ÑewroPharma

Programa de Innovación Terapéutica

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Salud Mental

MINISTERIO DE CIENCIA E INNOVACIÓN

Instituto de Salud Carlos III

Unión Europea

About us

NewroPharma is a platform of preclinical services created by *CIBERSAM*. We provide a comprehensive list of preclinical drug development services to pharmaceutical partners, biotech companies and academic research organizations covering every essential milestone in preclinical drug development

CIBERSAM is a translational research network on mental health supported by Carlos III Health Research Institute (ISCIII), and the Spanish Ministry of Science, Innovation and Universities. CIBERSAM comprises 23 clinical and basic research groups settled at multiple institutions: universities, hospitals, CSIC (Consejo Superior de Investigaciones Científicas), and other public research organizations. The *CIBERSAM* community accounts over 350 people, including psychiatrists, university professors, research fellows, technicians and administrative staff. *CIBERSAM* covers a broad spectrum of research initiatives on mental health, bridging between bench discoveries to patient care.

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About us

Our research structure

- Basic
- Pre-clinical
- Clinical

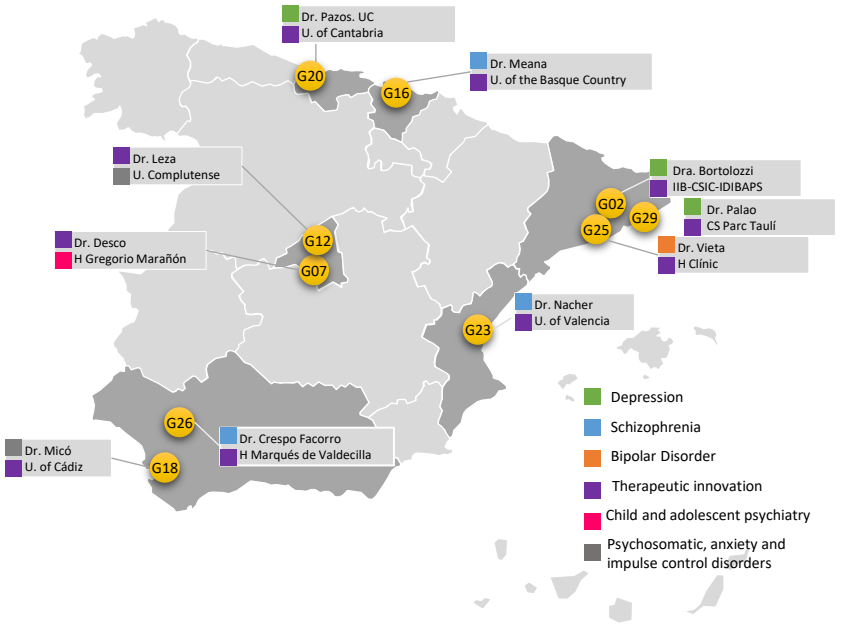
Translational

- Multidisciplinary
- Multicentre

Six Scientific Programmes oriented to the most significant pathologies:

- Depression
- Schizophrenia
- Bipolar Disorder
- Therapeutic Innovation
- Child and Adolescent Mental Disorders
- Psychosomatic, Anxiety and Impulse Control Disorders

Who and where we are



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Drug development plan

Patients

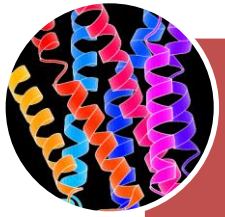
- Strong clinical understanding of human neuropsychiatric disorders: CIBERSAM

Human tissues

- Clinical evidences
- Human tissues
- Human genetics
- Human pathology

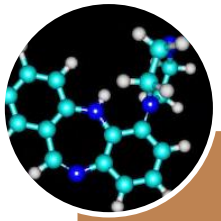
Target identification

- Case-control tissues
- Hypothesis-driven
- Hypothesis-free



Drug discovery

- Bioassay development
- Compound screening



Target validation

- Pharmacodynamics
- Mechanism of action
- Animal models of the disease
- *In vivo* studies
- Phenotypic screening

