Models of Neurodegenerative Disorders

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15th march 2023

Models of Neurodegenerative Disorders

Outline:

- What makes an animal model

- Suggested of models for degenerative brain disorders: **Alzheimer's**, Parkinson's, Huntington's and FAP

- Models for the BBB

What is a model?

Any experimental preparation developed for the purpose of studying a condition in the same or different species….

Typically models are preparations in animals that attempt to mimic a human condition

Purposes of an animal model

- Mimic the syndrome in its entirety
- Study potential therapeutic treatments

Other types of animal model

- Mimic only specific signs and symptoms

Models for neurodegenerative disorders

- Neurodegenerative disorders have no clear genetic causes, **the field has been guided by the discovery of mutated genes** that deterministically drive these disorders as well as genetic variants that alter risk.

- **These genetic guideposts**, along with the biochemical identification of proteins defining the pathological hallmarks of these diseases such as amyloid-β (Aβ), αsynuclein, and tau have **provided essential insights into the pathophysiology of neurodegenerative disorders, and provided the opportunity to create animal models for these diseases.**

- Genetic forms of the human disorders do not always perfectly phenocopy sporadic disease, but in many cases are excellent surrogates, providing intrinsic validity to genetic based models of neurodegenerative disorders.

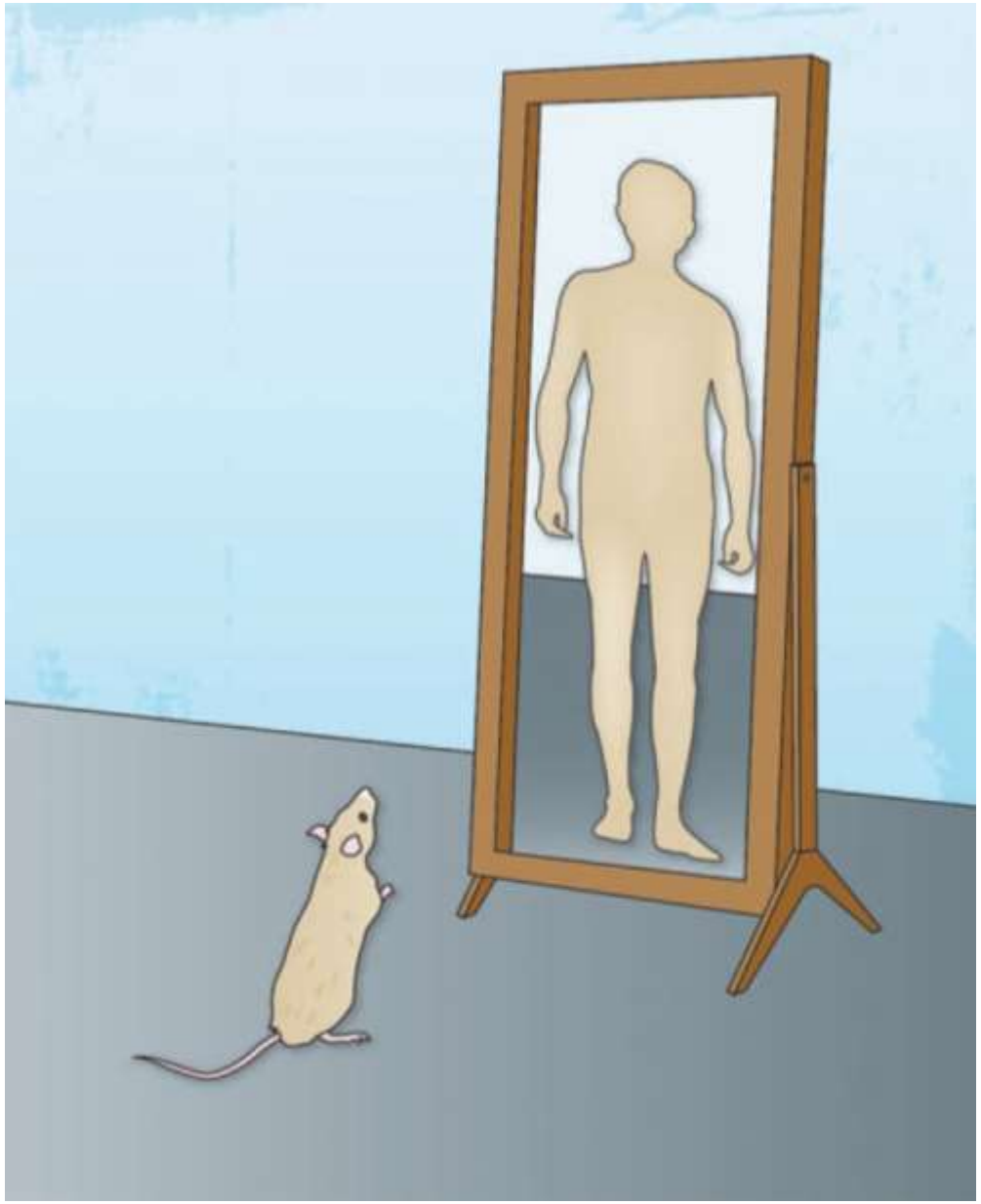
- ✓ Indeed, models based on genetic forms of these disorders have provided both insight into **molecular mechanisms** and the **temporality of changes of the human disease** and helped to identify candidate, potentially disease modifying, **therapies.**

Animal Models of Neurodegenerative Diseases Ted M. Dawson, Todd E. Golde, and Clotilde Lagier Tourenne
Nat Neurosci. 2018 October ; 21(10): 1370–1379. doi:10.1038/s41593-018-0236-8.

