IQVIA LABORATORIES

Validated Models and Assays for CNS Drug Discovery

Table of contents

Validated models for neuroscience drug discovery: A Guide to model selection and study design

Introduction

Neuroscience drug discovery presents a unique set of challenges that continue to obstruct the path to novel therapies. The central nervous system (CNS) is characterized by complex, region-specific biology, contributing to a limited understanding of many CNS disease pathologies. The highly selective blood-brain barrier (BBB) further complicates drug delivery. As a result of these factors, CNS drug development is associated with some of the highest attrition rates in the industry, often driven by gaps between promising preclinical findings and clinical outcomes. These challenges underscore the need for well-characterized, translationally relevant models and assays that can more accurately predict human biology and disease progression.

Partnering with specialized contract research organizations (CROs) can help address these challenges by providing access to validated models, advanced technologies, and deep scientific expertise. Our integrated offerings span *in vitro* screening, *in vivo* efficacy studies, and translational endpoints to enable a more integrated approach to study design and decision-making. In addition, our scientists provide expert guidance to support protocol development, data analysis, and interpretation, helping to ensure that studies generate meaningful, actionable insights.

IQVIA Labs Discovery Sciences delivered

15

small molecule candidates to neuroscience clients

IQVIA Labs Discovery Sciences worked on

86%

of new FDA-approved CNS drugs in the last 5 years

Over

100

neuroscience patents coauthored with our clients

IQVIA Labs Discovery Sciences offers a broad selection of validated cellular and animal models, with translational and disease-relevant readouts for your CNS drug discovery studies. This guide is intended to provide an in-depth overview of neuroscience models and assays, as well as valuable resources and key considerations for model selection and study design. Although the assays and readouts listed are recommendations for the specific models described here, this guide does not provide an exhaustive summary of our offerings. We can use other endpoints or develop custom options depending on the needs of your study.

Chapter 1: Neurodegenerative diseases

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease causing the progressive loss of upper and lower motor neurons responsible for voluntary muscle contraction. ALS is characterized by stiff muscles, muscle twitching, and gradual muscle atrophy, resulting in difficulty speaking, swallowing, and eventually breathing. About 90% of ALS cases have no known etiology, while the remaining 10% have a genetic cause. Mutations in more than 20 genes have been associated with familial ALS, with mutations in *SOD1*, *TARDBP*, *FUS*, and *C9orf72* genes responsible for the majority of familial cases. *In vivo* models of ALS based on these mutations display behavioral and pathological analogs of human disease related to motor neuron degeneration. Cellular models recapitulate mutation-associated marker expression and alterations in cell viability and function.

IN VIVO MODELS AND ASSAYS:

Transgenic SOD1-G93A mouse model

- Express a G93A mutant form of human SOD1 (superoxide dismutase 1)
- Display rapid, adult-onset motor neuron degeneration, progressive weakness, and paralysis

Behavioral endpoints: clinical scoring, fine motor and gait analysis, motor function

In vivo readouts: CMAP, EMG, MRI, MRS, PET (synaptic loss, neuroinflammation)

Ex vivo readouts/biomarkers: neurofilament light chain (NfL), neuromuscular junction (NMJ) analysis

Transgenic TDP-43 rNLS8 mouse model

- rNLS8 is an inducible, transgenic mouse model of ALS based on a human TDP-43 transgene
- Overexpression of TDP-43 lacking a clear nuclear localization signal is controlled by doxycycline administration; removal of doxycycline from the diet results in expression and accumulation of hTDP-43 ΔNLS in the cytosol of motor neurons
- Display dramatic reduction in motor function 2 weeks after stopping doxycycline treatment
- Increased NfL in cerebrospinal fluid (CSF) compared with controls, suggesting extensive axonal damage, as well as disintegration of the neuromuscular junction; examination of cellular pathology shows increased total TDP-43 and pTDP-43 in the spinal cord as well as cytosolic localization

Behavioral endpoints: clinical scoring, fine motor and gait analysis, motor function

In vivo readouts: CMAP, MRI, MRS and PET (synaptic loss, neuroinflammation)

Ex vivo readouts/biomarkers: NfL, NMJ analysis, TDP-43 pathology

IN VITRO MODELS AND ASSAYS:

hiPSC-derived motor neurons

- With disease-relevant mutations (TDP-43 G298S, SOD1 A5V, FUS R521G and C9orf72 repeat expansion, and wild type)

Cell-based assays: axotomy/axonal growth, marker expression, neurite outgrowth, C9orf72 foci formation, TDP-43 pathology

hiPSC-derived glutamatergic neurons

- Expressing TDP-43 M337V (homozygous and heterozygous)

Cell-based assays: marker expression, network activity (MEA), splicing of TDP-43 regulated genes (*STMN2, UNC13A, KCNQ2, HDGFL2, MYO8A*), TDP-43 phosphorylation/mislocalization

Patient iPSC-derived motor neurons

- Expressing relevant mutations including TDP-43 G298S, SOD1 A5V, FUS R521G, and C9orf72 repeat expansion

Cell-based assays: C9orf72 foci formation, TDP-43 pathology

Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurological disorder characterized by the accumulation of abnormal amyloid protein plaques and neurofibrillary tangles, contributing to neuronal loss and brain atrophy. This neurodegeneration results in increasing short-term memory impairment and general cognitive/functional decline over time. The definitive causes of AD are poorly understood, but models of disease pathology are valuable translational tools in drug discovery and development. Animal and cellular AD models are designed to capture key pathological features such as amyloid aggregation, tau pathology, and synaptic dysfunction, enabling investigation of disease progression and therapeutic mechanisms.

IN VIVO MODELS AND ASSAYS:

Transgenic 5xFAD mouse model

- The 5xFAD mouse is a transgenic model that overexpresses 2 key human proteins with mutations related to familial AD (FAD): amyloid precursor protein (APP) with Swedish (K670N/M671L), Florida (I716V), and London (V717I) mutations, and presenilin 1 (PSEN1) with M146L and L286V mutations
- Expression of both transgenes in 5xFAD mice is regulated by a Thy1 promotor to drive overexpression in neurons
- 5xFAD mice recapitulate many AD-associated cognitive and pathological changes

Behavioral endpoints: contextual fear conditioning, Barnes maze, radial arm maze, nest building

In vivo readouts: PET (neurometabolism), fUS, MRI, MRS

Ex vivo readouts/biomarkers: amyloid pathology (microdialysis and IHC), microgliosis, NfL

