EXPERIMENTAL MODELS FOR NEURODEGENERATIVE DISEASES

Report of the JPND Action Group

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JPND
research

The purpose of this Report is to recommend JPND actions in the area of Experimental Models for Neurodegenerative Diseases. The recommendations highlighted are the result of a consultation with leading European experts in the field, and are based on the analysis of the current status and urgent needs of pre-clinical research in the field of neurodegenerative diseases (ND). The actions conceived are aimed at facilitating the progress of European research and at expanding and strengthening current EC-supported actions. The potential actions to be fostered by JPND Member States span both the diffusion of knowledge to enable exploitation of available experimental models, as well as coordination of research activities and funding.

The Action Group was established following repeated consultations with the Members of the JPND Management Board (MB) and co-opting of experts in the cases of diseases under-represented in the initial group. The Action Group's mandate was to provide the JPND MB with an in-depth analysis of the data available in recent literature, grants awarded by the EU in the field, as well as identification of needs, opportunities and recommendations for potential and realisable actions.

Current research landscape

The shared view of Action Group members is that in the last decade, the study of the genetics of carrier families of ND provide a wealth of information on the components affecting disease mechanisms involved in ND pathogenesis and proved that, aside from the age of onset, the idiopathic forms of Alzheimer's and Parkinson's diseases, as well as frontotemporal lobar degeneration and amyotrophic lateral sclerosis, are clinically and neuropathologically similar to their most common familial forms. These phenotypic similarities have driven the development of a vast array of genetically-modified cell and animal models, based on the mutations described in the familial forms of ND. The studies conducted in experimental models have provided invaluable information on the pathogenesis and pathophysiology of these conditions. Major advances and new insights have been provided, for example, on the mechanisms governing the pathological aggregation of key proteins, the nature and processes of neuronal damage, the role of genetic determinants and the contribution of neuroinflammation in fuelling neuronal loss. Yet, the use of these models appears to have shed light only on partial aspects of the various disorders, thereby preventing a true translation into new treatments, diagnostics and prevention. The impressive amount of knowledge generated by experimental models has only marginally enriched the therapeutic armamentarium; in fact, although preclinical results were often encouraging, the enthusiasm raised faded when the new strategy was tested in the clinical setting.

Discussions held within the group were meant to provide a clear panorama on the general and specific criticisms of the models currently available. This analysis set the basis for the suggestion of innovative strategies to be applied in the field.

Opportunities and needs

Studies conducted in the past decades on experimental models have provided the basis for most of our current knowledge on the pathogenic and pathophysiological mechanisms of neurodegeneration. Nevertheless, a considerable hiatus remains between the significant advances made in the understanding of the pathogenic mechanisms of major ND and the identification of new and effective therapies. Therefore, the *discussion in the group has been mainly devoted to highlighting the limitations that hamper ND experimental models currently available.*

Age-related ND are largely human-specific neurodegenerative diseases; although aspects similar to those of human brain aging can be observed in aged non-human primates and some other higher-order animal species, these animals do not readily develop the full neuropathological or clinical phenotypes observed in humans. Yet, cell and animal models, even of genetic engineered non-mammalian species, such as *C. elegans*, *D. melanogaster* and zebra fish, proved useful for the dissection of the basic disease mechanisms and the screening of compounds targeting specific mechanisms involved in ND.

Genetically engineered mice have been, by far, the most popular and widely used animal models for the study of ND, but they might not be the most adequate species for mimicking human ND. Thus, research should also been carried out in other models and novel technologies should be generated, to facilitate the manipulation of their genome. In addition, reasons for the unsuccessful translation of preclinical research into clinical application may be also due to limited knowledge of the specific characteristics of the innumerable animal models generated.

The group agreed on the definition of the major unmet needs in the field of ND that still need to be addressed and how preclinical studies could provide the necessary results. Briefly, the major needs in the field of ND were summarized in the following points:

1. *Identifying markers for early diagnosis of ND*
2. *Identifying tests for a reliable measurement of the progression of the neurodegenerative diseases*
3. *Creating non-invasive methodologies for the study of brain dysfunctions*
4. *Identifying targets for treatments aimed at modifying (slowing, arresting, reverting) the progression of neuronal degeneration*
5. *Improving symptomatic treatments, with full characterization of the neuronal circuits causative of symptoms, particularly when clinical manifestations are heterogeneous*
6. *Identifying drugs able to modify/prevent the progression of ND*
7. *Identify environmental/alimentary factors which may increase the risk of onset for sporadic forms of neurodegenerative diseases*

To meet these needs, a number of elements of complexity must be taken into account when modelling a human neurodegenerative disease:

- **Genetics**: causative mutations and common variants increasing the risk for sporadic forms of neurodegenerative diseases;
- **Environment**: environmental toxins, stress, social interactions, infections, nutrition;
- **Aging**: dysmetabolism, hormonal factors, accumulation of damaging insults, genomic instability, immune derangement.

Most of the current models only take into account one of these factors and therefore do not reproduce the complexity of the diseases. Animal models are most suited for the combined analysis of these factors and the evaluation of their exact contribution to the development of the pathology.

Recommendations for Delivery

It is recommended that JPND considers taking initiatives in three domains:

1. **Call for collaborative projects**. A joint transnational call for collaborative projects may be launched, for the development of new or refined experimental models (*in vivo* or *in vitro*) of neurodegeneration aimed at overcoming the current limitations. The general objective is to generate *innovative tools* for preclinical investigation of neurodegenerative conditions. The multicentre approach will facilitate the establishment of specialized tests necessary for the validation of the models applied to the study of specific ND; different models will be applied in parallel to the study of ND mechanisms and, possibly, innovative treatments and housing of the models in different environments will increase the quality control of the results obtained.

2. **Support for the organization of interactive online workshops** (*webinars*) on experimental models for research on ND

3. **Support for the organization of a workshop to be held every three years** for a critical analysis of experimental models in use for the study of ND mechanisms and *screening* of therapeutic treatments. As a further action aimed at integrating EU research on neurodegeneration, the group recommends to promote and support the periodic organization (every 3 years) of "Portfolio Review on Animal Models" workshop aimed at assessing the changes in the state-of-the-art of experimental models for neurodegenerative diseases. An innovative format will be adopted, with selected scientists presenting the *pros* and *cons* of available models. An interactive discussion with participants will be granted, with the purpose of achieving a critical, unbiased and complete vision of the issue. Adoption of the web-based model will allow considerable reduction of costs. The Conference Proceedings will be published by JPND, thereby providing the scientific community with an updated, comprehensive and shared view on the models available.

Full Report

1. Introduction

This report was prepared by an Action Group set up by the Management Board of the EU Joint Programme on Neurodegenerative disorders (JPND) with the aim of providing an updated overview on the state-of-the-art of current models for the preclinical study of the molecular mechanisms involved in the manifestation of neurodegenerative diseases for the discovery of novel biomarkers of pathology and for the identification of treatments able to prevent or modify the progression of neurodegeneration.

Preliminary work had been carried out by JPND Working Group 2 and by consultation with Industries in a JPND workshop held in Milan on May 16th, 2012. The current report was generated by a group composed of:

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2. Terms of Reference

- Create a list of the models currently available and most utilized for the preclinical study of major ND and identify animal models other than rodents of potential interest for the study of ND
- Identify the gaps of current preclinical research on ND and scope the requirements to be considered in areas of unmet need.
- Identify current facilities for the creation, study and maintenance of animal and iPS/cell models for ND.
- Analyze current EU programs aimed at the generation of animal and iPS/cell models for ND.
- Establish guidelines/recommendations for calibrating and/or harmonising approaches to phenotypic validation in both animal and iPS/cell models for ND (for neuronal differentiation) and animal models.
- Provide a report and list of recommendations for actions to JPND Management Board.

3.   Methodology of Work

For a better organization of the preparatory work, after an initial phone conference, the group was subdivided into "disease-specific" subgroups that included the following experts:

- Parkinson models: F. Blandini, E. Hirsch, M.G. Spillantini
- Alzheimer and dementia models: M. Jucker, L. Nilsson and P. Hantraye
- Triplet disease models: P. Hantraye; B. Schneider and N. Deglon
- Amyothrophic lateral sclerosis models: B. Schneider
- Cell models: P. Marques Alves and O. Brustle

Each subgroup was requested to define, for each disease:
1. the *in vivo* and *in vitro* models available, with their characteristics in terms of phenotype and pathological alterations (neuronal degeneration/protein aggregation);
2. the commonalities of the shortcomings/deficits of each model;
3. the main objectives to pursue through the model:
    a. drug screening
    b. investigation of disease pathophysiology
    c. identification of novel biomarkers
4. which specific aspects should be taken into account to overcome the shortcomings identified at point 2 and, in general, which specific points should be addressed to improve preclinical research on that specific condition.

The work carried out by each group was then collated, circulated and discussed in a face-to-face meeting held in Milan on May 2, 2013. The document generated was then re-circulated to all members of the working group and sent for comments to other experts of the JPND community. We are grateful to J Lucas, Spain; Ignacio Torres Aleman, Spain; PH Jensen, DK; C. Shaw, UK; P. Giese, UK; D. Rubinsztein, UK; G. Mallucci, UK; S. Tabrizi, UK; D. Price, U.K.; L. Bué, France; B. Dermaut, France for their comments to the final document.