"**Mouse models** are one of the most important research tools for finding new treatments for **Alzheimer's Disease**" *

"Yet many of the potential disease-modifying treatments that have failed in clinical trials in recent years claimed to show some promise in mouse models"

Brain Res Bull . 2012 May 1; 88(1): 3–12. doi:10.1016/j.brainresbull.2011.11.017



Justice MJ, Dhillon P. Using the mouse to model human disease: increasing validity and reproducibility. *Dis Model Mech*. 2016;9(2):101–103. doi:10.1242/dmm.024547

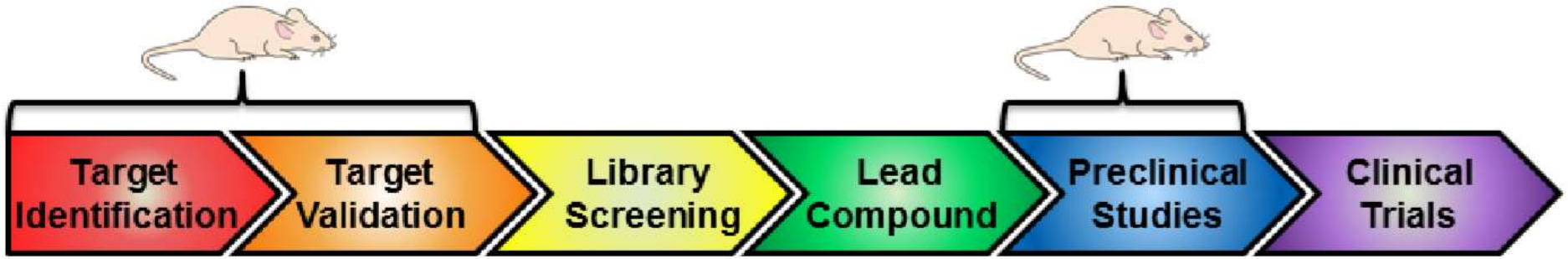


Fig. 4.
Roles for mouse models in the drug discovery pipeline. Mouse models have been very useful in the "basic science" stages of target identification and validation. Mouse models are also used for preclinical testing of lead compounds.

From Brain Res Bull . 2012 May 1; 88(1): 3–12.
doi:10.1016/j.brainresbull.2011.11.017

Alzheimer's Disease

Fig. 3.
Comparison of human and mouse Aβ. Mouse Aβ (mAβ) differs from human Aβ (hAβ) at positions 5, 10, and 13, which affects the aggregation properties of Aβ. β-secretease also has a greater preference for cleavage at the β' site in mouse Aβ, to produce $A\beta_{11\text{-}x}$ instead of $A\beta_{1\text{-}x}$.

From Brain Res Bull . 2012 May 1; 88(1): 3–12.
doi:10.1016/j.brainresbull.2011.11.017

The perfect model does not exist

Because **different models** may be most appropriate for addressing **different questions**, no one model should be considered the best.

- ❖ no existing mouse model exhibits all features of AD.
  - ❖ The ideal model of AD would develop the full range of clinical and pathological features of AD, including cognitive and behavioral deficits, amyloid plaques, neurofibrillary tangles, gliosis, synapse loss, axonopathy, neuron loss and neurodegeneration.
  - ❖ Different mouse lines develop these phenotypes to varying degrees and in different combinations.

- ❖ cognitive deficits and amyloid plaques are observed in almost all of the models, neurofibrillary tangles are generally seen only when human tau is also expressed, and neuronal loss is seen in only a few models. This is an issue for the use of mouse models **for preclinical drug testing**, where one would desire a model that incorporates most features of the disease.

- ✓ In general, however, this is less of a problem for studies aimed at dissecting mechanisms, when it can helpful to isolate some phenotypes from others.

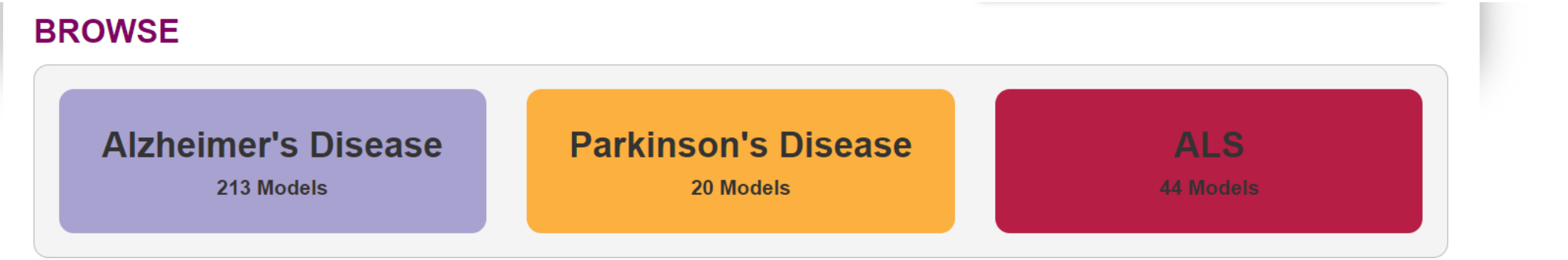
In this regard, AD mouse models are best viewed as reductionist tools for understanding the effects on brain function of genes/proteins that have been implicated in AD, and for identifying strategies to block them.