Transgenic Tg2576 mouse model

- Tg2576 mice overexpress a mutant form of APP, APPK670/671L, linked to early-onset FAD, developing amyloid plaques and progressive cognitive deficits
- Validation data show impaired cognitive functions in the contextual fear conditioning test starting at 5 months of age coinciding with increased cortical and hippocampal soluble beta amyloid levels
- Elevated insoluble beta amyloid levels and amyloid plaques evident at 13 months of age

Behavioral endpoints: contextual fear conditioning, open field testing

In vivo readouts: PET (neurometabolism), fUS, MRI, MRS

Ex vivo readouts/biomarkers: amyloid pathology (microdialysis and IHC), microgliosis, NfL

Transgenic PS19 mouse model

- PS19 mice express the P301S mutant form of human microtubule-associated protein tau (MAPT)
- Mice display cognitive decline, changes in brain metabolism, and significant tau pathology

Behavioral endpoints: contextual fear conditioning, open field testing

In vivo readouts: PET (neurometabolism), fUS, MRI, MRS

Ex vivo readouts/biomarkers: tau pathology, microgliosis, NfL

IN VITRO MODELS AND ASSAYS:

FAD patient iPSC-derived cortical neurons

- Expressing relevant mutations

Cell-based assays: synaptic activity (calcium flux assay), vesicle trafficking

hiPSC-derived glutamatergic neurons

- Expressing MAPT P301S or N279K (homozygous and heterozygous)

Cell-based assays: marker expression, network activity (MEA), tau pathology

hiPSC-derived microglia

- Expressing TREM2 R7H (homozygous and heterozygous), APO 4/3 (C112R/WT) and 4/4 (C112R/C112R)

Cell-based assays: cytokine/chemokine release, gene expression profiling, inflammasome activation, phagocytosis

Neuron-glia coculture

- Complex primary neuron coculture models of human iPSC-derived neurons, microglia, astrocytes, and oligodendrocytes

Cell-based assays: amyloid-induced toxicity, cytokine/chemokine release, neuron viability, network activity (MEA), NfL release

Huntington's disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the *HTT* gene, leading to the production of a mutant huntingtin protein with toxic gain-of-function properties. The disease is characterized by progressive degeneration of striatal and cortical neurons, resulting in motor dysfunction, cognitive decline, and psychiatric symptoms. Pathogenesis involves complex mechanisms including protein aggregation, transcriptional dysregulation, mitochondrial dysfunction, and impaired proteostasis. Effective animal models of HD have robust, gene dosedependent, progressive, and early-onset symptomology and neuropathology. Cellular models can be leveraged for highthroughput screening of candidate compounds for regulation of mutant HTT and reduction/reversal of disease phenotypes.

IN VIVO MODELS AND ASSAYS:

zQ175 knock-in mouse model

- Contains human mutant HTT (m*HTT*) allele with the expanded CAG repeat (~179 repeats) within the native mouse huntingtin gene
- Reflects an accurate representation of HD in humans from a genetic aspect
- Both homo- and heterozygous zQ175 mice exhibit first signs of motor symptoms from 3-4 months of age
- Disease progression occurs slowly compared with R6/2 mice, making zQ175 more suitable for the assessment of chronic treatments (motor deficits emerge at ~6 months of age)

Behavioral endpoints: disease severity, kinematic motor and gait analysis, learning and memory, motor function, tremor analysis

In vivo readouts: MRI, MRS, fUS, PET (dopamine receptors)

Ex vivo readouts/biomarkers: astrogliosis, gene expression, HTT quantification, microgliosis

Transgenic R6/2 mouse model

- Contains N-terminally truncated *mHTT* with CAG repeat expansion (~125 repeats) within the huntingtin gene exon 1
- Rapidly develops a progressive phenotype of HD-like symptoms starting as early as 6-8 weeks of age

Behavioral endpoints: disease severity, kinematic motor and gait analysis, learning and memory, motor function, tremor analysis

In vivo readouts: MRI, MRS, PET

Ex vivo readouts/biomarkers: astrogliosis, gene expression, HTT quantification, microgliosis neurotransmitter profiling

IN VITRO MODELS AND ASSAYS:

hiPSC-derived HTT 50CAG glutamatergic neurons

- Genetically engineered using CRISPR-Cas9 to carry a homozygous or heterozygous 50CAG repeat expansion in exon 1 of the *HTT* gene
- Express pan-neuronal and glutamatergic neuron markers from early on in differentiation

Cell-based assays: HTT detection/quantification, cell viability/morphology, network activity (MEA)

hiPSC-derived striatal or cortical neurons

- In-house differentiation of patient-derived stem cells, with a control, 17/18CAG, 45-48CAG, 45-50CAG, and 70CAG repeats

Cell-based assays: HTT detection/quantification, cell viability/morphology, marker expression

Human fibroblasts and lymphoblasts

- Immortalized patient- and control-derived cell lines expressing mHTT

Cell-based assays: HTT detection/quantification

SH-SY5Y cells transfected with human HTT fragments

- Human neuroblastoma cell line overexpressing mHTT

Cell-based assays: HTT detection/quantification, cell viability/morphology, polyglutamine (polyQ) inclusion/aggregation

Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and the accumulation of α-synuclein-containing Lewy bodies. Clinically, it presents with motor symptoms such as bradykinesia, rigidity, and resting tremor, as well as cognitive impairment and autonomic dysfunction. Disease pathogenesis is multifactorial, involving mitochondrial dysfunction, oxidative stress, impaired proteostasis, and neuroinflammation. *In vivo* and *in vitro* PD models are designed to recapitulate key aspects of disease pathology, including dopaminergic neuron loss, α-synuclein aggregation, and motor deficits. Models that can express multiple pathophysiologic and behavioral aspects of the disease are valuable for studying potential mechanisms of action in parallel. IQVIA Labs Discovery Sciences offers models validated for multiple endpoints to support evaluation of neuroprotective and disease-modifying strategies, as well as translational endpoints such as behavioral outcomes, biomarker changes (i.e., dopamine and metabolites; dopamine transporter [DAT] density), and target engagement.