Clinical trials

NewroPharma offers solutions at all preclinical stages of your drug R&D plan

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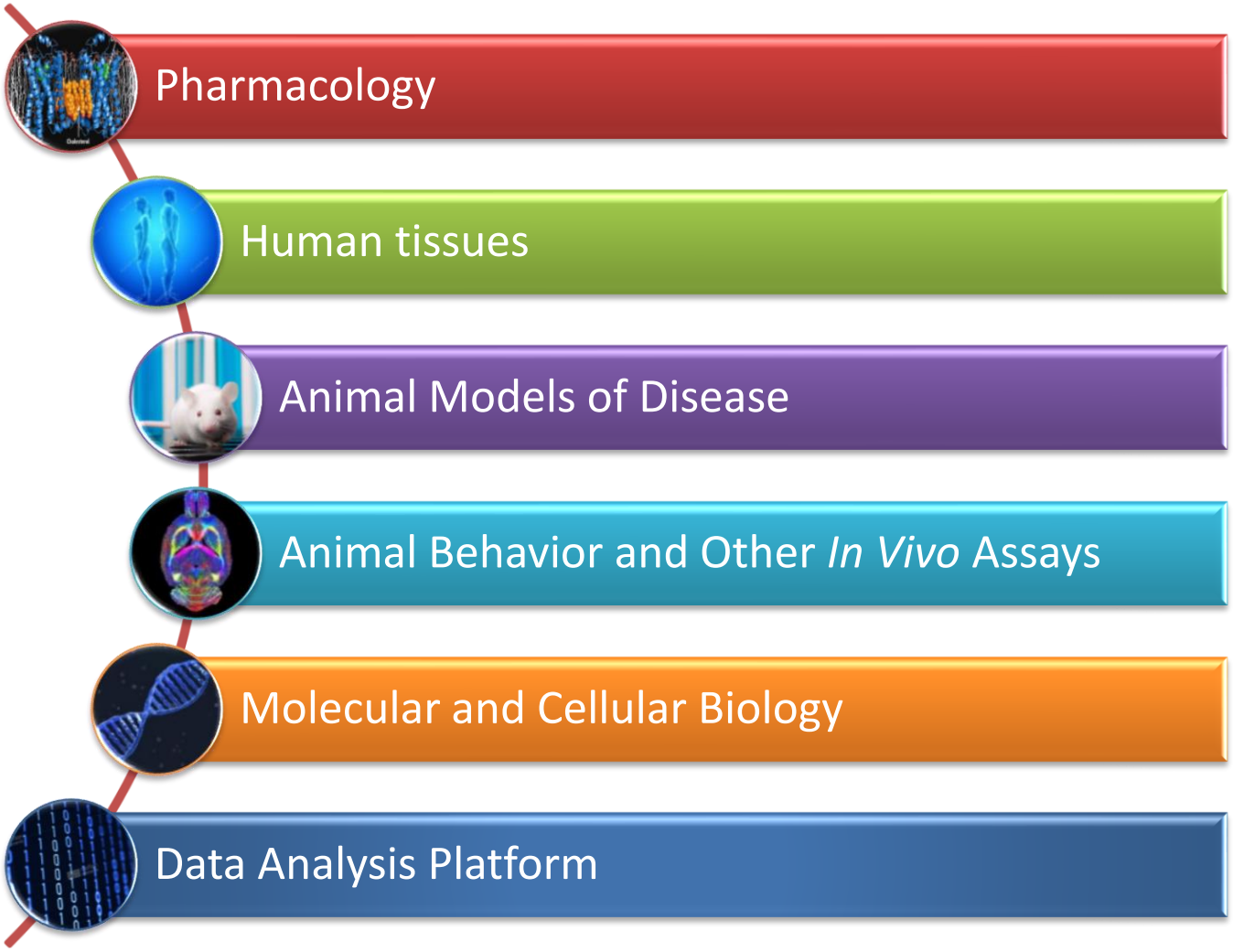
Catalogue of experimental methodologies



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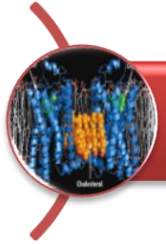

Pharmacology

❑ Facilities

- Category 3 Radioactive Laboratory (authorization for work with ^{35}S, ^{3}H, ^{14}C, ^{32}P and ^{33}P).
- *MicroBeta*^{2} 96-well microplate scintillation and luminescence counter (PerkinElmer) for semi-automated, high-throughput assays.
- QImaging Retiga R1 Camera for densitometric analysis.

❑ Radioligand assays

- Quantitative radioligand binding assays.
- Functional studies of GPCR signaling activity by [^{35}S]GTPγS binding studies.
- Profiling of ligand-elicited activation pattern of G-protein subtypes with Scintillation Proximity Assay (SPA) (description of biased drugs).
- Pharmacological profiling of ligands: determination of affinity, potency, efficacy (K_D, K_I, IC_{50}, ED_{50}, Emax) and intrinsic activity (full/partial/inverse agonist, neutral antagonist) parameters.
- Autoradiography with [^{3}H]- and [^{35}S]-labeled ligands for neuroanatomic distribution, quantitative, and functional assessments.
- Radioimmunoassay of drugs, hormones and other compounds
- Capacity of testing a wide variety of biological samples, including postmortem tissues and biopsy from human, non-human primates and rodents.