Brain Res Bull . 2012 May 1; 88(1): 3–12. doi:10.1016/j.brainresbull.2011.11.017

Animals models

Rodents

https://www.alzforum.org/research-models



Alzheimer's Disease

Animal Models currently available

Genetic models:
Transgenic

- ❑ **APP isoforms**
- ❑ **APP mutations**
- ❑ **PSEN**

- ❑ **Tau**

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 several mutations which results in tauopathy. The promoter is neuronal. Tau KO-mice are available.	Tau-inclusions (in some models PHF), gliosis, axonopathy, often pathology in spinal cord and the brain stem which then typically leads to motor disturbance, cognitive phenotypes in some models	Y, neuronal loss in some models (e.g. Tg4510) but not in most models	Tau inclusions of different morphology structure and biochemical composition, but different ultra-structure, in some models paired helical filaments essentially identical to those of Alzheimer's disease

Table 1

Representative mouse models of AD

This list is not comprehensive, but includes important prototypes, some commonly used models, and those that are available from repositories and thus are most easily available.

Tg Line	Gene/Isoform	Mutation	Promoter	Plaques (mo)	Cognitive deficits (mo)	Vendor Number	Reference
hAPP models							
PDAPP	hAPP695<751,770[1]	Ind	PDGF-B	6–9	6	n.a.	[27, 41, 49, 73, 110]
J20	hAPP695<751,770[1]	Swe, Ind	PDGF-B	6	4	JAX 006293	[114, 126]
Tg2576	hAPP695	Swe	HamPrP	9	10	Taconic 1349	[5, 163, 177]
APP23	hAPP751	Swe	Thy1	6 CAA: 12	3	n.a.	[21, 91]
TgCRND8	hAPP695	Swe, Ind	HamPrP	3 CAA: 11	3	n.a.	[29, 79]
TASD-41	hAPP751	Swe, Lon	Thy1	3 CAA: 7	6	n.a.	[140]
R1.40	hAPP YAC[2]	Swe	hAPP	14–15	16–17	JAX 005300	[66, 93, 94]
Aβ Models							
BRI-Aβ42A	BRI-Aβ42	n.a.	MoPrP	3	?	JAX 007182	[106]
hAPP/PS1 models							
PSAPP (Tg2576 × PS1)	hAPP695 PSEN1	Swe M146L	HamPrP PDGF-B	6	4	n.a.	[4, 40, 67]
APPswe/PS1ΔE9	m/hAPP695[3] PSEN1	Swe ΔE9	MoPrP MoPrP	6	6	JAX 004462	[82, 83, 146]
5XFAD	hAPP695 PSEN1	Swe, Lon, Flo M146L, L28V	Thy1 Thy1	2	6	JAX 008730	[89, 118, 122, 123]
2xKI	m/hAPP[3] PSEN1	Swe P264L	mAPP mPS1	6	9–12	n.a.	[25, 184]
Models with hTau							
TAPP (Tg2576 × JNPL3)	hAPP695 hTau-4R0N	Swe P301L	HamPrP MoPrP	8–15	motor deficits	Taconic 2469	[100]
3xTg	hAPP695 hTau-4R0N PSEN1	Swe P301L M146V	Thy1 Thy1 mPS1	6	4.5	JAX 004807	[12, 120, 121]
htau	hTau PAC[4]	Wild-type	hTau	–	12	JAX 005491	[3, 44, 128]

From Brain Res Bull . 2012 May 1; 88(1): 3–12.
doi:10.1016/j.brainresbull.2011.11.017

Alzheimer's Disease

Animal Models currently available

Genetic models:
Transgenic

- ❑ **ApoE**

TARGETED GENE	DESCRIPTION[1]	PHENOTYPE	NEURONAL DEGENERATION	PROTEINOPATHY / AGGREGATES
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

Alzheimer's Disease

Animal Models currently available

Genetic models:
Viral

TARGETED GENE	DESCRIPTION[1]	PHENOTYPE	NEURONAL DEGENERATION	PROTEINOPATHY / AGGREGATES
Viral vector-based models in rats	Lentiviral vectors overexpressing WT Tau or P301L Tau Intra-hippocampal injections through stereotactic intervention.	Progressive memory impairments (Morris tests)	Progressive pathology with time dependent 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	Yes NFT with PHF, positive Gallyas stained neurons (end stage)
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