4.   Purpose of the report

The main purpose of this work was ***to discuss the validity of preclinical research in the field of ND providing a complete panorama of the models currently available and a critical overview of their limitations***, thereby suggesting lines of intervention in the reach of the JPND community that may include funding of competitive calls and organization of initiatives aimed at harmonizing research activities in this field.

5.   Current Research Landscape

Background

In the neurodegenerative disorders that most frequently affect the general population, Alzheimer's disease (AD) and Parkinson's disease (PD), age is the main risk factor. Hence, prevalence and social costs of these diseases are bound to increase progressively and dramatically along with life expectancy. Despite the urgency to find new therapies capable of modifying disease progression, however, research efforts have not been conclusive.

In contrast to the unsuccessful search for new and effective therapies, the understanding of the pathogenic mechanisms underlying major neurodegenerative conditions has progressed considerably. The mechanisms governing the pathological aggregation of the key proteins, the nature and processes of neuronal damage associated with protein aggregate formations, and the role of neuroinflammation in fuelling the neurodegenerative process have been abundantly investigated and elucidated. Yet, this knowledge has only marginally enriched the therapeutic *armamentarium* of clinical neurologists. In fact, as new details of the mechanisms of neurodegeneration are elucidated, novel therapeutic agents designed to correct the biochemical or molecular defect are tested in animal models. Results are often encouraging, but when the new strategy is tested in the clinical setting - especially in large controlled clinical trials - enthusiasm fades away.

Current debate

The current debate aims at a better understanding of the reasons for the so far limited predictive ability of the available experimental models for the identification of novel therapies.

In humans, ND are largely age-related disorders. In non-human species, on the other hand, ND represent an extremely rare condition. However, aged mammals may develop neuropathological lesions: β-amyloidosis has been show to occur, spontaneously, in primates, bears and dogs, while neurofibrillary tangles have been reported in the brain of primates, bears and sheep, but not in aged laboratory rodents or non-mammalian species. In view of the significant similarities in the phenotype of the genetic and sporadic forms of ND (e.g. AD, PD, frontotemporal dementia, or amyotrophic lateral sclerosis, ALS) genetically modified animal models carrying the human genes found to be mutated in familial cases of ND were generated in order to study the mechanism of onset and progression of such pathologies..

Numerous transgenic lines of different species (including insects, nematodes or fish, besides rodents) succeeded in partially reproducing the lesions found in the different ND and were instrumental in dissecting basic disease mechanisms, as well as in the screening of compounds designed to target specific components of the said molecular mechanisms. Genetically engineered mice have been the most popular animal models for the study of ND. A revisitation of the results so far obtained in testing therapies in these engineered animals shows that these models have been quite predictive of the clinical outcome, and the translational failure of the studies done in

the animal has been more due to a misinterpretation of the model and inadequate preclinical studies than the incomplete nature of the model itself. This underlines the importance of a critical analysis of the exact potential of the models available for a better definition of their range of validity. Thus, the exercise carried out by the group was also aimed at a better definition of the key questions addressable with current models and on suggesting means to improve their predictive power.

Human ND have complex clinical features. Can they be modelled in animals?

Perhaps with the exception of SOD-1 mutant ALS mice, one of the major shortcomings of the available models is that they only partially recapitulate the complexity of the clinical features found in humans; more importantly, neuronal loss is apparent only in some of them. Large-scale efforts are on-going to humanize entire pathways in mice and to functionally annotate every mouse gene in the context of the whole organisms and of different environments. The knowledge that is rapidly accumulating will enable to distinguish the cause from the effect of the lesions - with the introduction of the human disease gene - and to create novel models bearing multiple transgenes; this may generate a mixture of lesions, which would better mimic the complexity of the human disorder. Thus, next generation of animal models must recapitulate the human ND more closely, in order to better understand the relevance of the interactions among different neural cells and circuits.

Failure of translation due to the inadequacy of the model or of the interpretation of the results?

The studies on immunotherapy of AD are generally considered the epitome of the failure of animal studies in the generation of innovative treatments for AD patients. The immunotherapy against β-amyloid, which showed some success in APP-transgenic mice, was rapidly tested in humans, but had to be discontinued due to the occurrence of meningoencephalitis in a subset of patients. Also in this case, it could be argued that the animal model had predicted that the immunotherapy would reduce the amyloid burden in the brain parenchymal (also observed in humans) and the inability of the model to indicate the risk of encephalopathy is questionable since a better evaluation of the effect of the treatment in mice would have shown to occur in the animal only in in rare, but the fact that the immune-therapy does not affect vascular amyloid, might have suggested angiopathy-related microbleeding.

The so far limited number of therapies tested in animal models and humans does not allow to draw definitive conclusions, however a critical revision of these preclinical studies points to a series of flaws related to the number of animals analysed, their gender (the vast majority of experimental studies use only male animals), their general health condition, which indicated that the lack of translation from animal to human might have been due to an insufficient depth of analysis and suggest that better protocols for animals studies should be implemented and more strict quality control system should be required for publication.

The analysis of the preclinical literature also indicates that in the description of a novel model the limitations are not generally acknowledged, while the predictive validity for humans is often

overstated. Thus, novel standards should be set up for the validation of novel animal models and comparative studies with the available models should be a requirement for the publication of any novel animal model.

Various explanations have been proposed for the frustrating gap separating basic from clinical research, but one general element clearly stands out: experimental models used in preclinical studies prove extremely useful to dissect pathogenic mechanisms, but a more critical analysis should be carried out and experimented to establish the models limitations when searching new treatments that should be transferred to patients.

Imaging

The fast technological advances in the field of imaging, particular with functional/molecular imaging, are imposing these technologies as a major support to neurological analysis for the diagnosis and prognosis of ND progression. Molecular imaging could represent a major asset for translational studies enabling to identify and test in models and humans specific marker of pathology. Yet, the tools currently available for animal imaging are not yet widely utilized in models of ND.

6. Review of existing models of neurodegeneration

Alzheimer's disease and tauopathies leading to dementia

Virtually all AD models are based on the use of transgenic methodologies targeting APP, presenilin, tau or AOPE genes, mostly in mice. Viral vector-based models are also available, in rats and mice, while toxic models are generally under-represented. A list of the principal cell and animal AD models available is reported in annex A. Such a list may also be complemented with the list published at http://www.alzforum.org/res/com/tra/default.asp.

Problems/pitfalls/limitations of experimental models for AD and taupathies

- Important phenotypes of Alzheimer's disease like macroscopic atrophy, tauopathy or neuronal loss are very modest or nonexistent in Aβ-driven transgenic mouse or rat models.

- Although aged non-human primates show atrophy and may show neuronal loss, tau-pathology is very rare and typical AD clinical symptoms do not occur. Moreover the occurrence of these pathologies is somewhat unpredictable, making these models not usable in practice.

- Lack of functional phenotypes comparable to dementia in patients, except in non-human primate models where the same tests can be administered (e.g. DNMS, CSST, memory load, CANTAB battery) and have been shown to be altered (aged monkeys).

- Limited possibilities to monitor disease progression or therapeutic response in living mice e.g. with biomarkers or imaging. Possibilities are greater in larger animals, but still limited.

- Models often do not relate to the biology of aging.

- Therapeutic response in models often poorly translates into clinical trials with patients, due to inadequate preclinical studies and misinterpretations of the models (e.g. mouse models of Aβ but not AD).

- Models have emanated from a neurocentric view of disease, but it is not clear if Alzheimer's disease neurodegeneration is a cell-autonomous process.

- Very few models in which the synthesis of pathogenic protein is regulated by endogenous promoter and is expressed at physiological levels.

- Few animal models have been developed to address vascular dementia.

- Mice are bred on different genetic backgrounds, often heterogeneous, and transgenes can be unstable across generations. Bad experimental procedures and design of studies with animal models will impact on pathogenic and functional phenotypes of any given transgenic model. These problems have led to poor reproducibility of findings between laboratories and an often confusing scientific literature.

- Standardized functional tests and readouts are lacking. Available tests (especially cognitive) are generally not validated for longitudinal (repeated) use. Monitoring therapeutic efficacy at various time-points following treatment can be an issue (test-retest problems).

- Considerable inter-individual variability leading to the need of using large cohorts

- Models poorly replicate the biochemical complexity (truncations and post-translational modifications of Aβ or Tau) and often do not reflect the biochemical resilience of pathology, e.g. Aβ-deposits in human brain. The nature of the construct used and of the animal recipient can alter the biochemical nature of the aggregates (i.e. overexpressing a human mutated tau protein in a mouse, rat or primate brain will yield biochemically different mixture of protein aggregates). How these biochemical differences influence the pertinence and the predictability of the model generated is still largely unknown and may have major consequences for the use of these animal models in pathophysiological studies or in the development and validation of predictive biomarkers (both imaging and peripheral markers). For example, the amyloid PET tracer PiB poorly recognizes amyloid aggregates in mouse models (and also poorly in primates!) whereas it is claimed to be specific of amyloid plaques in humans with AD.

