



NEUROFIT

PRECLINICAL RESEARCH

INDEX

1. NEUROLOGY PROGRAM p. 2
2. PSYCHIATRIC & COGNITIVE DISORDERS p. 3
3. INFLAMMATION PROGRAM p. 4
4. NEUROCELLULAR MODELS p. 5
5. SPASTICITY p. 6
6. NERVE CONDUCTION STUDY p. 7
7. NERVE FIBER MORPHOMETRY ANALYSIS p. 7

NEUROFIT offers a comprehensive list of disease models in rodents for assessing the efficiency of drug candidates. Studies include quantitative behavioural measurements to evaluate the impact of the treatment on the disease symptoms.

1. ■ NEUROLOGY PROGRAM

Diseases	Therapeutic screening target	Models
Alzheimer's disease	<ul style="list-style-type: none"> Procognitive drugs 	<ul style="list-style-type: none"> Scopolamine - induced cognitive deficit Methyllycaconitine - induced cognitive deficit LPS - induced cognitive deficit Cognitive deficit in aged mouse
	<ul style="list-style-type: none"> Disease modifier drugs 	<ul style="list-style-type: none"> icv Aβ - induced cognitive deficit
Parkinson's disease	<ul style="list-style-type: none"> Disease modifier / symptomatic drugs 	<ul style="list-style-type: none"> 6-OHDA - induced hemiparkinson lesion
	<ul style="list-style-type: none"> Anti-akinesia effect 	<ul style="list-style-type: none"> Haldol - induced akinesia
Epilepsy	<ul style="list-style-type: none"> Anticonvulsive drugs 	<ul style="list-style-type: none"> PTZ - induced convulsion
Peripheral neuropathies	<ul style="list-style-type: none"> Neuroprotectant, neuroregenerative drugs (Nerve repair / regeneration) 	<ul style="list-style-type: none"> Sciatic nerve crush Diabetes - induced neuropathy (STZ - rats)
Neuropathic pain	<ul style="list-style-type: none"> Pain relief drugs 	<ul style="list-style-type: none"> Diabetes - induced neuropathic pain (STZ - rats) Formalin test Acute pain

NEUROFIT offers a variety of well-accepted behavioural tests to assess the potential anxiolytic, antidepressant, antipsychotic or procognitive effect of test compounds. In addition, animal models have been established in order to better mimic the clinical symptoms of these disorders. Assays and models are validated with drugs used in the clinical setting (reference compounds).

2. ■ PSYCHIATRIC & COGNITIVE DISORDERS

TEST	ANIMAL MODELS
<p>■ ANXIETY</p> <ul style="list-style-type: none"> ■ Light-dark test ■ Marble burying ■ Elevated plus maze 	<ul style="list-style-type: none"> ■ CCK-4 - induced panic anxiety ■ Yohimbine - induced panic anxiety
<p>■ DEPRESSION</p> <ul style="list-style-type: none"> ■ Marble burying ■ Forced swimming test 	
<p>■ SCHIZOPHRENIA</p> <ul style="list-style-type: none"> ■ Hyperactivity in open-field 	<ul style="list-style-type: none"> ■ MK-801 - induced hyperactivity ■ D-amphetamine - induced hyperactivity ■ Phencyclidine - induced hyperactivity
<ul style="list-style-type: none"> ■ T-maze 	<ul style="list-style-type: none"> ■ MK-801 - induced cognitive deficit ■ Phencyclidine - induced cognitive deficit <ul style="list-style-type: none"> □ Acute □ Subchronic PCP followed by withdrawal
<p>■ CATALEPSY</p>	<ul style="list-style-type: none"> ■ Haldol - induced catalepsy
<p>■ COGNITIVE & MEMORY DEFICIT</p> <ul style="list-style-type: none"> ■ Object recognition test 	<ul style="list-style-type: none"> ■ Scopolamine - induced cognitive deficit
<ul style="list-style-type: none"> ■ T-maze 	<ul style="list-style-type: none"> ■ MK-801 - induced cognitive deficit
<ul style="list-style-type: none"> ■ Passive avoidance test 	<ul style="list-style-type: none"> ■ Phencyclidine - induced cognitive deficit ■ Methyllycaconitine - induced cognitive deficit ■ Natural forgetting ■ LPS Neuroinflammation - induced cognitive deficit

NEUROFIT provides *in-vivo* models for inflammation, contact allergic and autoimmune diseases.