Alzheimer's Disease

Animal Models currently available

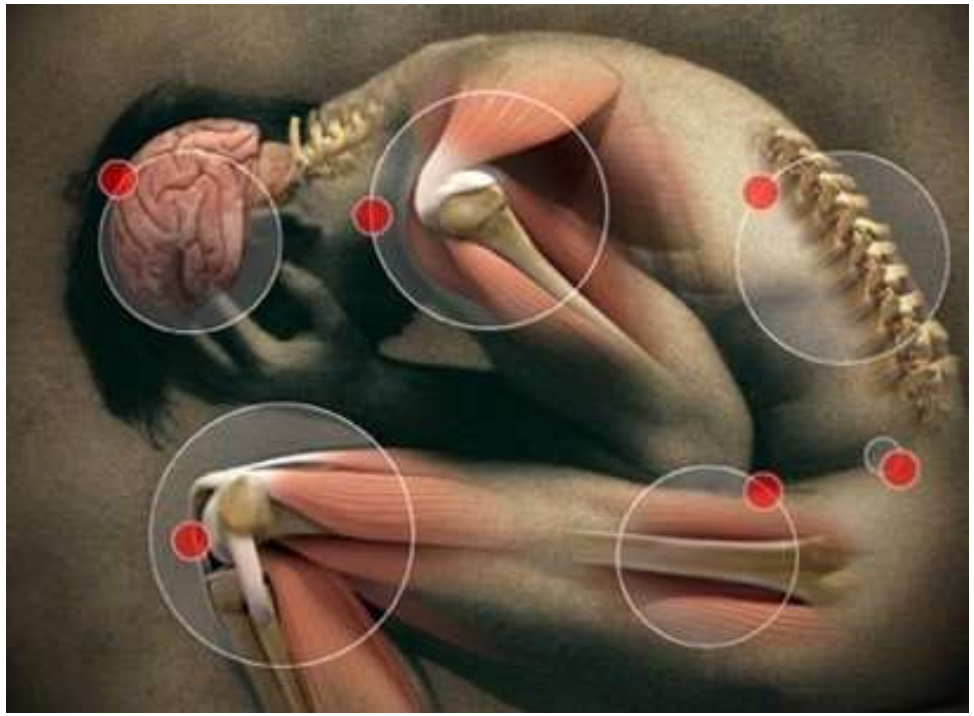
Non-genetic models

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 done with synthetic Aβ and Tau	The phenotypes are similar, albeit accelerated, compared to the uninjected hosts (see above)		Same as APP or Tau transgenic mice

Parkinson's disease

Resulting symptoms

- Muscular rigidity → extreme pain
- Postural instability
- Resting tremor



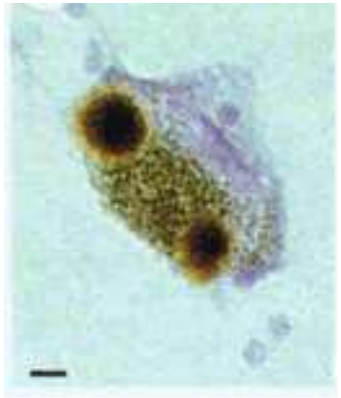
Doença de Parkinson - PD

- 2nd most common neurodegenerative disorder of the CNS

- 90-95 % sporadic

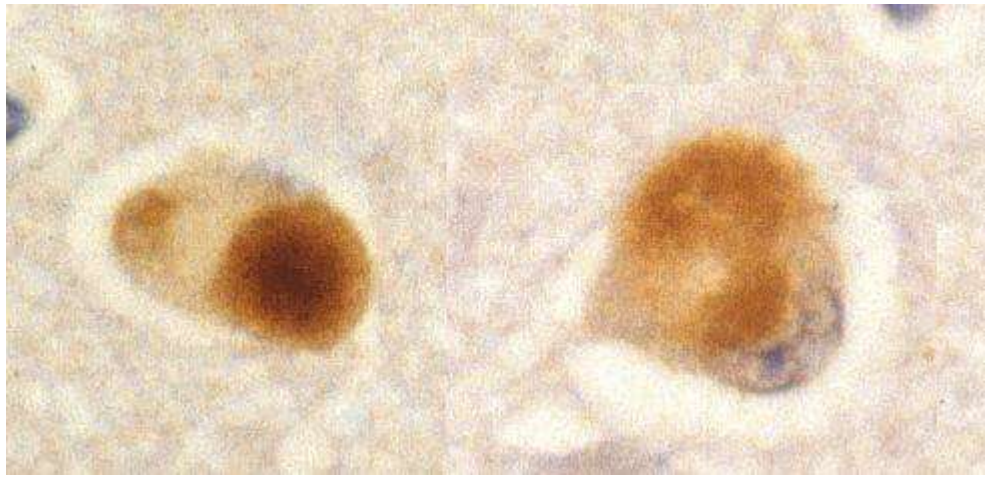
Patologia - PD

- Presence of lewy bodies in the substancia nigra (intracellular inclusions of aggregated α-synuclein)
- Dopaminergic neurons affected