- Theragnostic marker responses in patients often differ from those originally measured in animal models that were used to develop the drug

- Tauopathy models often develop severe motor phenotypes at an early age limiting their usefulness to study pathology and cognition in aged animals in rodents.

Current EU or National programs for the generation of novel animal models of AD and taupathies

Fondation Plan Alzheimer (France) is actively financing a call for projects on new animal and cellular models of Alzheimer and related disorders (however the financial resources are very limited).

The Network of Centres of Excellence in Neurodegeneration (COEN) initiative launched a call in 2011 for the creation of innovative animal models to be applied to the study of neurodegenerative diseases (http://www.neurodegenerationresearch.eu/initiatives/network-of-centres-of-excellence/results-of-first-funding-call/).

Repositories for animal models of experimental models of AD and taupathies

The Jackson Laboratory (JAX®mice), Taconic, QPS Austria Neuropharmacology, EMMA

Suggested actions to improve modeling of AD and taupathies

- Develop models based on recent genetic discoveries of neurodegenerative disease and use them to decipher pathogenic mechanism.
- Develop and use new animal models to explore pathogenic mechanisms of already established major risk factors that remain unclear or partly unclear (for AD e.g. aging, gender, head trauma, ApoE-isoform).
- Develop more models in which proteins are expressed at physiological levels and synthesized at correct subcellular location e.g. knock-in based models, or use the paradigm of seeded induction of proteopathies.
- Develop more conditional transgenic models as to enable intervention and to prove cause-effect relationships
- Further exploit sensors, reporter technologies and optogenetics for better monitoring and to reveal cause-effect relationships with respect to pathogenesis, synaptic, neuronal and cognitive function in living animals.
- Further explore viral vector-based animal models (including non-rodent and primate models) to study proteopathic lesion-behavior or lesion-neurodegeneration relationships.
- A greater focus on pathogenic mechanisms and phenotypic markers of early-stage disease in animal models i.e. before proteinaceous deposits appear in histology.
- Validate the hypothesis of toxic oligomers and small aggregates in mouse models of neurodegenerative disease
- Stimulate development, testing and validation (reproducibility, sensitivity) of standardized phenotypic readouts with proven relevance to human studies in a few selected animal models (including non-rodent) possibly a multi-center based approach
- In general, stimulate use of emerging technologies and specifically development of new transgenic technologies focusing on the brain (viral and non-viral gene transfer techniques)
- Initiate adequate comprehensive preclinical studies to replicate and translate promising findings from exploratory preclinical studies

Parkinson's disease

Both *toxic* and *transgenic* models are currently available for PD research. Toxic models are the classic – and oldest - experimental PD models and imply the use of compounds acting as mitochondrial toxins and/or proxidant agents, all provided with selective toxicity for dopaminergic neurons (i.e., MPTP, paraquat, rotenone, 6-hydroxydopamine, etc.). These compounds can be administered to the animals, mostly rodents or primates, either systemically or through stereotaxic injections directly into the nigrostriatal pathway (i.e. 6-hydroxydopamine). Depending on the compound and the route of administration, different sets of PD-like pathological and phenotypic features can be reproduced.

Transgenic models have become available more recently, with the advent of the "genetic era" of PD that followed the identification – in 1997 - of the first PD-linked mutation in the gene encoding for alpha-synuclein. The discovery of monogenic forms of PD has provided formidable insights into the disease pathogenesis, while the recent burst of genome-wide association studies has provided evidence that familial and sporadic forms of PD share common genetic backgrounds. These considerations have prompted the development of new animal models, rodents in particular, which recapitulate monogenic mutations, associated with *toxic gain of function* or *loss of function* in the gene product, observed in the major forms of autosomal dominant or recessive PD.

A list of the principal cell and animal PD models available is reported in annex B.

General problems/pitfalls/limitations of experimental PD models

- Lack of analysis of aging effect
- Lack of models using enriched environment conditions
- Lack of models grown in non-sterile environment
- Lack of behavioral analysis relevant to humans
- Lack of models for symptoms that do not respond to dopaminergic treatment
- Lack of models with progressive neuronal loss associated to alpha synuclein deposits and neuroinflammatory processes
- Lack of models representing end -stage pathology with dopaminergic and non-dopaminergic degeneration
- Lack of models analyzing the link between genetic and environmental factors

Current EU or National programs for the generation of novel animal models
Clinique de la souris, Strasbourg; Institute of Developmental Genetics, Neuherberg: generation of models of inherited diseases.

Repositories for PD animal models
The Jackson Laboratory (JAX®mice), Taconic, QPS Austria Neuropharmacology

Suggested actions to improve modeling of PD

- Developing models with progressive degeneration
- Taking into account aging effect, also in terms of defective DNA repair (genomic instability) and proteostatic deficiencies
- Analyzing the role of positive and deleterious environmental factors on the current models
- Developing models that combine neuronal loss and alpha-synuclein pathology
- Developing models mimicking the symptoms that do not respond to current treatments (e.g. non dopaminergic lesions) and test treatments on those models
- Developing models in non-human primates that allow a better behavioral analysis
- Developing new models to explore the link between PD risk-associated polymorphisms and susceptibility to environmental toxins
- Developing models suitable for high-throughput screening for drug discovery

Amyotrophic Lateral Sclerosis

Various transgenic models of ALS have been generated, mostly in mice, based on the knowledge accumulated in the study of the familial forms of the disease. Targeted genes include those encoding superoxide dismutase 1 (*SOD1*), (TAR)-DNA-binding protein 43 (*TDP-43*) and DNA/RNA-binding protein Fused in Sarcoma (*FUS*). A toxic model based on the systemic administration of neurotoxic amino acid beta-methylamino-L-alanine (BMAA) has also been described. Invertebrate models that replicate some aspects of the ALS pathology are also available. A list of the principal cell and animal ALS models available is reported in annex C.

General problems/pitfalls/limitations of experimental ALS models

- Until now, there is few/no demonstration that animal models of ALS pathology can be used to successfully identify effective disease-modifying treatments. The animal and cellular models lack the proof of their relevance to human disease.

- Not all models display clear neurodegeneration.

- Mechanisms are still poorly elucidated.

- There are confounding variables in the interpretation of these animal models: the presence of the endogenous non-mutated protein, variations in the expression level of the transgene and the species used.

- In ALS, the SOD1 model is quite remarkable as it reproduces major features of the disease. This is still not so clear for models based on FUS and TDP43 mutations. However, there are relatively few examples of successful therapeutic approaches in the SOD1 model of familial ALS, translating into significant and reproducible effects on animal survival. Conversely, some of the positive effects observed in this model did not translate into effective therapy, most likely because of false positive data.

- With FUS/TDP-43, various animal models exist with no real consensus on the phenotype produced.

- No animal model exists for the prevalent ALS-mutated gene C9ORF72. These animal models are likely to be under development.

Repositories for ALS animal models
The Jackson Laboratory (JAX®mice), Taconic, QPS Austria Neuropharmacology.

Suggested actions to improve modeling of ALS

There is a clear need for better guidance regarding the use of these animal models:

- Identifying the most relevant gene and the most relevant readout to test therapeutic intervention (e.g. the relevance of animal survival in the SOD1 ALS model is being questioned)

- Better understanding of the link between identified genes in familial ALS and sporadic

forms of the disease. What is the contribution of SOD1, FUS, TDP-43 and C9ORF72 to sporadic forms?

- Implementing experimental approaches aimed at understanding the role of protein aggregation and propagation of the disease in the CNS.

- Developing means to monitor early changes in animal models of the ALS pathology (electrophysiology, sensitive behavioral tests, etc.)

- The role of neuroinflammation (astrogliosis, microgliosis) should be further investigated using appropriate animal models of ALS, as it may play a crucial role in the established pathology.

- Non-human primate models are important to translate gene therapy into effective treatments.

Huntington' disease

Animal models of Huntington's disease (HD) include both toxic and genetic models. Toxic models are based on the use of neurotoxins, such as mitochondrial toxin 3-nitropropionic acid or excitotoxins (kainate, ibotenate, quinolinate), which can induce selective degeneration of striatal GABAergic projection neurons, without affecting striatal interneurons, thereby mimicking the neuropathological lesion of HD. Following the unraveling of HD genetic background, numerous transgenic models targeting the *huntingtin* (Htt) gene have been developed, providing new experimental opportunities. A list of the principal cell and animal HD models available is reported in annex D.

General problems/pitfalls/limitations

Rodent models

- multiplicity of genetic backgrounds in mice

- problems of transgenic line genetic drift

- lack of major neuronal degeneration (i.e. cell loss) in transgenic models (mouse, rat). Most (if not all models) do not replicate the complex neuropathological characteristics observed in patients. Number of huntingtin-containing intranuclear inclusions is much higher than that observed in patients. In general few intracytoplasmic (neuropile) aggregates in contrast to the human condition. The atrophy of the caudate and later putamen seen in presymptomatic patients cannot be seen in rodent models where the striatum is one structure in contrast to primate brain where the caudate is separated from the putamen by the internal capsule. In addition, the natural progression of the disease (striatal degeneration followed by basal ganglia degeneration and cortical involvement) is not replicated.