***IN VIVO* MODELS AND ASSAYS:**

MPTP mouse model

- A chemically induced, gold-standard animal model of PD created by peripheral administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)
- Characterized by decreased dopamine, 3,4-Dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) content in the striatum, as well as loss of dopaminergic neurons in the substantia nigra
- Animals display changes in interlimb coordination, speed of movement, hind limb joint angles, and limb trajectories

Behavioral endpoints: fine motor kinematic analysis, motor function

In vivo readouts: PET (neurometabolism, dopamine transporter), MRS, striatal dopamine and metabolites (microdialysis)

Ex vivo readouts/biomarkers: neuroinflammatory biomarkers, dopaminergic cell counts

6-OHDA rat model

- Chemically induced PD model created using unilateral injection of 6-hydroxydopamine (6-OHDA) into substantia nigra or median forebrain bundle of wild-type or nude rat
- 6-OHDA model in nude rat enables cell therapy studies
- Leads to unilateral loss of dopamine cells and decreased dopamine and dopamine metabolites (DOPAC and HVA) in the striatum
- Animals display alterations in gait, including changes to forelimb movements, interlimb coordination, and knee and ankle angles

Behavioral endpoints: fine motor kinematic analysis, rotation asymmetry, cylinder test

In vivo readouts: PET (dopamine transporter), MRI, striatal dopamine and metabolites (microdialysis)

Ex vivo readouts/biomarkers: neuroinflammatory biomarkers, dopaminergic cell counts

AAV Alpha-synclein overexpression rat/mouse model

- Biological transduction model in which overexpression of alpha-synuclein in the substantia nigra is induced by unilateral stereotaxic injection of adeno-associated virus (AAV) vectors
- Available in both rats (male Sprague-Dawley [CD] rats) and mice (male C57BL/6J mice)

Behavioral endpoints: fine motor kinematic analysis, motor function

In vivo readouts: PET (dopamine transporter), striatal dopamine and metabolites (microdialysis)

Ex vivo readouts/biomarkers: neuroinflammatory biomarkers, dopaminergic cell counts, alpha-synuclein pathology

PINK1/PARK2 knockout mouse model

- PINK1 and Parkin proteins, encoded by the *PINK1* and *PARK2* genes, respectively, have been linked to familial PD and are vital to mitophagy processes
- Mitophagy, the removal of damaged mitochondria, is a key pathological process in PD
- *PINK1/PARK2* KO mice display impaired gait at 2 months and decreased locomotion and rearing in the open field test at 6 months
- Increased NfL in plasma/CSF, indicating neuronal damage, and increased glial fibrillary acidic protein (GFAP) in CSF, indicating astrogliosis

Behavioral endpoints: fine motor kinematic analysis, open field test

In vivo readouts: MRI, MRS

Ex vivo readouts/biomarkers: plasma/CSF NfL, GFAP, dopaminergic cell counts

IN VITRO MODELS AND ASSAYS:

Human dopaminergic neurons (ReNcell VM)

- Immortalized human neural progenitor cell line differentiated into dopaminergic neurons

Cell-based assays: alpha-synuclein aggregation, Parkin translocation (mitophagy), TOM20 loss (mitophagy

TMEM175 ion channel cell line

- Express transmembrane protein 175 (TMEM175), a cation permeable ion channel located on the endosomal and lysosomal membrane

Cell-based assays: high-throughput patch clamp, FRET screening

hiPSC-derived glutamatergic neurons

- Expressing disease-relevant mutations (SNCA A53T, PRKN R275W, PINK1 Q456X, GBA R159W) and wild type

Cell-based assays: marker expression, cell viability, network activity (MEA)

hiPSC-derived dopaminergic neurons

- Expressing disease-relevant mutations (SNCA A53T, TMEM175 M393T and Q65P, LRRK2 G2019S, GBA N370S) and wild type

Cell-based assays: marker expression, cell viability, network activity (MEA)

Chapter 2: Autoimmune/neuroinflammatory diseases

Multiple sclerosis

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disorder of the CNS characterized by immune-mediated damage to myelin and subsequent neuroaxonal degeneration. It presents with a wide range of symptoms, including sensory disturbances, motor weakness, visual impairment, fatigue, and cognitive dysfunction, following either a relapsing-remitting or progressive disease course. Disease pathogenesis is complex and involves aberrant immune activation, blood-brain barrier disruption, inflammation, and neurodegenerative processes. *In vivo* and *in vitro* MS models are designed to recapitulate key features of the disease, including demyelination, neuroinflammation, and immune cell infiltration. Models that capture both inflammatory and neurodegenerative components are particularly valuable for evaluating therapeutic strategies targeting multiple aspects of disease progression.

IN VIVO MODELS AND ASSAYS:

Experimental autoimmune encephalomyelitis rat/ mouse model

- Experimental autoimmune encephalomyelitis (EAE) models are standard models for brain inflammation and demyelination studies
- EAE models are induced via injection of myelin oligodendrocyte glycoprotein (MOG) or proteolipid protein (PLP)
- Recapitulate the autoimmune features of MS, including chronic inflammatory demyelination, lymphocyte infiltration, and a relapsing-remitting disease course
- Disease course and the effect of novel therapeutics are then examined over several weeks

Behavioral endpoints: clinical scoring, kinematic motor and gait analysis, motor function

In vivo readouts: MRI, PET (neuroinflammation)

Ex vivo readouts/biomarkers: demyelination, neuroinflammation, NfL, gene expression profiling (including Nanostring), T-cell and B-cell immunoassays, cytokine profiling

Cuprizone-induced demyelination mouse model

- Induces demyelination in CNS through ingestion of cuprizone, a copper chelator
- Disrupts copper-dependent enzymatic function, resulting in oligodendrocyte death and region-specific demyelination
- Rapid and spontaneous remyelination occurs on removal of cuprizone from the diet
- Commonly used to study mechanisms of demyelination and remyelination in a non-immune-mediated context

Behavioral endpoints: clinical scoring, kinematic motor and gait analysis, motor function

In vivo readouts: dtMRI

Ex vivo readouts/biomarkers: demyelination, neuroinflammation, NfL, RNA expression

Lysolecithin-induced demyelination rat/mouse model

- Involves focal injection of lysolecithin (LPC) into specific areas of white matter to induce acute, severe demyelination through activation of macrophages and microglia
- Enables evaluation of remyelination mechanisms and therapeutic interventions

Behavioral endpoints: clinical scoring, kinematic motor and gait analysis, motor function

In vivo readouts: dtMRI

Ex vivo readouts/biomarkers: demyelination, NfL

IN VITRO MODELS AND ASSAYS:

Neuron-oligodendrocyte coculture

- Coculture of human iPSC-derived neurons and oligodendrocytes
- From day 7 of coculture, myelin basic protein (MBP, a marker of mature oligodendrocytes)-positive cells can be identified
- Colocalization of MBP with neurofilament H, a marker of neurites, demonstrates active myelination
- Can be used to investigate the ability of novel compounds to affect myelination/demyelination

Cell-based assays: marker expression, network activity (MEA), neuron viability

Neuron, oligodendrocyte, glia coculture

- As above, with addition of human iPSC-derived microglia and/or astrocytes to add an immune component

Cell-based assays: marker expression, network activity (MEA), neuron viability

Neuroinflammation

Mounting evidence indicates that the CNS is neither isolated nor passive in its interactions with the immune system. Although acute **neuroinflammatory** responses can be protective, chronic or dysregulated inflammation contributes to the progression of numerous neurological disorders, including AD, PD, and MS. Key mechanisms include cytokine release, immune cell infiltration, and disruption of the blood-brain barrier, leading to neuronal dysfunction and damage. *In vivo* and *in vitro* models of neuroinflammation are designed to capture these processes, enabling the study of inflammatory signaling pathways and evaluation of therapies aimed at modulating immune responses in the CNS.