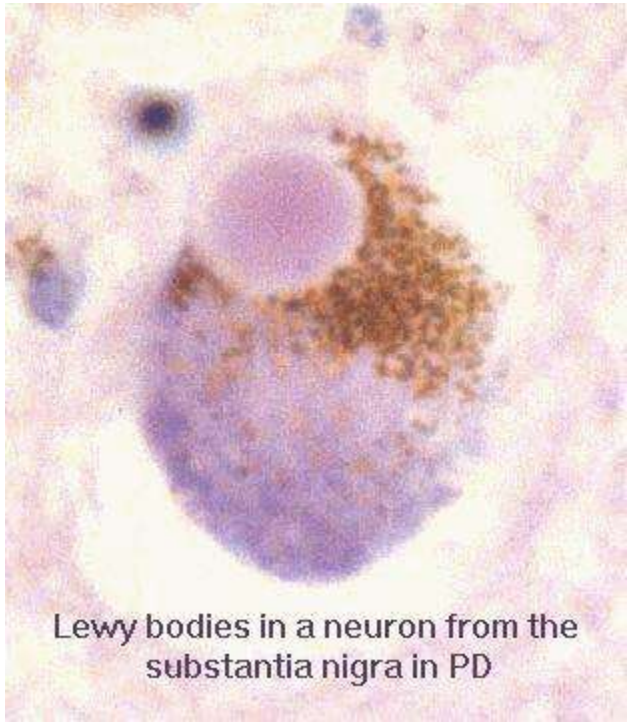


www.chemsoc.org

Lewy Bodies in neurons in substancia nigra



www.medweb.bham.ac.uk



Parkinson's Disease

Animal Models currently available

Genetic models

Transgenic

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

Parkinson's Disease

Animal Models currently available

Genetic models

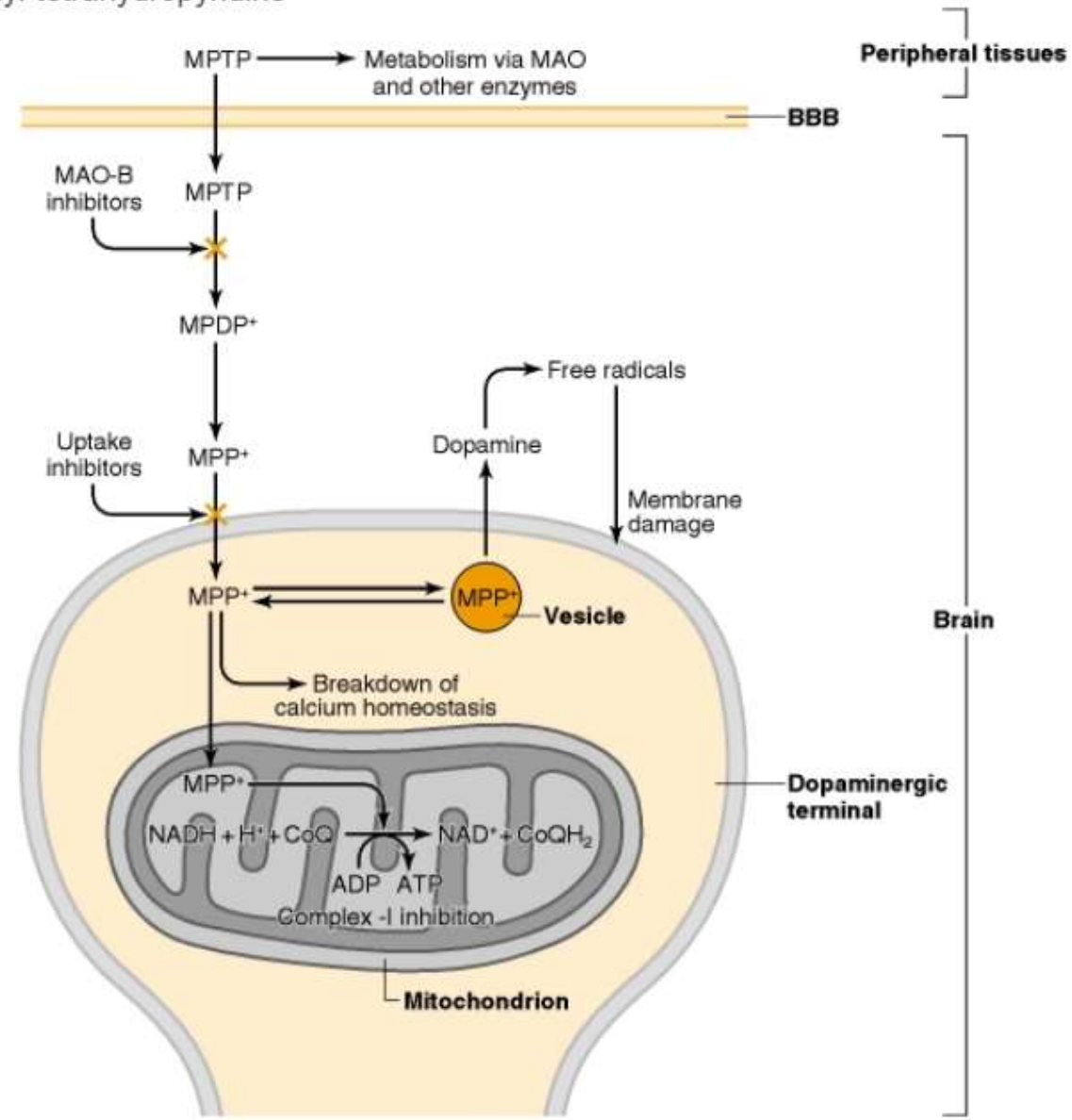
Viral

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 behavior can be analyzed	Loss of dopaminergic neurons	Alpha synuclein inclusions
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

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 6-hydroxydopamine	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 pump (rats) Intra-gastric or oral administration (mice) for investigation of PD-	Severe phenotype including akinesia, GI dysfunction, gait and balance disorders but not specific for dopaminergic neurons Less severe phenotype; impaired performances at the rotarod test; GI dysfunction (reduced fecal	Loss of dopaminergic and non dopaminergic neurons (widespread lesions). Glial cells also affected Moderate SNc lesion (oral>intragastric);	Alpha synuclein and tau pathology Trans-synaptic transmission of synuclein

Methyl-phenyl-tetrahydropyridine



Sian *et al.*, Basic Neurochemistry: Molecular, Cellular and Medical Aspects, 1999

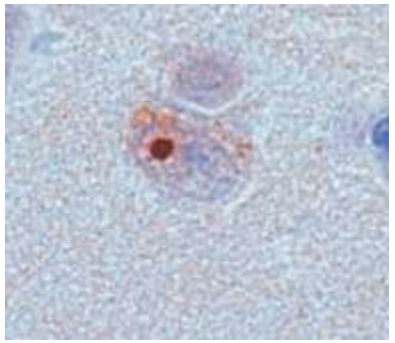
Non-genetic model(Pharmacological) -MPTP

- **MPTD crosses the blood brain barrier.**
- **Astrocytes and endothelia convert MPTP into the active neurotoxic MPP+ (1-methyl-4-phenylpyridinium), which is taken up by dopaminergic neurons through the dopamine transporter.**
- **MPP+ interacts with the mitochondrial complexes I-III-IV, inhibiting the electron transport chain and inducing ATP depletion and oxidative stress.**
- MPP+ is also linked to impairment of glutamate uptake by astrocytes and neuronal apoptosis.
- No typical Lewy bodies are observed in a MPTP primate model, **but a-synuclein immunoreactivity is enhanced** in the nigrostriatal system and in many brain areas.