- Very mild phenotype with the full-length mutant Htt

- peripheral symptoms complicating behavioral analysis (diabetes, obesity, blindness)

- lack of major motor impairments reminiscent of HD (whether dyskinesias are a primate specificity is still a question to be addressed)

- lack of major cognitive impairment reminiscent of HD

- limited appropriate translational follow-up (cognition, motor syndrome and imaging)

- limited models to investigate the cell-type specificity of the pathology

- the current models do not take into account aging phenomenon and susceptibility factors which might account for the large variability of the onset of appearance of the symptoms

Primate Transgenic model

- Too preliminary data on only few cases reported so far, to be conclusive.

- lack of major neuronal degeneration in the transgenic model. Only scarce behavioural data available on this model

- only scarce histopathological data available on this model

- model not easily available

Primate somatic gene transfer model

- Neuronal degeneration restricted to the injected areas and therefore not able to replicate the characteristic time-course of the neurodegeneration observed in patients (striatal degeneration followed by basal ganglia degeneration and cortical involvement)

- major motor impairments reminiscent of HD (dyskinesia, chorea, dystonia) are present, but no cognitive impairment demonstrated so far in this model

In vitro/iPS models

- iPS cells: limited number of individual lines often used to model disease mechanisms and the high incidence of line-to line variation.

- iPS cells: Instability of the CAG expansion

- hESCs are currently the only type of hPSCs available as clinical grade lines

- Further characterization of hESCs and iPSCs is needed

Repositories for animal models

The Jackson Laboratory (JAX®mice), QPS Austria Neuropharmacology.

Suggested actions

- Further production and characterization of hESCs and iPSCs is needed
- Develop pertinent non-human primate models of HD displaying both cognitive and motor symptoms
- Develop animal models suitable for the development and validation of translational imaging biomarkers pertinent for clinical follow-up and early detection of neuronal dysfunction (MR spectroscopy, diffusion MR spectroscopy, PET ligands for neurodegeneration, neuroinflammation, aggregates). This should be done in rats or large animal models because of the limited resolution (PET) and/or sensitivity (NMR) of the imaging modalities considered.
- Select, assess and validate behavioral tests for longitudinal assessment of lesion progression and of potential therapeutic efficacy of innovative treatment. In particular,

assess the test-retest effects of these behavioral tasks to allow longitudinal assessment at various time-points during experiments (e.g. baseline, lesion, post-treatment early, post-treatment late). N.B., most cognitive tasks have a strong test-retest effect (improvement in the performance due to previous learning of the task that prevent using the task more than 2 times in the same animal).

7.   Comparative analysis of gaps/challenges and proposed corrective actions

A final, comparative analysis of the characteristics of all the ND animal models examined by the action group, led us to identify the following gaps/challenges and potential corrective actions that may be undertaken:

Need to use behavioural tests relevant to the human disease: multi-center based development, testing and validation of standardized phenotypic readouts, with proven relevance to human studies, should be encouraged. Reliable behavioral tests for longitudinal assessment of lesion progression and of potential therapeutic efficacy of innovative treatments should also be developed.

Thorough investigation of the relationship between risk factors & genetic determinants: new models to further explore established risk factors (aging, gender, head trauma, ApoE-isoform), as well as models exploring the link between disease risk-associated polymorphisms and susceptibility to (or protection by) environmental factors should be developed. The aging effect, also in terms of defective DNA repair (genomic instability), should be specifically addressed. The links between identified genes, in familial forms of ND, and the corresponding sporadic form should be further investigated. More conditional transgenic models, as to enable intervention and to prove cause-effect relationships, should also be developed. Finally, development of new transgenic technologies (viral and non-viral gene transfer techniques) should be encouraged.

Addressing specific aspects of ND: as ND are chronic conditions that evolve over many years, it is crucial to develop models with progressive neuronal degeneration. Another crucial issue is the development of models mimicking symptoms that do not respond to current treatments (e.g., non-dopaminergic defects in PD). Development of models suitable for high-throughput screening for drug discovery should also be encouraged.

Deeper understanding of proteotoxicity mechanisms: as proteotoxicity has been recognized as a major component in the neurodegenerative process, shared by all the ND considered in this analysis, models based on seeded induction of proteopathies should be investigate, to fully understand the role of protein aggregation and the propagation of disease in the central nervous system (CNS). Models in which proteins are expressed at physiological levels and correct subcellular locations (e.g. knock-in based models) should also be developed; neurotoxicity by oligomers and small aggregates should also be specifically investigated in rodent models.

Implementation of innovative imaging techniques: a major gap to bridge is the lack of imaging techniques allowing the investigations of pathogenic mechanisms in living animals. To this aim, reporter and optogenetic technologies for investigation of cause-effect relationships with respect to synaptic, neuronal and cognitive function, should be implemented. A major endpoint should be the development and validation of translational imaging biomarkers pertinent to clinical follow-up and early detection of neuronal dysfunctions.

Identification of early markers of neurodegeneration: this is a major issue when considering the potential transfer of basic neuroscience products to the clinical setting, as it is widely accepted that any given neuroprotective intervention would be more effective if applied in the very early stages of the disease. Therefore, greater attention should be dedicated to pathological and phenotypic markers of early-stage disease in ND animal models.

Further understanding the role of neuroinflammation: neuroinflammation has been recently identified as a major component in the neurodegenerative process; the characteristics and the time-course of the neuroimmune response accompanying/sustaining the cell loss process are still largely unclear. Development of innovative models - combining, for example, neuronal loss, protein pathology and neuroinflammation - should be encouraged.

8. Conclusions

Fragmentation of EU research

The long debate on the current models underlined the necessity of collaboration among EU Countries for the generation of the models needed in the field and the difficulties to align all Countries on specific research topics: the group proposed that all Countries should align on the necessity of answering the three major open questions:

1. How to slow or halt the progression of ND
2. How to increase the efficiency of currently available tools to measure the progression of neurodegenerative process
3. How to measure the efficacy of innovative symptomatic treatments

The means of this ideological alignment should be left as open to the different Countries.

The group also agreed on the fact that brain complexity demands the use of in vivo animal models which should fully comply with the 3Rs requirement and a shared view on the ethical use of live organisms; in addition the group agreed on the necessity to exploit the widest possible range of animal models (from invertebrates to non-human primates) to solve the above questions.

Cell and animal repositories

In the analysis of the infrastructures with interest in animal models or active repositories of cell and animal models already available, the group identified numerous facilities promoted by the

European Strategy Forum on Research Infrastructures (ESFRI), including *EATRIS* (European Advanced Translational Research Infrastructure in Medicine); *BBMR* (Biobanking and Biomolecular Resources Research Infrastructure); *Infrafrontiers* (European Infrastructure for Phenotyping and Archiving of Model Mammalian Genomes). Further commercial transgenic animal repositories were identified. Therefore, the group did not recommend generating any novel JPND-supported facility. The group recommended to actively interact with the ESFRI facilities to find measures of implementation of common means.

Actions to integrate EU research

Because of the complexity of brain functioning and of the ND, the group strongly recommends the undertaking of research initiatives aimed at synergizing the expertise spread all over EU by funding collaborative projects and conferences.

9.   Recommendations for delivery

It is recommended that JPND considers taking initiatives in three domains:

1. **Call for collaborative projects**. A joint transnational call for collaborative projects may also propose new experimental models (*in vivo* or *in vitro*) of neurodegeneration aimed at overcoming the current limitations. The general objective is to generate *innovative tools* for preclinical investigation of neurodegenerative conditions. The multicentre approach will facilitate the establishment of specialized tests necessary for the validation of the models applied to the study of specific ND, different models will be applied in parallel to the study of a ND mechanisms and, possibly, innovative treatments and housing of the models in different environments will increase the quality control of the results obtained.

Premise

A serious limitation of modern neurosciences is the frequent failure to translate the wealth of information provided by preclinical research into commensurate gains in new treatments, diagnostics and prevention. This limitation is particularly critical in the field of neurodegenerative disorders. Studies conducted in experimental models have provided invaluable information on the pathogenesis and pathophysiology of disorders such as Alzheimer's disease, Parkinson's disease and others neurodegenerative conditions less frequent in the general population. Major advances and new insights have been provided, for example, on the mechanisms governing the pathological aggregation of key proteins, the nature and processes of neuronal damage, the role of genetic determinants and the contribution of neuroinflammation in fuelling neuronal loss. Yet, this knowledge has only marginally enriched the therapeutic armamentarium of clinical neurologists. As new pathogenic mechanisms are pinpointed, novel therapeutic agents designed to correct the biochemical or molecular defect are tested in animal models; results are often encouraging, but enthusiasm usually fades when the new strategy is tested in the clinical setting.

Unmet therapeutic needs for ND disorders are:

- progression of neuronal degeneration with identification of targets for neuroprotection;
- symptomatic treatments with identification of neuronal circuits causative of symptoms.

