Animal models of Parkinson’s disease

Miquel Vila, MD, PhD
Vall d’Hebron Research Institute
Autonomous University of Barcelona
Barcelona, Spain
Cross-species platform to study PD

- Yeast
- C. elegans
- Drosophila
- Zebrafish
- Mouse
- Rat
- Non-human primate

Increasing complexity, costs, handling & manipulation difficulty
Modeling PD in experimental animals

What do we need from a model?

- **Construct validity:** The ability of a model to reproduce what it claims to be reproducing.

- **Face validity:** The ability of a model to reproduce the clinical and pathological features of the human disease.

- **Predictive validity:** The ability of a model to discriminate between clinically effective and ineffective therapeutic strategies.

- **Feasibility, reproducibility, stability**

- **Goal driven**
Modeling PD in experimental animals

What should we be modeling?

- **Symptomatology**
  - Motor deficits
  - Non-motor symptoms (olfactory deficits, cognitive alterations, anxiety/depression, sleep disturbances, gastrointestinal dysfunction, autonomic dysfunction,...)
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- **Circuit/System alterations**
  - DA (SNpc/VTA)
  - NA (LC), Ach (DNV, PPN, NBM), Serotonin (DR)
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- **Neuropathology**
  - Cell death, Lewy-like pathology, inflammation
    - Substantia nigra
    - Extranigral: brain & periphery
Modeling PD in experimental animals

- **Neurotoxic models**
  - 6-OHDA
  - MPTP
  - Rotenone, paraquat, other

- **Genetic models**
  - Genetically-modified animals (Tg/KO)
  - Viral vector-mediated gene delivery

- **Seeded/protein-based models**
  - Synthetic pre-formed fibrils (PFF)
  - Pure recombinant species
  - Human brain-derived material
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- Neurotoxic models
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Main applications
- Pre-clinical testing of new symptomatic therapies, dopamine-replacement strategies and transplantation approaches
- Study of levodopa-induced motor fluctuations and dyskinesias
- Molecular mechanisms of neuron cell death

Main features
- Acute nigrostriatal neurodegeneration
- Behavioral abnormalities responsive to levodopa
- No Lewy-like pathology
Modeling PD in experimental animals

Garcia-Pardo et al. ACS Nano (2021)
Genetic models
- Genetically-modified animals (Tg/KO)

PD-causing genes
(aSyn, LRRK2, GBA, Parkin, PINK1, DJ-1, ATP13A2,...)

Other genes
(Ndufs2 KO, TFAM KO, VMAT2 KO, Nurr1, Pitx3 KO, ...)

Main features (Tg/KO PD genes)
- Limited and inconsistent nigrostriatal degeneration
- Occurrence of aSyn pathology (including peripheral)
- Subtle phenotypic effects (if any)
- Model some non-motor symptoms

Main applications (Tg/KO PD genes)
- Biology/function of PD-linked proteins
- Early/prodromal stages of the disease
- Interaction genetic factors-environment (in combination with toxins)
### Modeling PD in experimental animals

- **Genetic models**
  - Genetically-modified animals (Tg/KO)
  - Viral vector-mediated gene delivery

<table>
<thead>
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<th>Main features (AAV-aSyn models)</th>
<th>Main applications</th>
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<td>✓ Models dopaminergic neurodegeneration (rats)</td>
<td>✓ Mechanisms of aSyn-mediated aggregation, spreading &amp; neurotoxicity</td>
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<td>✓ Accumulation (and spreading?) of aSyn aggregates</td>
<td>✓ Therapies aimed at reducing aSyn levels</td>
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<td>✓ PD-like motor deficits</td>
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α-Synuclein protein expression

Striatal dopamine release

AAV-hα-Syn

Substantia nigra

Striatum

Non-viral inhibitory oligonucleotides

Pre-treatment

Treatment

Result

Triplet monoamine receptor inhibitor

Indatraline

Target: Monoamine neurons

Vehicle

IND-1337-ASO

Vall d’Hebron

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➤ **Seeded/protein-based models**
  - Synthetic pre-formed fibrils (PFF)
  - Pure recombinant species
  - Human brain-derived material