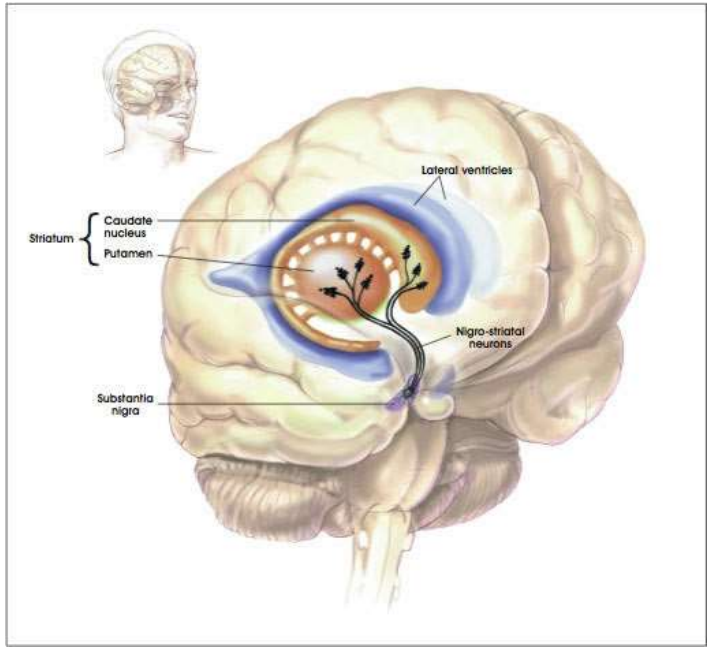
Animal models of neurodegenerative diseases Article in Revista Brasileira de Psiquiatria · December 2012 DOI: 10.1590/1516-4446-2013-1157

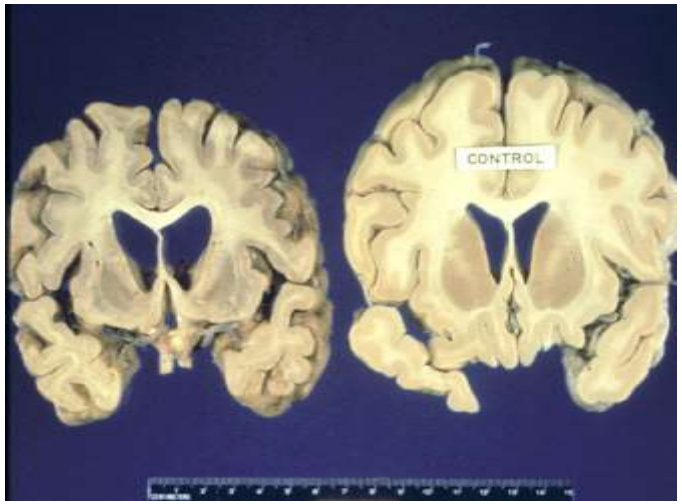
Huntington's disease

- Polyglutamine disease → mutation encoding for an addition of Q amino-acids
- Accumulation of the misfolded protein Huntingtin
- Formation of toxic inclusions in brain cells
- Degeneration of glutamatergic striatal neurons