In order to meet these needs, a number of elements of complexity must be taken into account when modelling a human ND disease:

- Genetics: causative mutations and common variants increasing the risk for sporadic forms of ND diseases;
- Environment: environmental toxins, stress, social interactions, infections, nutrition;
- Aging: dysmetabolism, hormonal factors, accumulation of damaging insults, genomic instability, immune derangement.

Most of the current models only take into account one of these factors and therefore do not reproduce the complexity of the diseases.

Specific objectives of the call

Within the remit of this call, neurodegeneration applies to Alzheimer's disease and other dementias, Parkinson's disease and related disorders, motor neuron disease, Huntington's disease, and other rare neurodegenerative disorders. Different types of expertise from specialized laboratories will be required to reproduce the complexity of the human disease of interest and to address the unmet needs mentioned above; proposed models will have to show a clear translational value and substantial innovative potential.

Support may be sought for projects addressing the following specific objectives:

- development of animal models characterized by progressive neurodegeneration and protein aggregate deposition/propagation (combining specialists of cell death and protein aggregation); models should reproduce the topography of the lesion, neuronal loss and dysfunction, as observed in the human disease;
- new models for the investigation of combined risk/protective factors for neurodegenerative diseases, including aging, common genetic and epigenetic variants, sex, inflammation-like, immune-related signalling, environmental factors (including prenatal events, social interactions, enriched environment, etc.). Models combining different factors will be required;
- development and validation of models able to monitor disease onset and progression, using novel or established biomarkers relevant to the human diseases and clinical settings;
- development of models suitable for high-throughput screening of factors influencing disease risk and innovative drugs;
- implementing the use of neuronal, neuronal-like cells or inducible pluripotent stem (iPS) cells, generated from different sources, to investigate phenotypic heterogeneity by analysing cell death processes, cell physiology and pathology;
- development, testing and validation of models mimicking specific symptoms that do not respond to current treatments

− the use of models suited for molecular imaging is highly recommended.

<u>Priority for the call</u>: high

<u>Time and budget scale</u>: 3-year projects; 15 million (minimum); min number of partners per project: 3 (from different countries)

<u>Added value to already existing initiatives</u>: no initiative in the field is currently ongoing

<u>Possible involvement of already existing initiatives</u>: none

<u>Possible involvement of industry</u>: strongly encouraged.

2. **Support for the organization of interactive online workshops** (*webinars*) on experimental models for research on ND

3. **Support for the organization of a workshop to be held every three years** for a critical analysis of experimental models in use for the study of ND mechanisms and *screening* of therapeutic treatments. As a further action aimed at integrating EU research on neurodegeneration, the group recommends to promote and support the periodic organization (every 3 years) of "Portfolio Review on Animal Models" workshop aimed at assessing the changes in the state-of-the-art of experimental models for neurodegenerative diseases. An innovative format will be adopted, with selected scientists presenting the *pros* and *cons* of available models. An interactive discussion with participants will be granted, with the purpose of achieving a critical, unbiased and complete vision of the issue. Adoption of the web-based model will allow considerable reduction of costs. The Conference Proceedings will be published by JPND, thereby providing the scientific community with an updated, comprehensive and shared view on the models available.

Alzheimer's disease and TAUopathies leading to dementia

ANIMAL MODELS CURRENTLY AVAILABLE

1. Genetic models

TARGETED GENE	DESCRIPTION[1]	PHENOTYPE	NEURONAL DEGENERATION	PROTEINOPATHY / AGGREGATES
APP	Most models overexpress human APP in mice or rats. Transgene is usually cDNA alternatively a genomic fragment and harbor one or several mutations causing Alzheimer's disease. There is also at least one APP knock-in model and a few inducible lines. The promoter is often neuronal.	Age-dependent accumulation of Aβ-deposits, secondary pathology of neuritic dystrophy, microgliosis and astrocytosis, usually mild cognitive phenotypes at least in aged animals with severe deposition	No (but, abnormal changes of nerve endings and disturbed signaling in vicinity of plaques)	Diffuse and cored plaques and cerebral amyloid angiopathy, Some models show all of these phenotypes, while in others are one or two of these phenotypes are predominant
Presenilin (PS)	Overexpression or knock-in of mutant presenilin; knock-out of presenilin	Overexpressing mice show elevated Aβ42, few other phenotypes In knock-out there is gross morphological changes, in brain-condition KO there is effects on neuronal differentiation leading to structural changes in brain	Dark stained neurons reported in PS-knock-in mice. Brain atrophy in PS- conditional-knockout	No
APPxPS	Bigenic mice expressing both mutant human APP and PS1 or PS2	More aggressive Aβ-pathology as compared to APP, and an earlier age-of-onset due to a change in Aβ42/40 ratio	No (but, abnormal changes of nerve endings and disturbed signaling in vicinity of plaques)	Diffuse and cored plaques and cerebral amyloid angiopathy. Some models show all of these phenotypes, while others show only some type of proteinopathies
Tau	Overexpression of human Tau in mice or rats. The transgene usually harbor one or	Tau-inclusions (in some models PHF), gliosis, axonopathy, often pathology in spinal	Y, neuronal loss in some models (e.g. Tg4510) but not in most models	Tau inclusions of different morphology structure and biochemical

	several mutations which results in tauopathy. The promoter is neuronal. Tau KO-mice are available.	cord and the brain stem which then typically leads to motor disturbance, cognitive phenotypes in some models		composition, but different ultra-structure, in some models paired helical filaments essentially identical to those of Alzheimer's disease.
ApoE	Human ApoE ε2 /ε3 / ε4 knock-in or astroglial overexpression	Differential effects on central and peripheral cholesterol metabolism in ApoE-models, and on Aβ-deposition in crossed APP/ApoE-models	No	No
Primates/ Prosimian	Caribbean vervet monkeys; lemurs; cotton-top tamarins; rhesus monkeys; squirrel monkeys; chimpanzees; baboons	Tau lesions have been very rarely described. Aβ-deposits occur in old age and dependent on the species. Rhesus monkeys primarily develop cerebral Aβ-amyloid angiopathy. Aged monkeys (baboons, rhesus) show memory deficits (DNMS test), executive function alterations (CSST, WCST tests), and motor deficits. In aged rhesus, correlations have been shown between impaired cognitive and motor functions and volumetrics and microstructural alterations in cortical and subcortical areas, respectively. These primates do not show the typical clinical symptoms of AD-patients.	Variable and unpredictable	Varies between models but amyloid angiopathy, diffuse Aβ deposits are common and there is also neuritic plaques although more sparse. Neurofibrillary tangles with PHF have been observed in some primates
Dogs	Beagles	Develop Aβ pathology Neuritic dystrophy, gliosis, ventricular dilation, cortical and hippocampal atrophy. Degeneration of smooth muscle cells in association with Aβ amyloid angiopathy. Reports of cognitive deficits in old dogs being dependent on Aβ-pathology and age.	No (?)	Amyloid angiopathy, diffuse Aβ deposits, few amyloid plaques.
Viral vector-based	Lentiviral vectors overexpressing WT Tau or P301L Tau Intra-	Progressive memory impairments (Morris tests)	Progressive pathology with time dependent	Yes NFT with PHF, positive Gallyas stained neurons

			appearance of AT8 then AT100 staining and spreading of this pathology from hippocampus to remote brain (neuronally-connected) regions. Biochemical abnormalities in blood and LCR	(end stage)
models in rats	hippocampal injections through stereotactic intervention.			
Viral vector-based models in mouse	AAV6 vectors overexpressing mutant forms of Tau (P301S or the 3PO) mutation Tau Intra-entorhinal cortex injections through stereotactic intervention.	Morris water maze behavioral tests demonstrated mild memory impairment tests)	Starting at 2 months and increasing by 6 months post-injection, hyperphosphorylated tau pathology, in addition to dystrophic neurites. Neuronal loss in tau-expressing regions, Neuroinflammation around plaques, and in regions expressing mutant tau.	Yes NFT with PHF
Ts65Dn, model of Down syndrome	Segmental trisomy of chromosome 16	Synaptic and cognitive phenotypes that depend on APP gene dosage	Degeneration of cholinergic neurons	No

[1]Expression of mutant gene, overexpression of WT gene, knock-out, etc.