IN VIVO MODELS AND ASSAYS:

Acute or chronic lipopolysaccharide administration (rat mouse)

- Acute and chronic lipopolysaccharide (LPS) administration induces systemic immune activation that triggers microglial activation and proinflammatory cytokine release in the CNS
- Acute LPS exposure is typically used to study rapid inflammatory responses
- Chronic administration models sustained neuroinflammation relevant to neurodegenerative disease progression and therapeutic intervention

Behavioral endpoints: learning and memory, locomotion, motor function

In vivo readouts: PET (neuroinflammation, neurometabolism)

Ex vivo readouts/biomarkers: astrogliosis, microgliosis, cytokine/chemokine levels, NfL

Mouse primary microglia/astrocytes

- Culture of microglia or astrocytes for assessment of cellular neuroinflammatory mechanisms
- Neuroinflammatory response induced by stimuli such as LPS, or disease-relevant triggers including β-amyloid or α-synuclein

Cell-based assays: cytokine/chemokine release, gene expression, inflammasome activation, phagocytosis

Human post-mortem isolated microglia/astrocytes

- Culture of microglia or astrocytes isolated from human post-mortem brain tissue

Cell-based assays: cytokine/chemokine release, gene expression, inflammasome activation, phagocytosis

hiPSC-derived microglia/astrocytes

- Culture of microglia or astrocytes derived from human stem cells

Cell-based assays: cytokine/chemokine release, gene expression, inflammasome activation, phagocytosis

Neuron-glia coculture

- Coculture of human iPSC-derived neurons, microglia, astrocytes and/or oligodendrocytes

Cell-based assays: cytokine/chemokine release, gene expression, inflammasome activation, phagocytosis

Chapter 3: Mental disorders

Autism

Autism spectrum disorders (ASD) are a group of neurodevelopmental conditions characterized by impairments in social communication and the presence of restricted, repetitive behaviors. The etiology is multifactorial, involving genetic, epigenetic, and environmental influences that impact synaptic development and neural circuit function. Preclinical models of ASD aim to recapitulate alterations in neuronal connectivity, synaptic signaling, and behavioral phenotypes relevant to social interaction and cognition.

IN VIVO MODELS AND ASSAYS:

BTBR T+tf/J mouse model

- Inbred strain exhibiting several symptoms of ASD, including reduced social interactions and anxiety
- Decreased whole brain and striatum volume, as well as statistical variation in metabolite levels

Behavioral endpoints: learning and memory, locomotion, anxiety-like behavior, social behavior

In vivo readouts: MRI, MRS

Anxiety

Anxiety disorders encompass a range of conditions characterized by excessive fear, worry, and heightened stress responses that interfere with daily functioning. Pathophysiology involves dysregulation of neural circuits governing threat processing, including the amygdala, hippocampus, and prefrontal cortex, as well as alterations in neurotransmitter systems such as GABA and serotonin. *In vivo* models are designed to assess behavioral and physiological responses to stress, supporting the evaluation of anxiolytic mechanisms and therapeutic candidates.

IN VIVO MODELS AND ASSAYS:

Symptomatic behavior induction in naïve mouse/rat

- Anxiety-like behaviors assessed using tests and behavioral measures, including:
  - » Marble burying test, which gauges the level of anxiety in a mouse or rat when it encounters unfamiliar objects
  - » Elevated plus maze, which examines anxiety-like behaviors in the context of heights and open space
  - » Chronic social defeat stress, in which naïve rodents are exposed to an intruder animal in a cage separated into two compartments
- Can be used to assess novel anxiolytic compounds or other therapies

Behavioral endpoints: anxiety-like behaviors, locomotion

In vivo readouts: compound/neurotransmitter quantification (microdialysis)

Depression

Depression is a mood disorder characterized by persistent low mood, anhedonia, and cognitive impairment, often accompanied by changes in sleep, appetite, and energy levels. Its pathogenesis is complex, involving dysregulation of monoaminergic signaling, neuroinflammation, hypothalamic-pituitary-adrenal (HPA) axis dysfunction, and impaired neuroplasticity.

IN VIVO MODELS AND ASSAYS:

Symptomatic behavior induction in naïve mouse

- Depression-like behaviors induced using behavioral protocols such as chronic stress
- Open field or elevated plus maze used to assess anxiety-like behavior
- Swim test or tail suspension used to assess depression-like behavior
- Sucrose preference test used to assess anhedonia
- Can be used to assess novel antidepressant compounds

Behavioral endpoints: depression-like behaviors, locomotion

In vivo readouts: compound/neurotransmitter quantification (microdialysis)

Schizophrenia

Schizophrenia is a chronic psychiatric disorder characterized by positive symptoms such as hallucinations and delusions, negative symptoms including social withdrawal and reduced motivation, and cognitive deficits. The disease is associated with disruptions in dopaminergic, glutamatergic, and GABAergic signaling, as well as altered neurodevelopment and synaptic function. *In vivo* and *in vitro* models aim to replicate key behavioral and neurobiological features, enabling investigation of disease mechanisms and the development of antipsychotic and disease-modifying therapies.

IN VIVO MODELS AND ASSAYS:

MK-801- or phencyclidine-induced symptoms in rat

- Administration of MK-801 or phencyclidine (PCP) to rats induces several behavioral deficits that recapitulate both positive and negative symptoms of schizophrenia
- Exhibit sensorimotor gating deficits including prepulse inhibition of startle

Behavioral endpoints: learning and memory, locomotion, startle reflex

In vivo readouts: PET, phMRI

Ketamine-induced psychosis in rat/mouse

- Administration of subanesthetic doses of ketamine are used to recapitulate both acute schizophrenia symptoms and underlying glutamatergic/dopaminergic dysfunction

Behavioral endpoints: learning and memory, locomotion

In vivo readouts: compound/neurotransmitter quantification (microdialysis)

Amphetamine-induced hyperactivity in rat/mouse

- Administration of amphetamine triggers locomotor hyperactivity and stereotyped behavior in rats and mice
- Based on theory that excessive synaptic dopamine produces symptoms of schizophrenia

Behavioral endpoints: learning and memory, locomotion

In vivo readouts: compound/neurotransmitter quantification (microdialysis)

Chapter 4: Pain

Acute and inflammatory pain

Acute pain is defined as a normal physiological response to external noxious stimuli and serves as a protective early warning system for the body. Inflammatory pain is usually caused by tissue injury, arthritis, or tumor growth. Tissues that are damaged due to infection, tumor growth, or injury typically show an inflammatory response, which triggers a pain reaction. Pro-inflammatory molecules activate nociceptors, evoking responses such as allodynia and hyperalgesia. Inflammatory pain models target both acute and chronic inflammatory pain depending on the stimulus, which includes carrageenan and capsaicin.