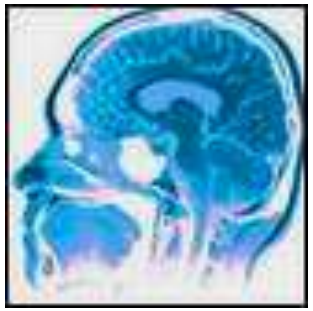


polyQ inclusion
In neocortex





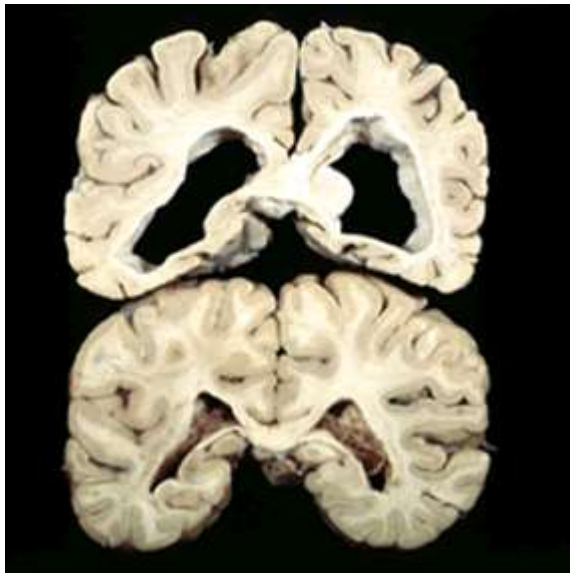
Huntington - HD

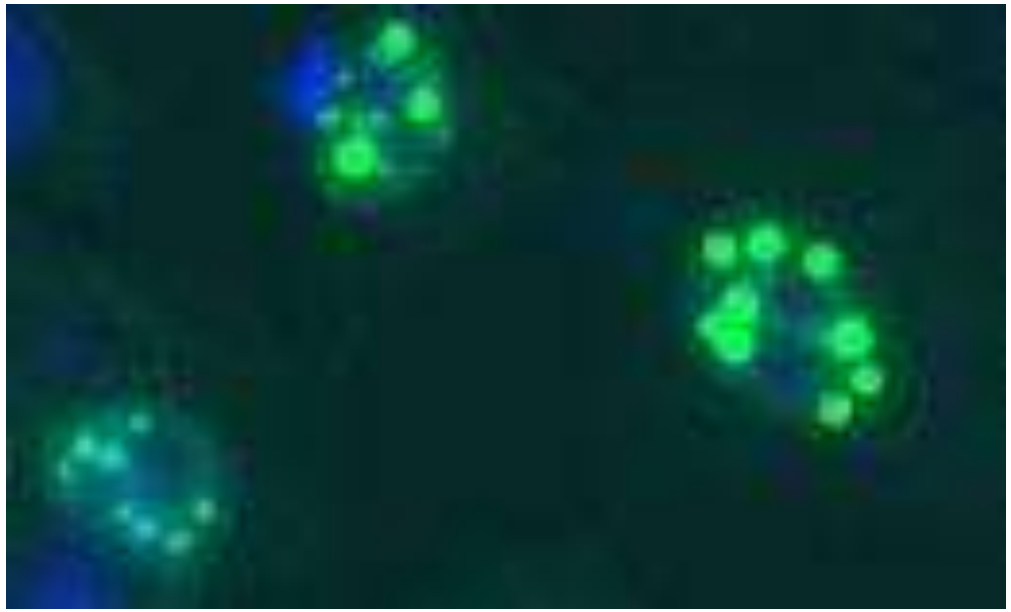


- Autosomal dominant disorder
- Most common form of polyglutamate repeats disorder (polyQ)
- Over 40 repetitions of glutamine CAG in the huntingtin gene

Huntington - HD

- Affects the CNS, specially the striatium and the cortex
- Degeneration of neurons, with intracellular inclusions of huntingtin

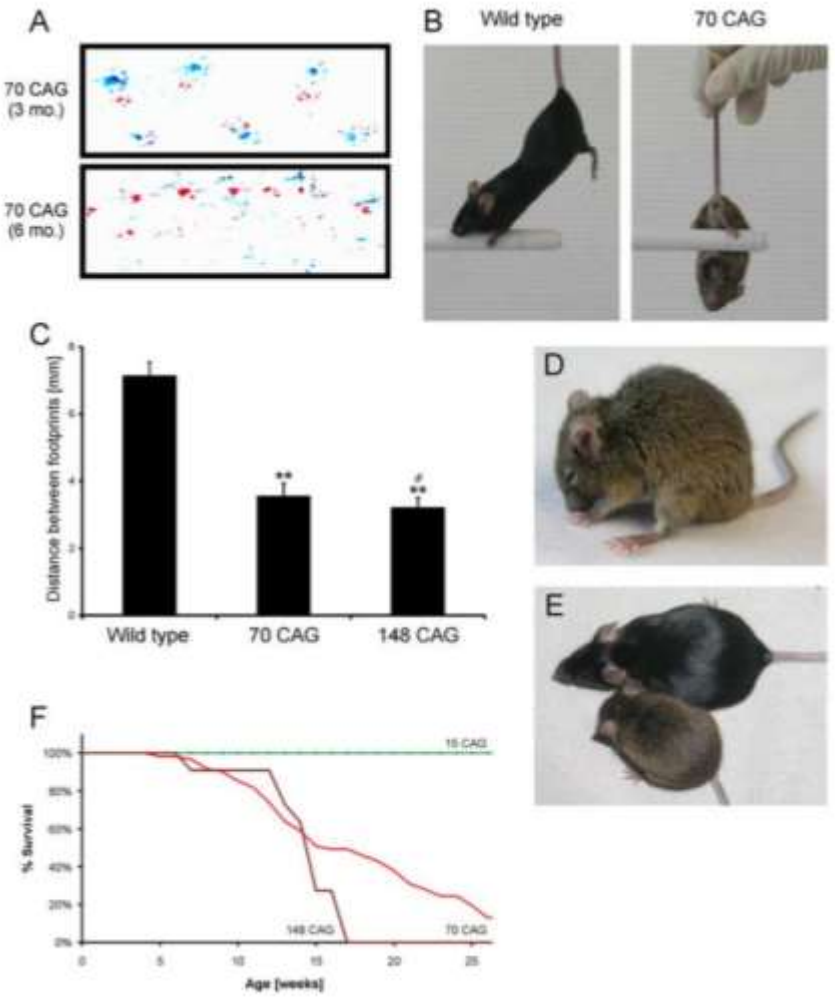




Intracellular inclusions of de huntingtin

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.

Huntington's Disease

Animal Models currently available

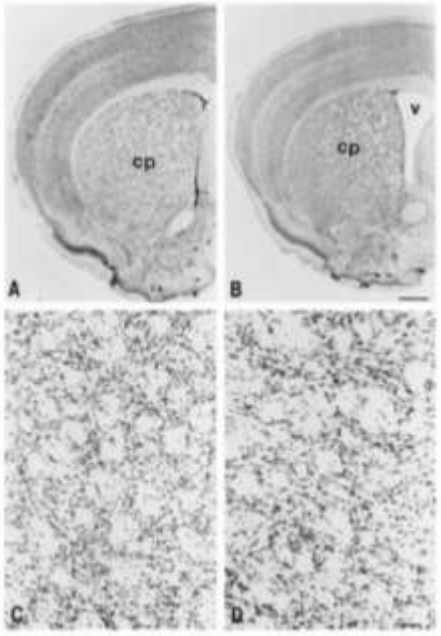
Cell, Vol. 87, 493–506, November 1, 1996

Exon 1 of the *HD* Gene with an Expanded CAG Repeat Is Sufficient to Cause a Progressive Neurological Phenotype in Transgenic Mice

Laura Mangiarini,[1] Kirupa Sathasivam,[1] Mary Seller,[1] Barbara Cozens,[7] Alex Harper,[2] Colin Hetherington,[3] Martin Lawton,[4] Yvon Trottier,[5] Hans Lehrach,[6] Stephen W. Davies,[7] and Gillian P. Bates[1]

Promotor + Exon 1 Q115-150

Neuronal loss, gliosis and inflamation



Huntington's Disease

Animal Models currently available

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.