2. Non genetic models –toxic models

TOXIN	SYSTEMIC/LOCAL ADMINISTRATION[2]	PHENOTYPE	NEURONAL DEGENERATION	PROTEINOPATHY / AGGREGATES
Seeding models	Infusion of minute amounts of brain extracts of Aβ- or Tau- lesion-containing brains into young APP or Tau transgenic mice. Recently, the infusion have been	The phenotypes are similar, albeit accelerated, compared to the uninjected hosts (see above)		Same as APP or Tau transgenic mice

| | done with synthetic Aβ and Tau | | | |

²Brief description of the procedure

CELLULAR MODELS CURRENTLY AVAILABLE

1. Induced pluripotent stem cells (iPSC)

CELL TYPE	DESCRIPTION	NEURONAL DEGENERATION	PROTEINOPATHY / AGGREGATES
DA neurons	iPSC model for sporadic PD (Soldner 2009)	None	None
neurons	iPSC model for fAD PS1 A246E and PS2 N141I (Yagi 2011)	None	Increased amyloid beta42 secretion
neurons	iN model for fAD (PS1, PS2) (Qiang 2011)	None	None
cortical neurons	iPSC model for AD (trisomy 21: increased APP dosage)(Shi 2012)	None	insoluble intracellular and extracellular amyloid aggregates, hyperphosphorylated tau protein in cell bodies and dendrites
neurons	iPSC model for sAD and fAD (duplication of APP) (Israel 2012)	None	higher levels of the pathological markers amyloid-ß(1–40), phospho-tau(Thr 231) and (aGSK-3b). Accumulation of large RAB5-positive early endosomes.
neurons, astrocytes	iPSC model for Alzheimer's disease (Kondo 2013)	None	Y; Aß oligomer accumulation leads to ER and oxidative stress

2. Genetic models

CELL SOURCE	DESCRIPTION	PHENOTYPE: (NEURONAL DEGENERATION; PROTEINOPATHY / AGGREGATES)	REFERENCE
Adults with Down Syndrome	Overexpression of APP (ref. Shi et al., 2012)	Secretion of the pathogenic peptide fragment amyloid-β42 (Aβ42), which formed insoluble intracellular and extracellular amyloid aggregates and hyperphosphorylation of tau protein	Shi et al., 2012
mtDNA-depleted SHSY5Y neuroblastoma and Ntera/D1 (NT2) human teratocarcinoma cells	mtDNA from the AD patients	elevated production of ROS; increased basal cytosolic calcium concentration and impaired intracellular calcium homeostasis, and abnormal mitochondrial morphology.	Ghosh et al, 1999

3. Non genetic-Toxic models

CELL SOURCE	DESCRIPTION OF THE TOXIC STIMULUS	PHENOTYPE: (NEURONAL DEGENERATION; PROTEINOPATHY / AGGREGATES)	REFERENCE
Human Neuroblastoma cell line (SH-SY5Y)	Toxic Aβ oligomers	Formation of senile plaques	Dodel et al., 2011
Primary cultures of cortical neurons	Aβ 1-40 peptide	Formation of senile plaques, accumulation of ROS, neuronal death.	Fonseca et al., 2009

Parkinson's disease

ANIMAL MODELS CURRENTLY AVAILABLE

Genetic models

TARGETED GENE	DESCRIPTION[1]	PHENOTYPE (Y/N; BRIEF DESCRIPTION)	NEURONAL DEGENERATION (Y/N; BRIEF DESCRIPTION)	PROTEINOPATHY/ AGGREGATES (Y/N; BRIEF DESCRIPTION)
Transgenic				
Alpha synuclein	Mice or rats overexpressing full length or part of alpha synuclein mutated (A30P, A53T, A30P/A53T) or wild type	Little behavioral effect. In some models alteration of gastrointestinal function.	In most of the models no neuronal loss but alteration in dopamine transmission.	Alpha synuclein deposits widespread in the brain
LRRK2	Mice overexpressing mutant (R1441G or G2019) or WT LRRK2	Little or no motor defects	no	Modest increase in total and phosphorylated tau
Models for the other genes mutated in PD (parkin, Pink1, DJ1)	KO transgenic mice	Little behavioral effect (mostly moderate decline in locomotor activity)	In most of the models no neuronal loss but alteration in dopamine transmission; mitochondrial defects (parkin, Pink1 mutants); increased susceptibility to pro-oxidant toxins (DJ1-mutants)	no
Aphakia (ak)	Mice deficient in Pitx3	Dysfunction due to dopaminergic degeneration	Loss of dopaminergic neurons in the substantia nigra but less in VTA	no
MitoPark mouse	Mitochondrial transcription factor A (Tfam) KO in midbrain DA neurons, causing reduced mtDNA	Delayed, progressive reduction of locomotor activity, ameliorated by L-DOPA	Adult-onset nigrostriatal degeneration with reduction of dopamine levels.	Intracellular inclusions positive for mitochondrial protein markers (no synuclein)

	expression and respiratory chain defects			
Engrailed mouse	Ablation of homeobox transcription factors Engrailed-1 and Engrailed-2 (required for survival of SNc dopaminergic neurons): *En1+/- ;En2-/-*	Subtle motor deficits	Loss of SNc neurons and striatal dopamine	no
c-Rel mouse	Mice carrying null mutation in DNA binding protein c-Rel (part of the NFkB complex): *c-Rel–/–*	Age-dependent locomotor and gait-related deficits responsive to L-Dopa	Age-dependent loss of SNc dopaminergic neurons and striatal terminals; reduction of striatal dopamine and homovanillic acid levels	Increased alpha-synuclein immunoreactivity in the SNc
Nurr1 mouse	Heterozygous KO of transcription factor Nurr1 (required for development and maintenance of dopamine neurons): *Nurr1 +/–*	Decreased rotarod performance and locomotor activities	Age-dependent nigral cell loss and reduction in striatal dopamine and dopamine-mediated signaling. Increased vulnerability to MPTP	no
Atg-7 mouse	Conditional deletion of autophagy-related (Atg) gene 7 in SNc neurons	No PD-like phenotype	Age-related loss of dopaminergic neurons and striatal dopamine	Accumulation of low-molecular-weight alpha-synuclein and ubiquitinated protein aggregates
		Virus-induced		
Alpha synuclein	Cav-viruses over expressing human alpha synuclein in mice or rats	Unilateral injection of the virus in the striatum. Loss of dopaminergic neurons. The advantage of the Cav is that as compared to other viruses it is less immunogenic. Rotation	Loss of dopaminergic neurons	Alpha synuclein inclusions

		behavior can be analyzed		
Alpha synuclein	AAV expressing full length alpha synuclein in rat or monkeys	Unilateral injection of the virus in the striatum or the cerebral cortex. Loss of dopaminergic neurons. When injected in the cerebral cortex it can be combined with a 6-OHDA lesion of nigral dopaminergic neurons	Loss of dopaminergic neurons of alteration of cortical neurons depending on the site of injection. It can mimic end stage Parkinson's disease in which Alpha synuclein inclusions are found in the cerebral cortex.	Yes when injected in the striatum. It can also mimic end stage Parkinson's disease in which Alpha synuclein inclusions are found in the cerebral cortex.
LRRK2	Cav-viruses or AAV over expressing human wild type or mutated LRRK2 in mice or rats	Unilateral injection of the virus in the striatum. Loss of dopaminergic neurons. The advantage of the cav is that as compared to other viruses it is less immunogenic. Rotation behavior can be analyzed	Loss of dopaminergic neurons	N

<u>Non-mammalian models</u>

Drosophila	Overexpression of wt or mutant human alpha-synuclein	Progressive loss of climbing activity	Age-dependent and selective loss of dopaminergic neurons	Fibrillary inclusions containing alpha-synuclein
	Parkin or PINK1 KO or overexpression of mutated forms	Loss of climbing activity	Mitochondrial defects and moderate dopaminergic degeneration	no
	DJ1-beta (homolog of human DJ1) KO	?	Enhanced susceptibility to pro-oxidant toxins	no
	LRRK2 KO or overexpression of mutated forms	no	no	no
Zebrafish	Parkin or PINK1 KO	Moderate reduction in locomotor activity	Moderate loss of dopaminergic neurons, reduced mitochondrial complex I activity and increased susceptibility to toxins	no

| | DJ1 KO | no | Increased susceptibility of dopaminergic neurons to toxins | no |
| | Deletion of functional domain WD40 of LRRK2 | Locomotor defects | Loss of dopaminergic neurons | no |

[1]*Expression of mutant gene, overexpression of WT gene, knock-out, etc.*

Non-genetic (toxic/pharmacological) models

TOXIN	SYSTEMIC/LOCAL ADMINISTRATION[2]	PHENOTYPE (Y/N; BRIEF DESCRIPTION)	NEURONAL DEGENERATION (Y/N; BRIEF DESCRIPTION)	PROTEINOPATHY / AGGREGATES (Y/N; BRIEF DESCRIPTION)
6-OHDA	Local Nigral, MFB or striatalstereotaxic injection	Apomorphine/amphetamine-induced rotations; the striatal injection produces a progressive partial degeneration over about 4 weeks, while nigral/MFB injection causes complete, fast evolving lesions (within 1 week). Good for analysis of LID.	Yes, there is loss of DA neurons, but not of other neurons. The striatal injection produces immediate terminal damage, followed by delayed loss of nigral cell bodies; nigral microglial activation precedes actual loss of DAergicneurons	no
MPTP	Systemic i.p. or s.c.via osmotic pump in mice, i.p., i.m. or intra jugular in monkey. Recently it has been administered intra-nasaly	Little phenotype in mice and rather a hyperactivity, akinesia and rigidly in monkey, resting tremor in green monkeys and transient rest tremor in macaques. Can be combined with lesions of other neurotransmitter systems such as cholinergic neurons in the PPN to produce gait and balance disorders or norepinephrine neurons in the locus coeruleus to produce intellectual impairment. Good for analysis of LID.	Yes, loss of dopaminergic neurons reproducing the selective vulnerability seen in human. Neuroinflammatory processes in monkey but not in rodents.	no
Rotenone	Systemic i.v., s.c. or i.p. via osmotic	Severe phenotype including akinesia, GI dysfunction, gait and	Loss of dopaminergic and non dopaminergic	Alpha synuclein and