3. ■ INFLAMMATION PROGRAM

Diseases	Therapeutic screening target	Models
Multiple sclerosis	Disease modifiers / neuroprotectant	<ul style="list-style-type: none"> ■ Relapsing-Remitting EAE in Dark-Agouti rats ■ MBP-EAE in Lewis rats
Rhumatoid arthritis	Immunomodulators, Anti-inflammatory, Immuno-suppressors	<ul style="list-style-type: none"> ■ Pristane - induced Rheumatoid arthritis ■ Collagen - induced Rheumatoid arthritis
Allergic contact dermatitis	Immunomodulators, Immuno-suppressors, Anti-inflammatory drugs	<ul style="list-style-type: none"> ■ Oxazolone - induced allergic contact ■ Ovalbumin - induced allergic contact
Acute inflammation	Immunomodulators, Immuno-suppressors, Anti-inflammatory drugs	<ul style="list-style-type: none"> ■ Carrageenan mouse air pouch model
Neuroinflammation	Cognitive enhancers, Immunomodulators, Immuno-suppressors, Anti-inflammatory drugs	<ul style="list-style-type: none"> ■ LPS - induced neuroinflammation <ul style="list-style-type: none"> ▫ cytokine release ▫ cognitive deficit

NEUROFIT offers a comprehensive list of neurocellular models and assays for evaluating neurotoxicity, neuroprotection or neurotrophic activity of new test compounds.

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

4. ■ NEUROCELLULAR MODELS

<p>RAT PRIMARY CULTURES</p>	<ul style="list-style-type: none"> ■ Cortical, Hippocampal, Mesencephalon neurons ■ Spinal cord motoneurons ■ Purified sensory neurons : embryos and adults ■ Schwann cells - sensory neurons co-culture ■ Rat nerve-human muscle co-culture ■ Oligodendrocyte precursor
<p>MODELS</p>	<ul style="list-style-type: none"> ■ Parkinson's models: survival of mesencephalic dopaminergic neurons intoxicated with MPP+ or with 6-OHDA ■ Alzheimer's model: survival of hippocampal neurons intoxicated by $\left. \begin{array}{l} A\beta 1-40 \\ A\beta 1-42 \end{array} \right\}$ ■ ALS model (Excitotoxicity): survival of neurons intoxicated with glutamate or NMDA ■ Co-culture nerve/muscle: effects of test compounds on co-culture innervation ■ Multiple sclerosis: Oligodendrocyte precursor proliferation ■ Stroke: survival of neurons intoxicated with glutamate or NMDA ■ Peripheral neuropathies: survival of DRG neurons and status of neurite network intoxicated with cisplatin, vincristine or taxol; survival of spinal motor neurons intoxicated with vincristine ■ Neuroinflammation : LPS - stimulated co-culture of glia cells and neurons (survival / cytokine release)
<p>ASSAYS</p>	<ul style="list-style-type: none"> ■ Viability and survival tests: LDH, Acid Phosphatase, MTT assays, ATP ■ Neurotrophic effect: neurite length and density, number of branch point neuritis ■ Co-culture: length of neurites, surface of innervated muscle fiber area, innervation rate, video samples ■ Cytokine/chemokine measurement: IL1α, IL1β, IL2, IL6, IL10, IL12p40, IL17, IL18, TNFα, TNFβ, IFNγ, PGE2, CCL5, CXCL10 ■ Nitric oxid measurement

NEUROFIT is able to characterize, evaluate and follow the impact of chemodenervation agents in both **rats** and **mice**.

The main and most sensitive read-out is the mesure of the amplitude of the compound muscular action potential (**CMAP**) in the injected muscle following the stimulation of the motor nerve

5. ■ SPASTICITY (HYPERTONIA)

Pharmacological treatments of spasticity available at Neurofit include:

<p>CHEMODENERVATION</p>	<p>by intramuscular BOTULINUM TOXIN or its derivatives :</p> <p>The procedure consists of injecting Botulinum neurotoxins (Botox®, Myobloc®, Dysport® and Xeomin®) into the muscle to disrupt the contraction and allow muscle relaxation via inhibition of the exocytosis of synaptic vesicles containing acetylcholine.</p>
<p>NEUROLYSIS</p>	<p>with PHENOL or ETHANOL injection :</p> <p>The procedure involves a perineural injection of Phenol (or other neurolytic agents) onto the sciatic nerve and the evaluation of the amplitude of the Compound Muscular Action Potential (CMAP) in the gastrocnemius (target muscle) following the stimulation of the sciatic nerve.</p>
<p>CONDUCTION BLOCK</p>	<p>with ANESTHETICS or CURARE-like compounds :</p> <p>The use of curare or curare-like agents as a non-destructive nerve block methods is common practice in anesthesiology to partially or completely block the motor nerve activity and consequently induces muscle relaxation.</p>

6. ■ NERVE CONDUCTION STUDY

NEUROFIT performs nerve conduction studies in various animal models of nerve injury, peripheral neuropathy and myopathy in order to evaluate the impact of drug treatment on nerve-muscle function.

Nerve conduction studies consist of:

- **compound muscle action potential (CMAP)**
also known as motor nerve conduction study
- **sensory nerve action potential (SNAP)**
also known as sensory nerve conduction study

7. ■ NERVE FIBER MORPHOMETRY ANALYSIS

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

- Axon size distribution, axon density
- g-ratio distribution (Myelin thickness)
- Debris clearance
- Regenerating axons
- Nerve caliber
- Intra Epidermal Nerve Fiber density (IENF)