Human tissues

In essence, ***NewroPharma*** is a platform of preclinical services. However, preclinical drug development may require (or at least benefit from) the inclusion of clinical samples for target discovery and validation. Our platform has access to three highly-valuable human sample resources:

- **Repository of case/control human samples**
- **Postmortem human brain collection**
- **Human olfactory neuroepithelium cells**

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DNA Collection

Repository of case/control human samples

- The **CIBERSAM's collection of DNA samples** is a large repository of clinical samples containing over 20,000 samples from psychiatric patients and controls.
- Cases with severe mental illnesses: major depression, bipolar disorder, schizophrenia.
- Age- and sex- matched controls. Cohorts of disease-free family members.
- DNA, mRNA, and platelet extractions are available.

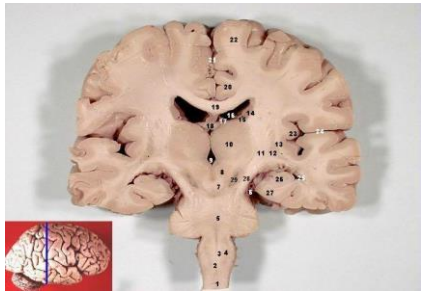
Human tissues

Brain Samples

Postmortem human brain collection

Our platform holds the Brain Collection of the University of the Basque Country (UPV/EHU). Specimens are obtained at autopsies performed in the Basque Institute of Legal Medicine, and transferred to the Collection under coded identification. Clinical histories and blood toxicological assessments allow retrospective search for ICD/DSM-diagnosed cases with mental illnesses. Controls are carefully selected to match those demographic features in the selected psychiatric cases. Currently, the collection accounts more than 1,600 brains. Frozen samples from the dorsolateral prefrontal cortex, and paraffin-embedded sections are available.

Groups of subjects by antemortem ICD diagnoses of severe mental illnesses	N
Subjects with **major depressive disorder**	91
Subjects with **schizophrenia**	157
Subjects with **bipolar disorder**	59
Subjects with addictive disorders	
Alcohol abusers	173
Heroin abusers	47
Polydrug abusers	46
Control subjects	**N**
Control subjects (free from psychiatric or neurologic conditions)	247
Suicide victims with mild psychiatric condition	54



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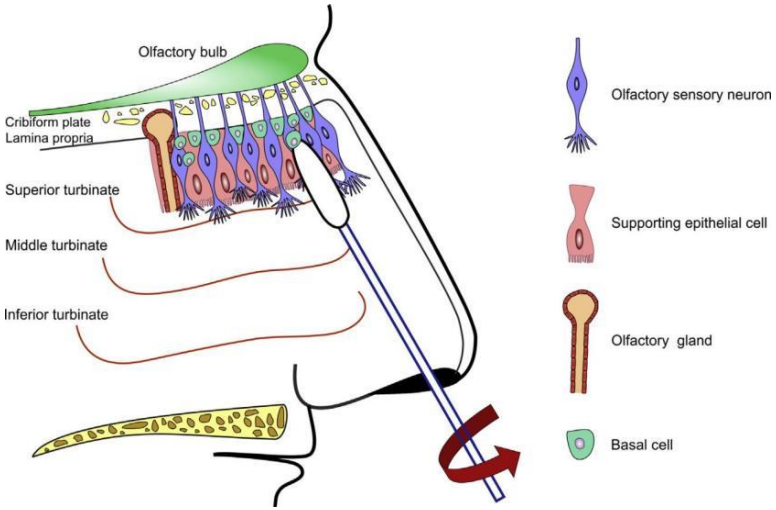
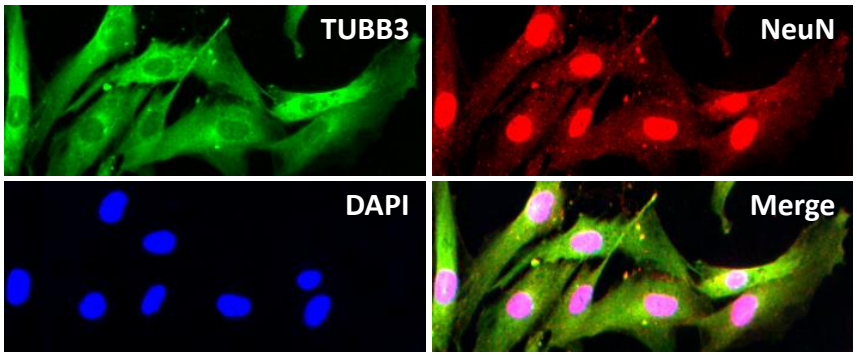
Olfactory neuroepithelium cells