	pump (rats)	balance disorders but not specific for dopaminergic neurons	neurons (widespread lesions). Glial cells also affected	tau pathology
	Intra-gastric or oral administration (mice) for investigation of PD-related GI dysfunctions	Less severe phenotype; impaired performances at the rotarod test; GI dysfunction (reduced fecal output following oral adm.)	Moderate SNc lesion (oral>intragastric);	Trans-synaptic transmission of synuclein pathology along the brain-gut axis (intragastric adm.)
Paraquat	Systemic i.p. (mice)	No clear motor deficits	Moderate SNc cell loss; decreased striatal TH immunoreactivity	Up-regulation and aggregation of synuclein in the SNc
Annonacin	Systemic i.v., via osmotic pump	Severe phenotype including akinesia, gait and balance disorders but not specific for dopaminergic neurons. Reproduces an atypical form of PD in the French Caribbean	Loss of dopaminergic and nondopaminergic neurons reproducing the pathology seen in an atypical form of PD in the French Caribbean	Tau pathology
l-trans-pyrrolidine-2,4-dicarboxylate(EAATsinhibitor)	Local Intra nigral	Rotation after unilateral lesion	Selective loss of DA neurons	Alpha synuclein pathology
LPS alone or associated to 6-OHDA	Local or systemic Intra nigral or i.p.	???	Loss of dopamine neurons neuroinflammatory processes	no

[2]*Brief description of the procedure*

CELLULAR MODELS CURRENTLY AVAILABLE

CELL TYPE	DESCRIPTION	NEURONAL DEGENERATION (Y/N; BRIEF DESCRIPTION)	PROTEINOPATHY/ AGGREGATES (Y/N; BRIEF DESCRIPTION)
SH-SY5Y	Human neuroblastoma cells	Sensitivity to PD-related toxins; mitochondrial defects, proteotoxicity and cell death triggered by transfection with PD-associated mutant genes	Synuclein aggregation can be triggered under specific conditions
PC12	Rat pheochromocytoma	Sensitivity to PD-related toxins	Synuclein aggregation can be triggered under specific conditions
MES	Hybrid rat mesencephalic-neuroblastoma cells	Sensitivity to PD-related toxins	Synuclein aggregation can be triggered under specific conditions
Primary neuronal cultures	Cultured dopaminergic neurons from embryonic mesencephalon	Sensitivity to PD-related toxins; synuclein overexpression-induced cell death	Synuclein aggregation can be triggered; cell-to-cell synuclein propagation can be observed
Cybrids	Hybrid cell lines obtained by fusing cells that lack mtDNA with platelet mtDNA from PD patients	Defects of the mitochondrial ETC	no
iPS	Induced pluripotent stem cells re-programmed from human fibroblasts	PD-related biochemical defects from donor cells are substantially maintained (*"brain in a dish"*)	Synuclein aggregation can be triggered

Amyotrophic Lateral Sclerosis

ANIMAL MODELS CURRENTLY AVAILABLE

Genetic models

TARGETED GENE	DESCRIPTION[1]	PHENOTYPE (Y/N; BRIEF DESCRIPTION)	NEURONAL DEGENERATION (Y/N; BRIEF DESCRIPTION)	PROTEINOPATHY / AGGREGATES (Y/N; BRIEF DESCRIPTION)
SOD1	At least three mutations: SOD1 G93A, G87R, G85R G93A fALS mice: 20 copies, hSOD1 promoter	Y; paralysis and premature death	Y; motoneuron degeneration, muscle denervation; implication of non-cell autonomous mechanisms; inflammation characterized by astroglial and microglial activation; muscle atrophy	Y: aggregated SOD1 can be detected with specific antibodies
TDP-43	Overexpression of WT and mutated forms	Y; Motor deficits but no paralysis	Y; +/- MN loss, depending on the line. Overall mild effects	Not in all cases Sometimes Ub+,TDP43 nuclear and cytsol aggregates
TDP-43	Conditional KO	Weight loss and age-dependent motor impairment	Y; MN degeneration	N
FUS	PrP-hFUS WT overexpression	Y; motor impairment, paralysis and death	Y: MN loss	Y
Transgenic mutated FUS rats		Motor axon degeneration, muscle denervation	Y; Motor axon degeneration, muscle denervation	Y
TDP-43	AAV based viral model	?	?	?

[1]*Expression of mutant gene, overexpression of WT gene, knock-out, etc.*

Non-genetic (toxic/pharmacological) models

Toxin	Systemic/Local Administration[2]	Phenotype (Y/N; brief description)	Neuronal degeneration (Y/N; brief description)	Proteinopathy / Aggregates (Y/N; brief description)
BMAA	Mainly systemic	Some motor phenotypes reported	?	?

[2]*Brief description of the procedure*

CELLULAR MODELS CURRENTLY AVAILABLE

Cell type	Description	Neuronal degeneration (Y/N; brief description)	Proteinopathy / Aggregates (Y/N; brief description)
iPS cells (SOD1, TDP-43)	Co-cultures MN/astrocytes/micro glial cells	Y; used to evaluate non-cell autonomous processes by co-cultivating cells carrying or not-carrying the pathogenic mutation	N
MN	Primary cultures with or w/o astrocytes		

Huntington's disease

ANIMAL MODELS CURRENTLY AVAILABLE

TRANSGENIC MOUSE MODELS

TARGETED GENE	DESCRIPTION[1]	PHENOTYPE (Y/N; BRIEF DESCRIPTION)	NEURONAL DEGENERATION (Y/N; BRIEF DESCRIPTION)	PROTEINOPATHY / AGGREGATES (Y/N; BRIEF DESCRIPTION)
HTT	Prion-N171-82Q	Abnormalities of sensorimotor gating Adipose tissue pathology Altered energy metabolism Balance and coordination alterations Body weight loss Brain atrophy Clasping behavior Diabetes mellitus Energy- and appetite-regulating hormones changes Gait alteration Grip strength impairment Internal organs pathology Locomotor impairment Neurogenesis impairment Neuronal loss Neuronal morphology alterations Neuronal physiology alterations Neurotransmiters level depletion Oxidative and nitrosative stress polyQ protein aggregates Premature death Procedural learning alteration Tremor	Y Atrophy and present of "dark cells" and atrophy of the brain,	Y Diffuse nuclear accumulation of Htt in the Striatum, cortex, hippocampus amygdala.
	NLS-N171-82Q	Muscle abnormalities	N	N
	R6/1	Affective function alteration Balance and coordination alterations Body weight loss Brain atrophy Clasping behavior Diabetes mellitus Exploratory behavior impairment Gait alteration Grip strength impairment Increased cerebral blood flow Internal organs pathology Locomotor impairment	Y Atrophy and present of "dark cells" and atrophy of the brain, no major cell death	Y Intranuclear and neuropil aggregates throughout brain fewer dendritic spines

		Muscle abnormalities Neurogenesis impairment Neuronal loss Neuronal morphology alterations Neuronal physiology alterations Overall condition Oxidative and nitrosative stress polyQ protein aggregates Premature death Reactive gliosis Seizure Spatial learning deficits Visual impairment		
	R6/2	Adipose tissue pathology Altered energy metabolism Auditory dysfunction Balance and coordination alterations Body weight loss Brain atrophy Clasping behavior Diabetes mellitus Disease duration Exploratory behavior impairment Gait alteration Grip strength impairment Hypothermia Internal organs pathology Locomotor impairment Muscle abnormalities Neurogenesis impairment Neuronal loss Neuronal morphology alterations Neuronal physiology alterations Neurotransmiters level depletion Oxidative and nitrosative stress polyQ protein aggregates Premature death Reactive gliosis Rousability Spatial learning deficits Tremor Visual discrimination learning impairment Visual impairment Brain Metabolic alterations seen using NMR spectroscopy and post mortem ATP and metabolite detections	Y Atrophy and present of "dark cells"	Y Intranuclear and neuropil aggregates throughout brain fewer dendritic spines
	YAC72	Locomotor impairment Clasping behavior Balance and coordination alterations Brain atrophy Neuronal morphology alterations	Y Atrophy and present of "dark cells"	N Very few NIs in the striatum

		Neuronal loss Reactive gliosis Neuronal physiology alterations		
	YAC128	Affective function alteration Balance and coordination alterations Body weight loss Brain atrophy Clasping behavior Declarative memory impairment Exploratory behavior impairment Gait alteration Grip strength impairment Immune response deficit Internal organs pathology Locomotor impairment Neuronal loss Neuronal morphology alterations Neuronal physiology alterations polyQ protein aggregates Procedural learning alteration Spatial learning deficits	Y	Y
	CAG140	Neuronal physiology alterations Locomotor impairment Exploratory behavior impairment Declarative memory impairment Loss of striatal markers (DARPP32)	Y Minor atrophy in age animals, Loss of striatal markers in medium size spiny neurons	Y Intranuclear and neuropile aggregates
	BACHD	Spatial learning deficits Declarative memory impairment Body weight loss Balance and coordination alterations Locomotor impairment Exploratory behavior impairment Brain atrophy Neuronal physiology alterations polyQ protein aggregates Body weight loss	Y Atrophy of the striatum in vivo using NMR (~14% at 12 months)	Y Almost impossible to detect with standard immunohistochemical protocols
	HD190QG	polyQ protein aggregates Neuronal physiology alterations	N	Y
	Q111	Neuronal physiology alterations	Y Atrophy of the striatum at 12 months (~20%)	Y

[1]Expression of mutant gene, overexpression of WT gene, knock-out, etc.