IN VIVO MODELS AND ASSAYS:

Chemical-induced acute pain mouse/rat model

- Administration of formalin, acetic acid, or capsaicin induces acute tonic pain

Behavioral endpoints: paw flinching/licking/biting, tail flick, mechanical allodynia, von Frey

In vivo readouts: fUS

Complete Freund's Adjuvant (CFA)-induced inflammatory pain rat model

- Subcutaneous injection of CFA into the paw simulates inflammatory pain
- Causes significant swelling, hyperalgesia, and mechanical/tactile allodynia lasting for 1-2 weeks

Behavioral endpoints: cold/heat/mechanical allodynia, paw withdrawal

IN VITRO MODELS AND ASSAYS:

Acute pain-related ion channel screening panel

- Ion channels, including voltage-gated sodium channels (Na_Vs), transient receptor potential channels (TRPs), and acid sending ion channels (ASICs), are expressed in HEK293 cell lines
- Screening efficiently identifies and characterizes compounds modulating the activity of pain-related ion channels

Cell-based assays: high-throughput patch clamp screening

Neuropathic pain

Neuropathic pain originates from damage or disease along the somatosensory nervous system, comprised of the dorsal root ganglia and other peripheral neurons. Neuropathic pain often emerges as a secondary disease such as cancer, metabolic disorders, viral infections, autoimmune disorders, trauma, hereditary neuropathies, inflammatory disorders, or as a side effect of chemotherapy. Symptoms severely deteriorate patients' quality of life, yet identifying safe, effective, and nonaddictive therapies remains a challenge.

IN VIVO MODELS AND ASSAYS:

Spinal nerve ligation rat model

- Also known as the Chung model
- Involves tight surgical ligation of the L5 and L6 spinal nerves
- Creates consistent peripheral neuropathy, enabling evaluation of new analgesics

Behavioral endpoints: tactile allodynia, locomotion, fine kinematic motor and gait analysis

In vivo readouts: fUS

Partial sciatic nerve ligation rat model

- Involves tight surgical ligation of one-third to one-half of the sciatic nerve at the upper thigh level
- Induces chronic neuropathic pain, enabling evaluation of new analgesics

Behavioral endpoints: tactile allodynia, locomotion, fine kinematic motor and gait analysis

In vivo readouts: MRI

Spared nerve injury rat/mouse model

- Highly reproducible model involving ligation and severing of the tibial and common peroneal branches of the sciatic nerve
- Sural nerve is left intact
- Partial denervation induces rapid, prolonged tactile hypersensitivity in the territory of the spared nerve

Behavioral endpoints: tactile allodynia, thermal allodynia, locomotion, fine kinematic motor and gait analysis

In vivo readouts: MRI

Chemotherapy-induced polyneuropathy rat model

- Mimics chemotherapy-associated polyneuropathy through administration of paclitaxel or oxaliplatin
- Traditional or surgical administration routes
- Severity of effects depends on dosage

Behavioral endpoints: thermal allodynia

In vivo readouts: CMAP, fUS, sNCV

***IN VITRO* MODELS AND ASSAYS:**

Chronic pain-related ion channel screening panel

- Ion channels, including Na_vs, voltage-gated potassium channels (K_vs), voltage-gated calcium channels (Ca_vs), and TRPs, are expressed in HEK293 cell lines
- Screening efficiently identifies and characterizes compounds modulating the activity of pain-related ion channels

Cell-based assays: high-throughput patch clamp screening

Migraine

Migraine is a complex neurological disorder characterized by recurrent episodes of moderate to severe headache, often accompanied by symptoms such as nausea, photophobia, and phonophobia. Its pathophysiology involves dysregulation of the trigeminovascular system, cortical spreading depression, and altered neurotransmitter signaling, including calcitonin generelated peptide (CGRP) pathways. These processes contribute to both peripheral and central sensitization, driving pain and associated symptoms.

***IN VIVO* MODELS AND ASSAYS:**

PACAP-induced migraine mouse model

- Infusion of pituitary adenylate cyclase-activating polypeptide (PACAP), a potential mediator of migraine pathogenesis
- PACAP stimulates PAC1 receptors, inducing cranial artery dilation
- Treated mice demonstrate facial and hindpaw sensitivity, as well as photophobia

Behavioral endpoints: light aversion

In vivo readouts: fUS

Chapter 5: Injury and ischemia

Stroke/ischemia

Stroke is a cerebrovascular disorder characterized by the interruption of blood flow to the brain, most commonly due to ischemia resulting from arterial occlusion, leading to oxygen and nutrient deprivation and subsequent neuronal injury. Ischemic stroke triggers a cascade of pathological events, including excitotoxicity, oxidative stress, inflammation, and disruption of the blood-brain barrier, which contribute to cell death and neurological deficits. Hemorrhagic stroke is caused by a ruptured blood vessel, leading to bleeding inside and around the brain. The extent and progression of injury depend on factors such as duration of ischemia, reperfusion, and collateral circulation. Preclinical models of stroke and ischemia are designed to recapitulate these processes, enabling the study of acute injury mechanisms, neuroprotection, and recovery, as well as the evaluation of therapeutic interventions targeting both the ischemic event and downstream damage.