- The human olfactory neuroepithelium is a direct source of neural progenitor cells, and their extraction requires minimal invasive procedures.
- We collect samples from psychiatric patients and control volunteers that will derive into neurons under the appropriate culture conditions.
- To find new biomarkers and/or targets for drug discovery, neuronal populations derived from the human olfactory neuroepithelium can be used in functional and biochemical assays



Structure of the human olfactory neuroepithelium and extraction procedure



Neurons derived from human olfactory neuroepithelium

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Animal Models of Disease

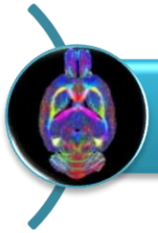
❑ Validated animal models of neuropsychiatric diseases available at ***NewroPharma***

Disease / symptom	Species	Model description	In-house key references
Depression / Stress	Rat and mouse	Stress associated with repeated exposure to corticosterone	*Mol Psychiatry.* 2016, 21: 328-338
	Rat and mouse	Unpredictable chronic mild stress (UCMS)	*Eur. Neuropsychopharmacol.* 2014, 24: 996-1003
	Rat and mouse	Peripubertal stress	PLoS One. 2014, 9: e94666
	Mouse	Small interfering RNA-induced infralimbic cortex overactivation	*Glia* 2019, 67: 1122-1137
	Rat and mouse	Restraint stress	*Neuropsychopharmacology* 2003, 28: 1579-1588.
	Rat and mouse	Social isolation	*Pain* 2013, 154: 2014-2023
	Rat and mouse	Early maternal deprivation	*Neuropharmacology* 2012, 62: 1944-1953
	Rat and mouse	Comorbid pain and depression model	*Anesthesiology* 2012, 117: 613-625
	Rat and mouse	Olfactory bulbectomy	*Mol. Pharmacol*. 2010, 77: 424-434
PTSD	Rat and mouse	Repeated (un)conditioned fear-inducing stimuli	*Am. J. Psychiatry*. 2011, 168:163-172
Schizophrenia / Psychosis	Rat and mouse	Poly(I:C)-induced maternal immune activation model	*Neuropharmacology* 2017, 116: 196-207
	Rat and mouse	Poly(I:C) + Social isolation two-hit model	*Neurobiol. Dis*. 2013, 59: 126-140
	Rat and mouse	Acute or repeated exposure to NMDA channel blockers	*Neuropharmacology* 2019, 158: 107745
	Mouse	*STXBP1* overexpression in GABA and glutamatergic neurons	*Transl. Psychiatry* 2013, 3: e221
Autism	Mouse	*CNTNAP2* gene full knockout	*Cell* 2011, 147: 235-246
	Mouse	*FMR1* gene full knockout	*Mol. Psychiatry* 2015, 20: 118-25
Alzheimer´s disease	Mouse	5xFAD overexpressing C57BL/6 mice	*Mol Neurobiol*. 2019, 56: 8628-8642
Parkinson´s disease	Mouse	Overexpression of pathogenic alpha and gamma-synuclein Overexpression of neuromelanin	*eBioMedicine* 2020, 59: 102944 *Nat Commun* 2019, 10:973
Chronic pain	Rat and mouse	Chronic constriction of the sciatic nerve	*Biol. Psychiatry* 2013, 73: 54-62
	Rat and mouse	Streptozotocin administration (STZ)	*Eur. J. Pain* 2018, 22: 127-141
	Rat and mouse	Freund's adjuvant-induced monoarthritis	*Int. J. Neuropharmacol.* 2015, 18: pyv019
Neuroinflammation	Rat and mouse	Lipopolysaccharide (LPS)-induced neuroinflammation	*Biol. Psychiatry* 2013, 73: 32-43
Multiple sclerosis	Mouse	Experimental autoimmune encephalomyelitis (EAE)	*J. Neuroinflammation* 2010, 7: 60