TRANSGENIC RAT MODELS

Targeted gene	Description[1]	Phenotype (Y/N; brief description)	Neuronal degeneration (Y/N; brief description)	Proteinopathy / Aggregates (Y/N; brief description)
HTT	A 1962 bp rat HD cDNA fragment carrying expansions of 51 CAG repeats under the control of 885 bp of the endogenous rat HD promotor	Slow progressive phenotypes with emotional, cognitive and motor dysfunction Accumulation of huntingtin aggregates and nuclear inclusions in striatal neurons Alterations using PET scan imaging (loss of D2 receptors)	Y Small striatal atrophy but no actual cell loss	Y
HTT	HD transgenic rat model using a human bacterial artificial chromosome (BAC), which contains the full-length HTT genomic sequence with 97 CAG/CAA repeats and all regulatory elements.	robust, early onset and progressive HD-like phenotype including motor deficits and anxiety-related symptoms neuropil aggregates and nuclear accumulation of N-terminal mutant huntingtin	Not described	Y
Tet/HD94	conditional mouse model Yamamoto*, Lucas* and Hen, Cell 2000	Balance and coordination alterations Body weight loss Brain atrophy Clasping behavior Neuronal loss polyQ protein aggregates	Y Atrophy and present of "dark cells" and atrophy of the brain,	Y Diffuse nuclear accumulation of Htt in the Striatum, cortex, hippocampus amygdala.

GENETIC OTHER MODELS

Targeted gene	Description[1]	Phenotype (Y/N; brief description)	Neuronal degeneration (Y/N; brief description)	Proteinopathy / Aggregates (Y/N; brief description)
HTT	Rat intrastriatal injection of AAV2-CMV-97Q-GFP	progressive formation of intracytoplasmic and ubiquitinated intranuclear aggregates in neurons	time-dependent loss of 97Q-GFP staining is observed between day 12 and day 35 after injection 12 d after infection, a population of striatal cells undergoes apoptotic	

			cell death	
HTT	rat Intrastriatal infection by Lentiviral vector expressing HTT171, 853, 1220-82Q	Earlier onset and more severe pathology occurred with shorter fragments, longer CAG repeats, and higher expression levels Neuronal dyfunction and neuronal loss Neuronal morphology alterations Neuronal physiology alterations polyQ protein aggregates	selective striatal lesions with relative sparing of striatal interneurons and severe loss of GABAergic medium size spiny neurons	Intranuclear inclusions and neuropile aggregates with aging Sequential appearance of ubiquitinated htt aggregates
HTT	Mice intrastriatal injection of AAV1/8-CBA-Htt365aa-100Q	neuropathology and motor deficits degenerating and shrunken Htt-labeled neurons in cortex layers 5 and 6 and in the dorsal striatum at 2 weeks Clasping phenotypeat 2 weeks	Decreased somal cross-sectional area of GABAergic neurons of the striatum, a decreased number of nissl-positive cells in the striatum	Striatal and cortical neurons infected with AAVHtt100Q had strong diffuse nuclear labeling or intranuclear aggregates
HTT	Rat intrastriatal injection of AAV1/2-Exon1 70/20/8Q	HTT immunostaining is diminished by 5–8 weeks neuronal cell death within the striatum, with marked striatal atrophy, enlargement of the ipsilateral lateral ventricle Fluoro-Jade B staining and reactive astrogliosis	Decrease neuronal immunoreactivity (NeuN, calbindin D28k, or DARPP-32) at 2 weeks, and complete loss at 5 weeks Complete loss of NPY, parv, and ChAT immunoreactivity	Accumulation of misfolded Htt between 1-5 weeks
HTT	Non-human Primate Intrastriatal infection by Lentiviral vector expressing Exon 1 -82Q	Locomotor impairment (dyskinesia) Neuronal loss Neuronal morphology alterations Neuronal physiology alterations polyQ protein aggregates	Y selective striatal lesions with relative sparing of striatal interneurons and severe loss of GABAergic medium size spiny neurons	Y Intranuclear inclusions mostly.
HTT	Transgenic Non-human Primate achieved using lentiviral vector technology exon-1 htt with a 147-glutamine repeat (147Q)	Locomotor impairment (dystonia and chorea) polyQ protein aggregates	Not described	Y

NON-GENETIC (toxic/pharmacological) MODELS

TOXIN	SYSTEMIC/LOCAL ADMINISTRATION [2]	PHENOTYPE (Y/N; BRIEF DESCRIPTION)	NEURONAL DEGENERATION (Y/N; BRIEF DESCRIPTION)	PROTEINOPATHY / AGGREGATES (Y/N; BRIEF DESCRIPTION)
3-nitropropionic acid	Repeated Systemic administration in rats	Y Locomotor impairment (dystonia) Cognitive deficits (perseveration) Neuronal loss Neuronal morphology alterations	Y selective striatal lesions with relative sparing of striatal interneurons and severe loss of GABAergic medium size spiny neurons	N

		Neuronal physiology alterations Metabolic alterations (MR spectrosocopy)		
3-nitropropionic acid	Chronic Systemic administration in non-human primates	Locomotor impairment Hyperkinetic syndrome followed by bradykinesia & dystonia Neuronal loss Neuronal morphology alterations Neuronal physiology alterations Metabolic alterations (MR spectrosocopy)	Y selective striatal lesions with relative sparing of striatal interneurons and severe loss of GABAergic medium size spiny neurons	N
Excitotoxins (Kainate, Ibotenate, Quinolinate)	Rat Stereotactic injections	Locomotor impairment Hyperkinetic syndrome followed by bradykinesia & dystonia Neuronal loss Neuronal morphology alterations Neuronal physiology alterations Metabolic alterations (MR spectroscopy)	Y selective striatal lesions with relative sparing of striatal interneurons and severe loss of GABAergic medium size spiny neurons	N
Excitotoxins (Kainate, Ibotenate, Quinolinate)	Non-human primate Stereotactic injections	Locomotor impairment Acute choreatic syndrome following apomorphine or L-DOPA systemic administration Neuronal loss Neuronal morphology alterations Neuronal physiology alterations Metabolic alterations (MR spectroscopy, FDG PET) Receptor binding losses (PET imaging)	Y selective striatal lesions with relative sparing of striatal interneurons and severe loss of GABAergic medium size spiny neurons with Ibotenate & quinolinate	N

[2]Brief description of the procedure

CELLULAR MODELS CURRENTLY AVAILABLE

CELL TYPE	DESCRIPTION	NEURONAL DEGENERATION (Y/N; BRIEF DESCRIPTION)	PROTEINOPATHY / AGGREGATES (Y/N; BRIEF DESCRIPTION)
Non-neuronal cell lines	HEK293, COS-7 and HeLa cells	exhibit some of the pathological features of HD, including Htt aggregation and cytotoxicity, but lack	

		neuronal markers	
Neuron and neuron-like cell lines	PC12 cells, N2a neuroblastoma cell lines	mHtt leads to inhibition of neurite outgrowth Higher cell death in mHtt-expressing cells	
Inducible rat neuroprogenitor cell line	HC2S2	mHtt-expressing cells show nuclear fragmentation and neuritic degeneration that are time-dependent Absence of cell death when cells are undifferentiated	Cytoplasm and nucleus
Inducible cell lines	PC12, ST14A, NG108-15, N2a, HN10	Greater susceptibility of postmitotic cells	Nuclear and neuritic localization
striatal cell lines	ST14A, striatal cell lines derived from a mouse model of HD	tedious and time-consuming to generate and differentiate into mature neurons	
Primary neurons from WT mice/rats	Transient transfection with plasmids expressing mutant/WT Htt fragments	Acute degeneration after a week, use to investigate Htt antibodies	
Primary neurons from WT rats	Infection wih LV expressing fragments of mutant/WT Htt	Progressive pathology, cell death between 6-8 weeks	Mainly nuclear inclusions appearing at 4 weeks
Primary cortical neurons from WT mice	Infection wih adenoviral vector vectors expressing full-length mutant/WT Htt		Accumulation of HTT aggregates at day 13, diffusely distributed cytoplasmic Htt
hESC and NCS from HD patients	Development of technologies that now provide unlimited access to hPSCs (hESCs and hiPSCs)	Source of cell therapy protocols	
iPSC and NSC from HD patients	Microarray profiling showed disease-associated changes in electrophysiology, metabolism, cell adhesion, and ultimately cell death Minor or no effect on differentiation and proliferation	Electrophysiological defects and cell death with large CAG expansion	

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