IN VIVO MODELS AND ASSAYS:

Transient middle cerebral artery occlusion (tMCAO) mouse/rat model

- Most widely used and widely validated animal model of ischemic stroke
- Occlusion in either Wistar rats or C57BL/6J mice followed by reperfusion results in lesion formation and a pathological response against which novel compounds can be investigated
- Following 90-minute occlusion, tMCAO in rats induces functional impairment

Behavioral endpoints: clinical scoring, fine kinematic motor and gait analysis, learning and memory, limb placement test

In vivo readouts: PET (neurometabolism), MRI

Ex vivo readouts: infarct volume, neuroinflammation

Permanent middle cerebral artery occlusion (pMCAO) rat model

- Model of ischemic stroke induced by electrocoagulation of the middle cerebral artery in spontaneously hypertensive rats
- Animals display functional impairment and changes in BBB integrity, as well as measurable lesions

Behavioral endpoints: clinical scoring, fine kinematic motor and gait analysis, learning and memory, limb placement test

In vivo readouts: PET (neurometabolism), MRI

Ex vivo readouts: infarct volume, neuroinflammation

Hypertensive rat model

- Spontaneously hypertensive rat (SHR) developed through inbreeding of Wistar rats with high blood pressure
- Models human essential hypertension and associated stroke risk
- Animals exhibit high systolic pressure from 5-6 weeks of age

Behavioral endpoints: learning and memory

In vivo readouts: PET (neurometabolism), MRI

Hind limb ischemia rat model

- Involves surgical induction of ischemia, typically through ligation of the femoral artery and vein
- Simulates human peripheral artery disease and limb ischemia

Behavioral endpoints: fine kinematic motor and gait analysis

In vivo readouts: Doppler imaging, fUS

Spinal cord injury

Spinal cord injury involves damage to the spinal cord that disrupts motor, sensory, and autonomic function below the site of injury. Pathophysiology includes an initial mechanical insult followed by secondary processes such as inflammation, excitotoxicity, and scar formation that exacerbate tissue damage. Preclinical models are used to study injury progression and evaluate therapies aimed at neuroprotection, regeneration, and functional recovery.

IN VIVO MODELS AND ASSAYS:

Spinal cord contusion rat model

- Spinal cord injury is induced in rats at the T10 thoracic position by mechanical contusion using the PinPoint Precision Cortical Impactor™
- Provides precise control of velocity, depth, and power of impact to generate accurate, reliable, and reproducible models of spinal cord injury
- Predictable recovery pattern

Behavioral endpoints: clinical scoring, fine kinematic motor and gait analysis, locomotion

Ex vivo readouts: infarct volume, nerve fiber density, neuroinflammation, NfL

Traumatic brain injury

Traumatic brain injury (TBI) results from an external mechanical force that disrupts normal brain function, leading to cognitive, motor, and behavioral impairments. Pathophysiology involves a primary injury followed by secondary processes such as inflammation, excitotoxicity, and blood-brain barrier disruption that contribute to ongoing neuronal damage. Preclinical models recapitulate injury mechanisms and aid in evaluating therapies aimed at neuroprotection and recovery.

IN VIVO MODELS AND ASSAYS:

Cortical contusion injury rat/mouse model

- Gold-standard method for testing novel TBI therapeutics
- Injury induced in rats or mice by mechanical contusion using the PinPoint Precision Cortical Impactor™
- Provides precise control of velocity, depth, and power of impact to generate accurate, reliable, and reproducible models of spinal cord injury
- Predictable recovery pattern

Behavioral endpoints: clinical scoring, fine kinematic motor and gait analysis, locomotion

In vivo readouts: MRI

Ex vivo readouts: infarct volume, neuroinflammation, NfL

Chapter 6: Rare and other diseases

Epilepsy

Epilepsy is a chronic neurological disorder characterized by recurrent, unprovoked seizures resulting from abnormal, excessive neuronal activity in the brain. Pathophysiology involves disruptions in excitatory and inhibitory signaling, network hyperexcitability, and, in some cases, neuroinflammation and structural abnormalities. *In vivo* and *in vitro* models are used to study seizure mechanisms and neuronal network dysfunction, and to evaluate antiepileptic and disease-modifying therapies.

IN VIVO MODELS AND ASSAYS:

Pentylenetetrazol (PTZ)- or kainic acid (KA)-induced seizure rat model

- Systemic administration of PTZ or KA produces seizures
- PTZ causes generalized seizures via disinhibition of GABAergic circuits
- KA increases activity of excitatory glutamatergic circuits, producing a model of tonic-clonic seizures

In vivo readouts: fUS, fMRI, EEG

In Vitro Models and Assays:

IN VIVO MODELS AND ASSAYS:

Seizure-related ion channel screening panel

- Ion channels, including Na_Vs, K^Vs, calcium-activated potassium channels (K_{Ca}s), and GABA, are expressed in HEK293 cell lines
- Manual and automated screening against preselected panels of ion channels related to epilepsy and seizures

Cell-based assays: high-throughput patch clamp screening

hiPSC-derived neurons

- Cultured GABAergic and glutamatergic neurons differentiated from human stem cells

Cell-based assays: marker expression, network activity (MEA)

Neuron-glia coculture

- Coculture of GABAergic and glutamatergic neurons and glia

Cell-based assays: marker expression, network activity (MEA)

Sleep

Sleep is a fundamental biological process essential for maintaining neurological function, including memory consolidation, synaptic plasticity, and metabolic homeostasis in the brain. Disruptions in sleep are associated with a range of neurological and psychiatric disorders, including neurodegeneration, mood disorders, and cognitive impairment, reflecting the critical role of sleep-wake regulation in brain health. Pathophysiology involves complex interactions between circadian rhythms, neurotransmitter systems, and neural network activity. Preclinical models are used to study sleep architecture, neural circuitry, and underlying mechanisms, as well as to evaluate therapeutic strategies targeting sleep and its role in neurological function.

IN VIVO MODELS AND ASSAYS:

Naïve rat/mouse or disease models

- EEG and home cage activity monitoring can be used to profile sleep/wake cycles
- Can be conducted in wild type or disease model animals
- Sleep/wake activity also used to characterize stimulant or sedative drug effects

Behavioral endpoints: home cage activity

In vivo readouts: EEG, EMG

Batten disease

Batten disease (neuronal ceroid lipofuscinosis, NCL) is a group of up to 13 rare, inherited neurodegenerative disorders characterized by progressive accumulation of lipopigments made up of fats and proteins in neurons, leading to cell dysfunction and death. Clinically, it presents with symptoms such as seizures, vision loss, cognitive decline, and motor impairment, typically beginning in childhood. Pathophysiology involves lysosomal dysfunction, impaired protein degradation, and neuroinflammation. In vivo models are used to study disease mechanisms, including lysosomal pathology and neuronal degeneration, and to evaluate potential therapeutic strategies.

IN VIVO MODELS AND ASSAYS:

CLN2/CLN6/CLN8 mouse models

- CLN6 and CLN8 are transmembrane NCL mutations existing on the endoplasmic reticulum
- CLN2 is a lysosomal enzyme with an NCL mutation

Behavioral endpoints: fine kinematic motor and gait analysis

In vivo readouts: MRS, MRI, dtMRI, PET (neurometabolism), fUS

Ex vivo readouts: retinal and photoreceptor analysis, lipofusin accumulation

Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a severe, X-linked genetic disorder characterized by progressive muscle degeneration due to mutations in the DMD gene, leading to the absence of functional dystrophin protein. While DMD is rare, it is the most common form of muscular dystrophy, with an incidence of 1 per 3,500 live male births. The disease presents with early-onset muscle weakness, loss of ambulation, and eventual respiratory and cardiac complications. Pathophysiology involves muscle fiber damage, chronic inflammation, and impaired regeneration.

