochemical analysis
- Histo-morphology

## **STEP 4: TEST BATTERY**

Pharmacological analysis with reference compounds.