Animal Models of Disease

- Other genetically modified animal models

Target gene	Protein effect	Species	Genotype	In-house key reference
TH:Cre	Expression of Cre recombinase under the tyrosine hydroxylase promoter	Rodents	Transgenesis	*Prog Neuropsychopharmacol Biol Psychiatry*. 2021, 104: 110030 *Biol Psy.* 2019, 85: 1021-1035
HTR2A	Serotonin 5-HT_{2A} receptor knockout	Mouse	Full knockout	*Nat. Neurosci*. 2012, 15: 1245-1254
GRM2	Metabotropic glutamate receptor-2 (mGluR2) knockout	Mouse	Full knockout	*Sci. Signal.* 2016, 9: ra5
GRIN2C	Knockout for the GluN2C subunit of the NMDA glutamate receptor	Mouse	Full knockout	*Transl. Psychiatry* 2020, 10: 427
CTNNB1	β-catenin conditional knockout	Mouse	Conditional knockout	*Mol. Neurobiol*. 2019, 56: 553-566
CTNNB1	Overexpression of β-catenin	Mouse	Transgenesis	*Mol. Neurobiol*. 2020, 57: 1704-1715
HTR1A	Overexpression of serotonin 5-HT_{1A} receptor in the postsynaptic terminals	Mouse	Transgenesis	*ACS Chem. Neurosci.* 2017, 8: 2393-2401
Thy1-YFP	Thy1-YFP insertion preceded by different promoters to allow selective labelling of neuronal populations	Mouse	Transgenesis	*J Neurosci*. 2020, 40: 5008-5018
Tac2:Cre & PACAP:Cre	Expression of Cre recombinase under the Tac2 or PACAP gene promoters	Mouse	Transgenesis	*Neuron* 2014, 83:444-454 *eLife* 2018, 7: e35960



Animal Behavior and Other *In Vivo* Assays

NewroPharma offers a wide variety of *in vivo* assays in rodents oriented to CNS drug development.

- **Behavioral tests in rodents**
- ***In vivo* neuroimaging**
- **Microdialysis**
- **Electrophysiology**
- **Deep brain stimulation**
- **Pupillometry**
- **Modulation of gene expression in rodent brain**

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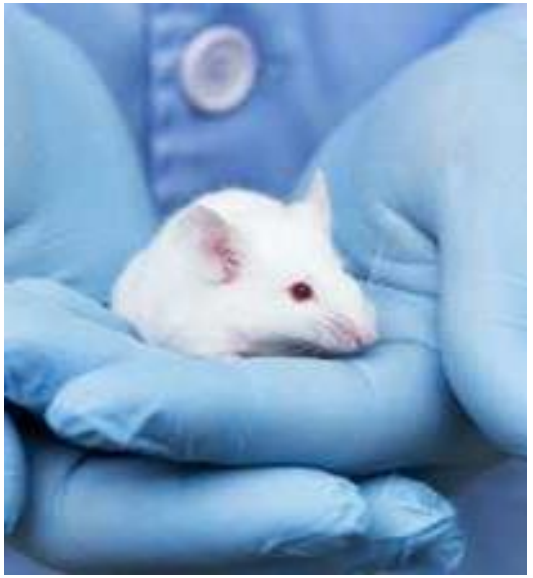
Behavioral tests in rodents (1/2)

Behavioral domain	Test name	Disease relevance
Cognitive abilities	Radial maze test (8 arms)	Disorders with cognitive deficit (e.g. dementia, schizophrenia, autism)
	Y-maze test	
	Morris water maze test	
	Passive / active avoidance test	
	Pattern separation test	
	Novel object recognition test	
	Touchscreen Operant Platform, optimized for: • Five-choice serial-reaction time task (5CSRTT) • Paired Associates Learning (PAL) • Pairwise Visual Discrimination (PVD) task	
	T-maze test	
	Fear conditioning test	
	Latent inhibition test	
Anxiety	Novelty-suppressed feeding test	Anxiety and depressive disorders
	Open field test	
	Elevated plus-maze	
	Light-dark box test	
	Zero-maze test	
	Marble burying test	
Depressive-like behavior	Sucrose /saccharin preference test	
	Splash test	
	Forced swim test	
	Tail suspension test	
	Conditioned place preference and conditioned place aversion	

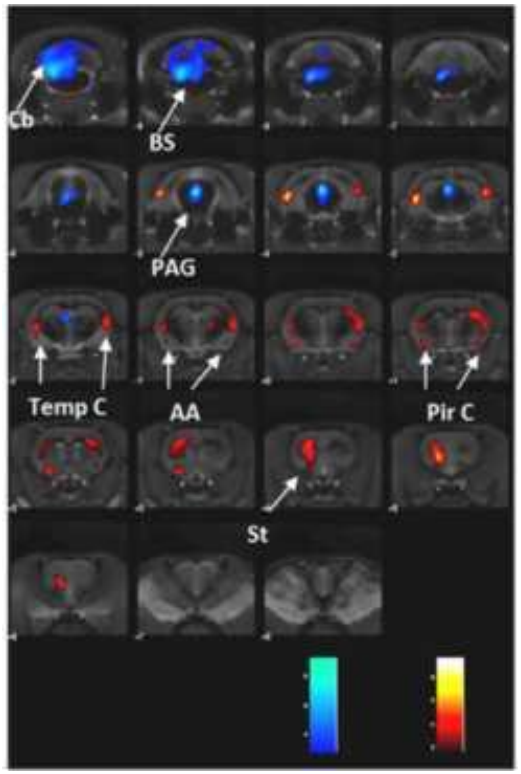


Behavioral tests in rodents (2/2)