IN VIVO MODELS AND ASSAYS:

X-linked muscular dystrophy (mdx) mouse

- Characterized by a nonsense point mutation in exon 23 of the dystrophin gene, leading to dysfunctional dystrophin protein
- Exhibit muscle necrosis but display a milder phenotype than humans

Behavioral endpoints: fine kinematic motor and gait analysis

In vivo readouts: MRI, MRS

Ex vivo readouts: creatine kinase, muscle fiber analysis

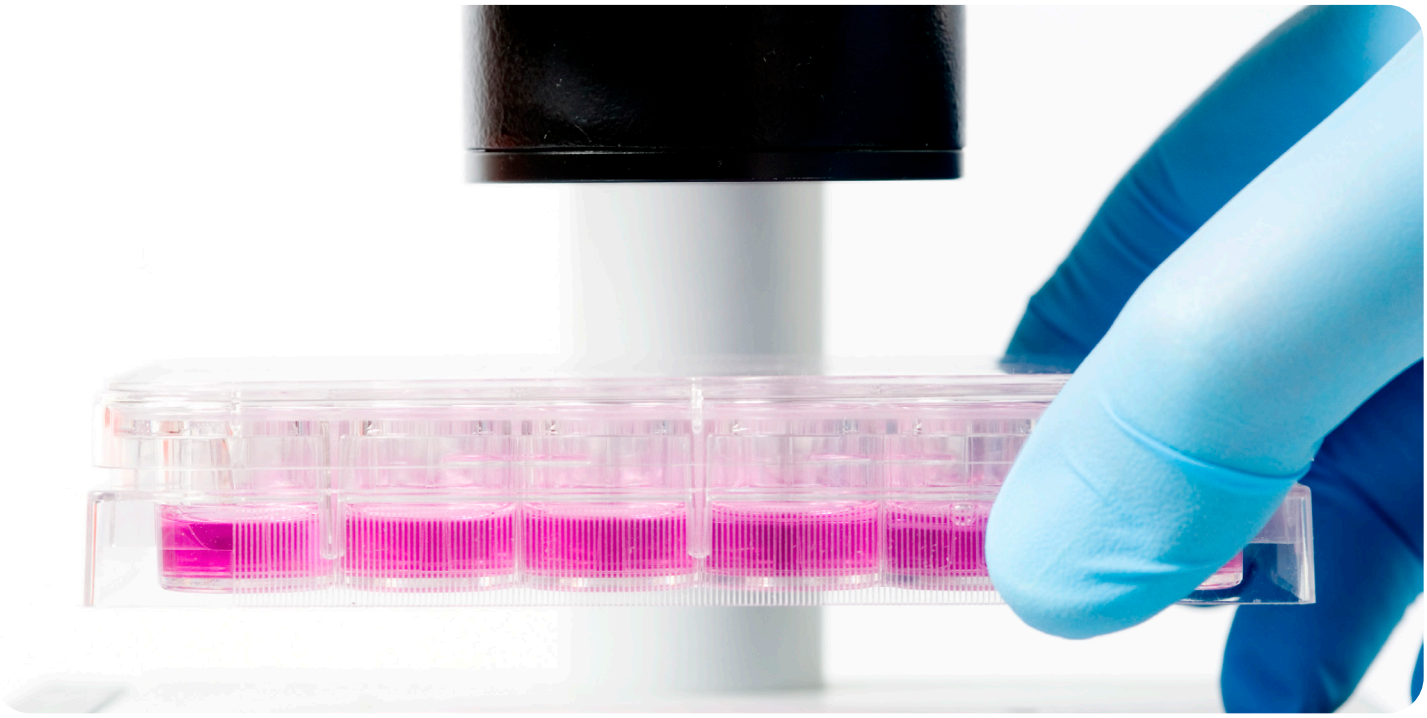
IN VITRO MODELS AND ASSAYS:

hiPSC-derived skeletal myocytes

- Skeletal myocytes differentiated from human stem cells expressing disease-relevant mutations (dystrophin exon 44/ dystrophin exon 52 deletion, wild type)

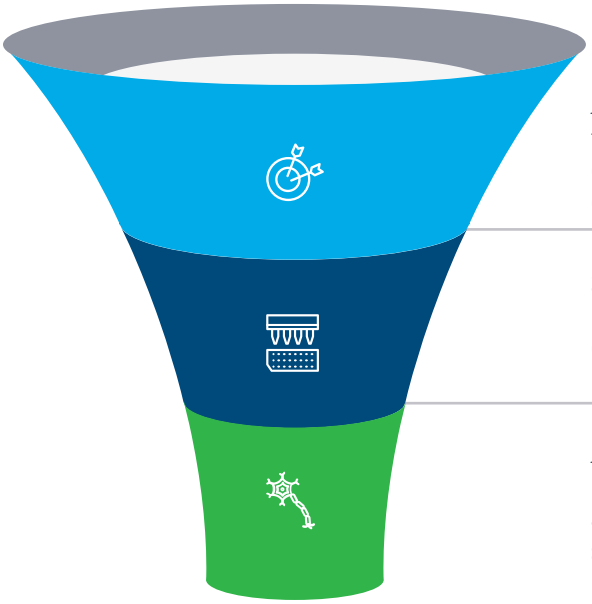
Cell-based assays: myoblast to myotube differentiation, dystrophin, creatine kinase, and utrophin quantification

Key considerations for study design



Designing effective studies for CNS drug discovery requires careful alignment between the therapeutic hypothesis and the models used to evaluate it. Given the complexity of CNS biology and the high rate of translational attrition, selecting the appropriate model or assay is critical to generating meaningful, predictive data. Study design should be guided by factors such as mechanism of action, target biology, disease relevance, and stage of development. No single model can fully capture the intricacies of CNS disorders, so a strategic, tiered approach that integrates multiple model systems is often necessary to build confidence in a therapeutic candidate.

A phase-appropriate framework can help guide model and assay selection:



Early target identification and screening
In vitro assays provide high-throughput, controlled systems to evaluate target engagement, pathway modulation, and preliminary toxicity during early discovery. These models are well suited for screening large compound libraries and refining candidate selection.