**Main features**
- Trans-synaptic spreading and accumulation of pathological aSyn (including peripheral)
- Nigral neurodegeneration (and beyond SN?)
- No PD-like behavioural motor deficits; potential to model aSyn pathology in multiple circuits and to produce non-motor symptoms

**Main applications**
- Mechanisms of aSyn spreading, aggregation and toxicity
- Therapies aimed at blocking aSyn spreading
Modeling PD in experimental animals

Highly melanized neurons are preferentially vulnerable in PD
Neuromelanin accumulates with age in the human brain

Rodents lack neuromelanin

- Neuromelanin is uniquely abundant in the human brain
- PD is an exclusively human disease
Synthesis of neuromelanin

Human-like neuromelanin production in AAV-TYR expressing rats

Human-like neuromelanin production in AAV-TYR expressing rats

Intracellular NM levels

0.5m
1m
2m
4m
12m
24m

Time post-AAV-hTyr injection (months)

Intracellular NM density (OD)

Human-like neuromelanin production in AAV-TYR expressing rats

**Progressive PD-like features**

- Hypokinesia
- Lewy-like pathology
- Nigrostriatal neurodegeneration
- Extracellular NM & Neuroinflammation

**Intracellular NM levels**

**Neuronal dysfunction**

**Neurodegeneration**
Pathogenic threshold of neuromelanin accumulation

Intracellular NM levels

Neuronal dysfunction
Neurodegeneration

Mechanisms of neuromelanin-linked PD pathology

Dopamine (DA) → DA semiquinone → DA quinone → Aminochrome

1. Lysosomal / Autophagy dysfunction
2. UPS impairment
3. Inclusion formation
- Reduced mitochondrial respiration
- Oxidative stress
- Impaired neurotransmission
- Neurodegeneration

Vila Movement Disorders (2019)
Therapeutic modulation of neuromelanin accumulation
Therapeutic clearance of neuromelanin

- Reduces Lewy body inclusion formation
- Attenuates nigrostriatal neurodegeneration
- Restores motor symptoms

Therapeutic reduction of neuromelanin production

- Reduces Lewy body inclusion formation
- Attenuates nigrostriatal neurodegeneration
- Prevents motor symptoms

Gonzalez-Sepulveda*, Compte* et al. Brain (in press)
Modeling brain-wide neuromelan accumulation in vivo

Vila (unpublished)
Neuromelanin-producing rodent PD models

- Tyrosinase-overexpressing rats/mice represent the **first rodent models** exhibiting age-dependent neuromelanin accumulation at levels up to those reached in elderly humans.

- Excessive production/accumulation of neuromelanin within neurons can ultimately compromise neuronal function and trigger **multisystem PD-like pathology with motor/non-motor deficits**.

- **Strategies to maintain or decrease intracellular neuromelanin** to levels below its pathogenic threshold in vivo can prevent, halt or delay PD-like neuronal dysfunction and degeneration.

- Intracellular neuromelanin levels **reach their pathogenic threshold in PD** patients and pre-PD subjects (i.e. ILBD).
In contrast with the situation for many other neurodegenerative diseases, PD benefits from a **wide range of available animal models**, in several species.

The utility of a given model will depend on the **question being asked** (goal-driven).

Proof of concept of a therapeutic strategy may require the **use of multiple models** and a **realistic experimental design** compared to the human situation (i.e. aligned with disease stages).

**Emerging aspects** to incorporate in PD animal modeling:
- Aging or age-related factors (e.g. neuromelanin)
- Sex (male sex bias in animal modeling)
- Co-pathologies (overlap of synucleinopathy with other proteinopathies?)
Neurodegenerative Diseases Research Group

Miquel Vila, MD, PhD
Senior Researchers
Ariadna Laguna, PhD
Marta Martínez-Vicente, PhD
Jordi Bové, PhD

Postdoctoral Researchers
Marta González-Sepúlveda, PhD
Helena Xicoy, PhD
Thais Cuadros, PhD
Iria Carballo-Carbajal, PhD

Predoctoral Researchers
Núria Peñuelas
Joan Compte
Alba Nicolau
Camille Guillard-Sirieix
Marina Lorente-Picon
Gerard Roch

Research Technicians
Annabelle Parent
Joana Cladera
Jordi Romero-Gimenez
Guillem Colell

miquel.vila@vhir.org