Behavioral domain	Test name	Disease relevance
Locomotor abilities	Rota-rod test	Neurologic disorders with psychomotor deficits
	Bar test (catalepsy)	Schizophrenia (evaluation of extrapyramidal effects)
Exploratory skills	Open field test	Autism, schizophrenia
Social interaction	Three-chamber sociability test	
	Reciprocal social interaction test	
Vocal communication	Ultrasonic vocalizations	Autism
Stereotyped/Repetitive behavior	Grooming/digging behavior	
	Spontaneous alternation in T-maze	
Sensorimotor skills	Prepulse inhibition (PPI) test	Schizophrenia
Nociception	Acetone test	Pain mechanisms; substance analgesic efficacy
	Paw pressure test	
	von Frey test	
	Dynamic weight bearing test	
	Hot plate test	
	Cold plate test	
	Spontaneous pain test	
	Conditioned place preference (CPP) test	

In vivo neuroimaging in rodents

Methodology	Instrument
Custom radiotracer synthesis for PET and SPECT	FASTlab Multi-Tracer Platform with $^{68}Ge/^{68}Ga$ generator; Agilent HPLC system
Cerebral glucose metabolism on PET-imaging	PET/CT (Argus, SEDECAL)
Computed Tomography (CT) Scan of the bones	PET/CT (Argus, SEDECAL)
Structural MRI for gray matter volumetric studies	Bruker 7T MRI Biospec 70/20 USR scanner
Structural MRI for white matter volumetric studies	Bruker 7T MRI Biospec 70/20 USR scanner
Nuclear Magnetic Resonance (NMR) spectroscopy	Bruker 7T MRI Biospec 70/20 USR scanner
SPECT scan for rodent brain studies	U-SPECT-II, Milabs
Fluorescence Molecular Tomography (FMT)	In-house developed equipment
Localization of molecular targets by single plane illumination microscope (SPIM) 3D micro-imager	SPIM 4D-Nature
C-arm X-ray imaging	Siemens Siremobil Compact L C-Arm X-Ray



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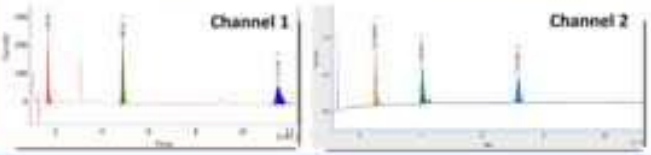
Microdialysis in awake rodents

- Quantitative *in vivo* studies of rodent brain extracellular neurotransmitter concentrations.
- Stereotactic implantation of single or multiple probe
- Four-animal automated station and 5-animal manual station available
- Ultra-low detection limit (0.1-0.5 fmol)
- Time resolution: 15 min bins
- Simultaneous detection of multiple neurotransmitters (monoamines, glutamate, d-serine, GABA, etc...) and their metabolites by HPLC and UPLC

CHROMATOGRAPHIC ANALYSIS (UHPLC-ECD)



✓**Simultaneous analysis of monoamines and metabolites.**

✓Channel 1: noradrenaline, dopamine and serotonin.
✓Channel 2: homovanillic acid (HVA), 5-hydroxyindole acetic acid (5-HIAA), and 3,4-di-hydroxyphenylacetic acid (DOPAC).

- ✓ **Analysis time <15 minutes.**
- ✓ **Detection limit of 0.1 - 0.5 fmol .**
- ✓ **Required sample volume 20 µL.**

MICRODIALYSIS ANALIZER

✓**Simultaneous analysis of glucose, lactate, pyruvate, glycerol, glutamate and urea.**

✓**Kinetic enzymatic analyser**
Sample volume: 0.2 – 1.0 µL.

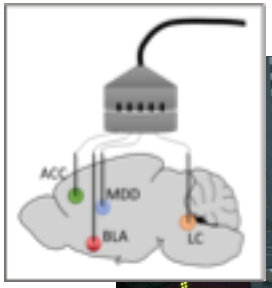
✓**Minimum sample volume:**
Sum of sample volumes per analyte + 2.0 µL.