Mechanism validation and lead optimization
Specialized assays, including functional, electrophysiological, or biomarker-driven approaches, help confirm mechanism of action and deepen understanding of target biology. These studies support hypothesis validation and inform compound optimization.

Preclinical efficacy and translational studies
In vivo models are essential for evaluating therapeutic efficacy, pharmacokinetics/pharmacodynamics, and safety of drug candidates in a physiologically relevant setting. Incorporating translational endpoints such as imaging, fluid biomarkers, or behavioral readouts can help bridge preclinical findings to clinical expectations.

Technical spotlight: *In Vivo* imaging services

In neuroscience drug discovery, preclinical imaging modalities can be used to investigate drug effects on disease pathophysiology in the brain, by enabling the assessment of gross anatomical structures alongside cellular and molecular structure and function. Small animal imaging technologies align directly with techniques used in clinical settings for diagnosis and disease monitoring, providing highly translational methods for in-depth insights at every level of drug discovery:

- **MRI** — MRI is a noninvasive imaging method that uses a magnetic field and radio waves to provide detailed 3D imaging of brain structure, volume, damage, and blood-brain barrier integrity. Depending on your study objectives, you can choose anatomical, diffusion, hemodynamic, pharmacological, or functional MRI, or a combination of these techniques
- **PET imaging** — Preclinical PET imaging studies employ radio-labelled ligands, also known as radiotracers, to image and quantify changes in functional physiological processes such as metabolism, neuroinflammation, and perfusion, as well as receptor occupancy. PET imaging studies can be performed in combination with CT, enabling output of combined data on radiotracer accumulation and atomic localization
- **SPECT imaging** — Preclinical SPECT imaging enables in vivo measurement of brain perfusion, inflammation, and biodistribution of novel compounds or cells. It is commonly used to study metabolic changes, cerebral blood flow, and oxygen levels and can be combined with CT scans and PET imaging to generate quantifiable data on therapeutic compound binding and brain function
- **fUS imaging** — fUS is a novel method that relies on real-time, noninvasive, and highly sensitive Doppler-based imaging of the brain. fUS technology provides ultrafast spatial and temporal resolution images and can be used for acute or chronic characterization of disease models, efficacy and target engagement studies in rodents. Our experts have pioneered the use of fUS in *in vivo* efficacy studies in CNS drug discovery

The noninvasive nature of preclinical imaging methods enables longitudinal tracking of drug effects in the same individual over time, providing highly translational, long-term data for *in vivo* efficacy and proof-of-concept studies. Imaging studies can also provide valuable monitoring data for safety and toxicology studies, as well as robust pharmacokinetic/pharmacodynamic data through assessment of biodistribution using labelled test compounds. Our team's extensive expertise in preclinical imaging techniques, paired with machine learning-driven data analysis tools, ensures every study yields high-quality data to support confidence in your lead candidate.

In addition to stand-alone studies, our preclinical CNS imaging services can be combined with other endpoints, including behavior, electrophysiology, microdialysis sampling, and *ex vivo* biomarker analysis, to build a comprehensive data package on drug efficacy.

ACCELERATING PROGRESS IN CNS DRUG DISCOVERY

Neuroscience drug discovery remains one of the most complex and high-risk areas of pharmaceutical development, shaped by biological intricacies, limited disease understanding, and persistent translational challenges. Overcoming these barriers with the use of well-characterized, translationally relevant models and assays that more accurately reflect human disease can improve confidence in preclinical findings. As the scientific toolbox continues to evolve, the ability to generate highquality, predictive data through thoughtful model selection and study design will be essential to advancing new therapies and improving clinical success rates.

A well-equipped preclinical research partner can drive your discovery program to its full potential. Working with an experienced CRO that offers validated models, integrated capabilities, and deep domain expertise can significantly enhance study quality, streamline development, and reduce risk. IQVIA Labs Discovery Sciences Laboratories combines a comprehensive portfolio of validated CNS models and assays with expert scientific support to help guide programs from early discovery through translational research.

For end-to-end support in model selection, study design, and data analysis for your neuroscience discovery program, click here to get in touch with IQVIA Labs Discovery Sciences Discovery Services experts today.

Glossary

CMAP
Compound muscle action potential; a form of EMG readout that measures evoked, grouped action potentials of several muscle fibers.

CT
Computed tomography; a noninvasive imaging method using a rotating x-ray source to generate detailed, cross-sectional scans of bones, blood vessels, and soft tissues.

EEG
Electroencephalography; a quantitative, noninvasive technique to assess changes in electrical activity and sleep state associated with neurological disease models.

EMG
Electromyography; measures muscle activation and is used to detect change in muscle activity and neuromuscular abnormalities. It can be used in conjunction with nerve conduction velocity test.

Fine kinematic motor and gait analysis
A high-precision system for quantification of minute changes in gait, movement, and balance.

fUS
Functional ultrasound imaging; a noninvasive method that measures transient changes in blood flow using Doppler-based imaging.

MEA
Multielectrode array; measures neuronal activity in cells using clusters of microscopic electrodes.

Microdialysis
A minimally invasive sampling technique used for measurement of free, unbound analyte concentrations. Can be used for measurement of compounds, neurotransmitters, hormones, and pathological proteins, as well as therapeutic antibodies and proteins.

MRI

Magnetic resonance imaging; a highly specialized and translational imaging method of brain activity and function.

- **dtMRI**
  Diffusion tensor MRI; enables quantitative study of white matter and demyelination

- **fMRI**
  Functional MRI; measures changes in brain activity in response to physical stimuli

- **MRS**
  Magnetic resonance spectroscopy; measures metabolite levels at multiple time points

- **phMRI**
  Pharmacological MRI; measures changes in brain activity in response to pharmacological stimuli

PET/SPECT nuclear imaging

Positron emission tomography/Single photon emission computed tomography; translational nuclear imaging techniques that use radio-labelled tracers and gamma-radioactive compounds, respectively.

- **CLINDE-SPECT**
  Uses [^{123}I]-CLINDE TSPO ligand to detect neuroinflammation

- **DAT-PET**
  Uses [^{123}I]-beta-CIT DAT ligand for imaging of dopamine receptor density

- **FDG-PET**
  Uses ^{18}F-fluorodeoxyglucose to detect changes in cerebral metabolism

- **FEPPA-PET**
  Uses ^{18}F-FEPPA TSPO ligand to detect neuroinflammation.

- **FMISO-PET**
  Uses ^{18}F-fluoromisonidazole to detect hypoxia

- **SynVest1 PET**
  [^{18}F]SynVesT-1 (SDM8) for imaging of synaptic density

sNCV

Sensory nerve conduction velocity; a test measuring the speed and strength of electrical signals in sensory nerves in response to stimulation.

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