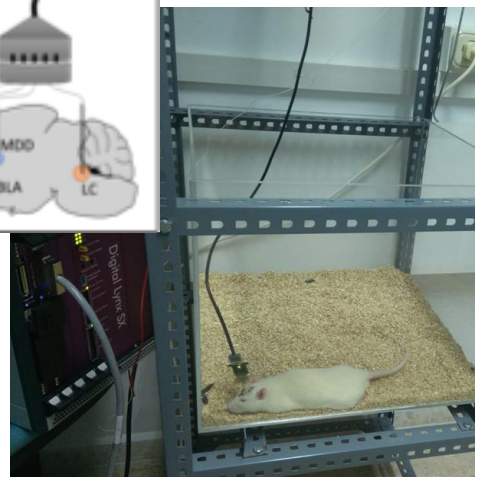
Animal Behavior and Other *In Vivo* Assays

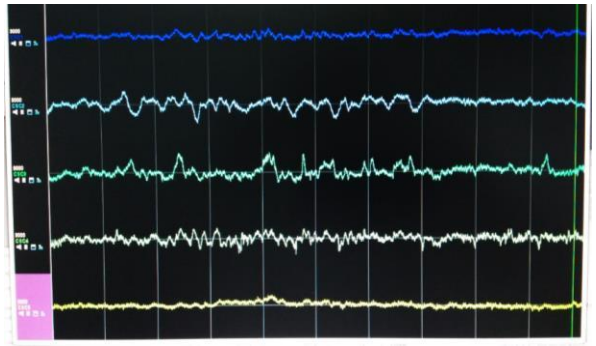
Electrophysiology

- *In vivo* recordings of anesthetized or awake animals.
- Single-units (Spikes), local field potentials (LFP) or electroencephalograms (EEG).
- Behavioral correlation in awake animals.
- Stereotactic implantation of stimulation and recordings electrodes.
- Applicable to rats and mice.

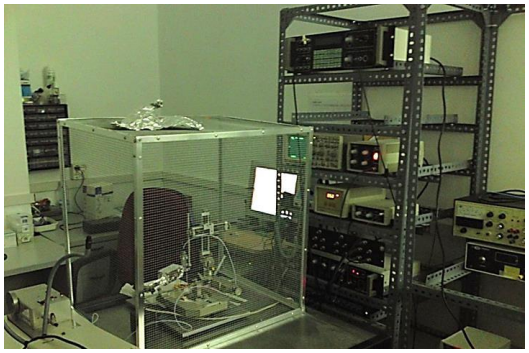
AWAKE ANIMALS

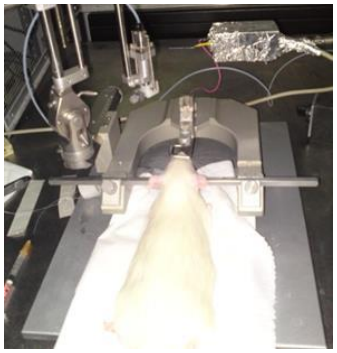


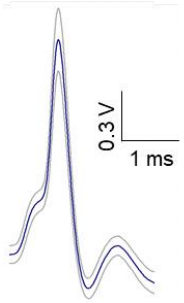




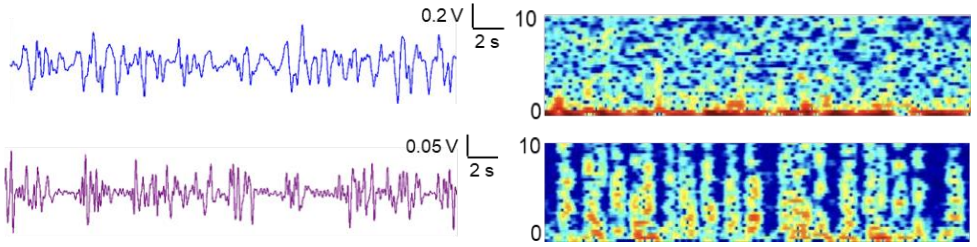
ANESTHETIZED ANIMALS







Single-unit (Spike)



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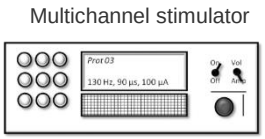
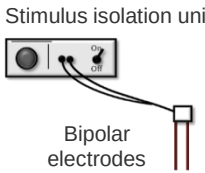
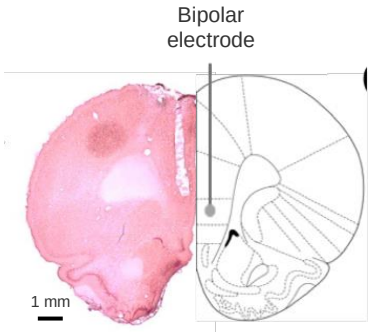
Deep brain stimulation

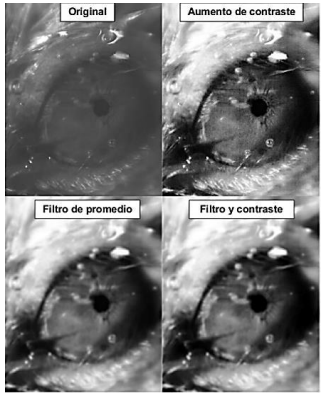
- Stereotactic implantation of single or multiple stimulation electrodes
- Low-frequency or high-frequency stimulation in selective brain regions
- Adjustable parameters: Frequency, amplitude, pulse width and stimulation duration
- Simultaneous stimulation of eight animals
- Applicable to rats and mice



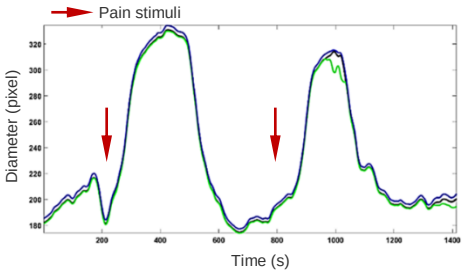




Pupillometry



- Fully-automated, time-resolved recording of pupil diameter in anaesthetized animals
- Sensitivity for monitoring sensorial signals involving the *locus coeruleus*



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Modulation of gene expression in rodent brain

- **Viral and plasmid vectors**
  - Stereotactic infusion of genes or modulatory gene sequences using adenovirus or plasmid vectors
  - Non-directed or cell-type targeted gene modulation
  - Applicable to rats and mice

- **Antisense oligonucleotides**
  - Stereotactic infusion or nasal spray administration of small oligonucleotides (ASO, miRNA, siRNA) to knockdown specific genes
  - Selective modulation of gene expression in monoaminergic cell populations using conjugated oligonuclotides
  - Applicable to rats and mice

- **DREADD** (Designer receptor exclusively activated by designer drugs)
  - Viral vectors introducing genes coding for artificially designed receptors with inhibitory or excitatory activity, and solely activated by exogenous ligands (e.g. clozapine N-oxide)
  - Allows selective activation of specific cell types, brain areas, or neuronal circuits.
  - Stereotactic inoculation
  - Applicable to rats and mice

Molecular and Cellular Biology

- **Gene expression quantification and modulation**
- **Protein analysis**
- **Neuroanatomy and microscopy**
- **Cell biology**

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Gene expression quantification and modulation

- Quantitative assessment of gene expression
  - Affymetrix mRNA microarrays
  - Quantitative RT-PCR systems: TaqMan RT-PCR microRNA and StepOne (Applied Biosystems); iCycler (Bio-Rad); CFX-connect (Bio-Rad)
  - Identification of bacterial 16S ribosomal RNA in peripheral tissues
  - Ribo-trap immunoprecipitation
- Epigenetic modulation
  - Chromatin immunoprecipitation (ChIP)

Protein analysis

- Protein expression analyses
  - High throughput ELISA in 96- and 384-well microplates
  - Descriptive and quantitative Western blotting
  - Radioimmunoassay
- Protein-protein interactions
  - Immunoprecipitation
  - Blue-native PAGE and 2-D PAGE
  - Capture ELISA

Molecular and Cellular Biology

Neuroanatomy and microscopy

- Histopathology (Nissl, H&E, Bielschowsky, Golgi, Luxol fast blue stains, among others)
- *In situ* hybridization with ^{33}P and ^{35}S and fluorescent (RNAscope) probes
- Immunohistochemistry and dual/triple/cuadruple immunofluorescence labeling
- Wide variety of bright field, epifluorescence, and laser-scan confocal microscopy
- Multiple neuroanatomical analyses
  - Qualitative and quantitative distribution profile
  - Co-expression (fluorescent ISH), co-localization (D/TIL), and protein interaction (proximity ligation assay, PLA) assessments

Cell biology

- Neuronal cultures derived from human olfactory neuroepithelium
- Primary cultures from rodent brain cells
  - Cortical and hippocampal cells
  - Neuronal, astrocyte and microglial cell cultures
  - Neurosphere cultures
- Identification of bacterial colony forming units in mammal tissues

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Computational methods and resources

- Bioinformatic analysis of *omic* data
  - GWAS, EWAS
  - Analysis of transcriptomic data
  - Collection, analysis and interpretation of proteomic data (MASCOT)

- Complex statistical modelling with multiple covariates
  - Statistical packages: *SPSS* (IBM), *JMP* (SAS), *R* (*R*-project), *GraphPad Prism*.

- Quantitative analysis of neuroimaging (structural and functional MRI, PET) data.
  - Imaging software: *MMWKS*, *SPM*, *PMOD*, *ITK-SNAP*, *ImageJ*

- Computational methods for mechanism-based drug discovery
  - Molecular dynamics simulations of the structure and function of G Protein-Coupled Receptors
  - Drug efficacy
  - Biased agonism

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