Huntington's Disease

Animal Models currently available

viral

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

Huntington's Disease

Animal Models currently available

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	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*

Familial amyloid Polyneuropathy

Animal Models currently available

Familial Amyloid Polyneuropathy – older (initial)

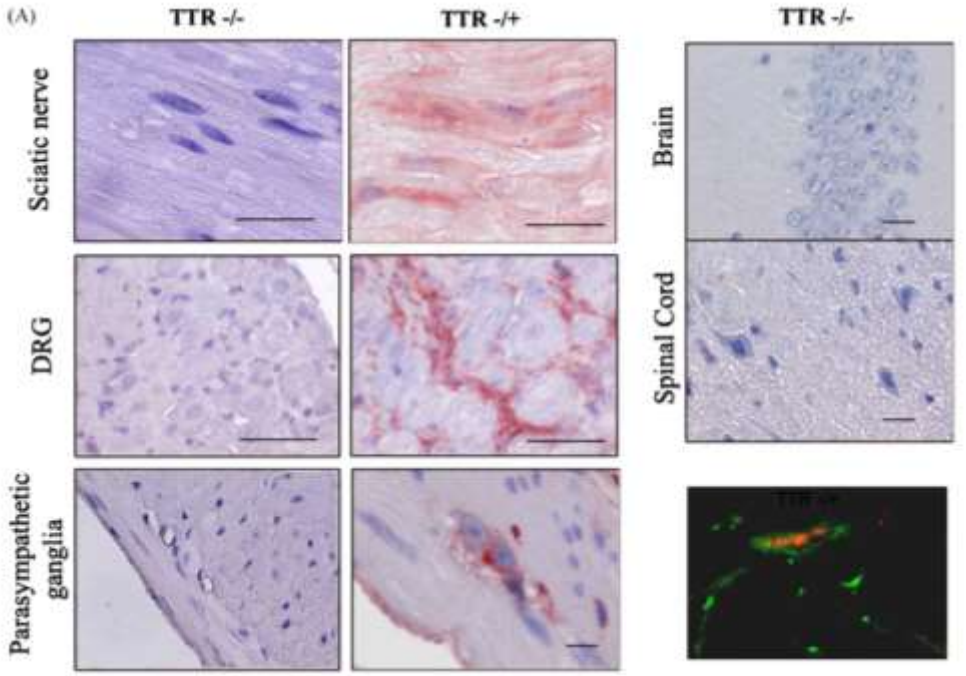
Transgenic strain	Genetic background	Promoter	[Human TTR] mg/dl	Tissue expression	TTR amyloid deposition Start age (months) / organs affected	References
MT1-TTR Met30	(C57BL/6xC3H)F1 x Balb C	Mouse metalothionein	0.25-1.4	Intestine, testis, brain, heart	negative	Sasaki et al., 1986, 1989
MT2-TTR Met30	C57BL/6	Mouse metalothionein and h TTR promotor	1.0-4.8	Liver, brain, kidney, heart, lung, skeletal muscle	4/6 heart, GI tract, kidney, skin, thyroid, vesicular glands	Wakasugi et al., 1987 Shimada et al., 1989 Yi et al., 1991
0.6-hTTR Met30	C57BL/6	0.6 kb hTTR regulatory regions	0.2-3.0	Liver, yolk sac	15 np	Yamamura et al., 1987 Shimada et al., 1989
6-hTTR Met30	C57BL/6	6 kb hTTR regulatory regions	0-17.1	Liver, yolk sac, choroids plexus, kidney	9 kidney, esophagus, heart, stomach, intestine, lung, spleen	Nagata et al., 1995
6-hMet30 (knock-out for mTTR)	MF1x129/Sv/Ev	6 kb hTTR regulatory regions	20-60	Liver, yolk sac, choroids plexus, kidney	11. esophagus, stomach, heart, lung, liver, bladder, intestine, kidney, thyroid, spleen	Yokoi et al., 1996 Episkopou et al., 1993
TTR Ser84	C57BL/6xC3H	7 kb hTTR regulatory regions	25	Liver, yolk sac, choroids plexus	negative	Waits et al., 1995
TTR Pro55	C57BL/6xDBA/2	All known hTTR regulatory regions	1-3	Liver, eye, brain	negative	Teng et al., 2001
TTR Pro55	C57BL/6	Sheep metalothionein	20	Liver, intestine	negative	Sousa et al., 2002 (s)
TTR Pro55 (knock-out for mTTR)	C57BL/6x129/SV/EV	Sheep metalothionein	5	Liver, intestine	4-8 skin, intestine	Sousa et al., 2002 (s)
TTR WT	C57Bl/6xDBA/2	All known hTTR regulatory regions	100-350	Liver, eye, brain, kidney, heart, skeletal muscle, stomach	18 Heart, kidney	Teng et al., 2001

Newer models

V30M/Hsf1 model:

"The mouse models **do not recapitulate the human whole features, since the peripheral nervous tissue is spared.**

- A new mouse model expressing the human transthyretin V30M in a heat shock transcription factor 1 (Hsf1) null background.
- The lack of HSF1 expression leads to an extensive and earlier non-fibrillar TTR, evolving into fibrillar material in distinct organs **including the peripheral nervous system**.



Santos SD, Lambertsen KL, Clausen BH, et al. CSF transthyretin neuroprotection in a mouse model of brain ischemia. *J Neurochem*. 2010;115(6):1434–1444. doi:10.1111/j.1471-4159.2010.07047.x

However, there is a lack of mouse models to replicate the early neuropathic manifestations of FAP.

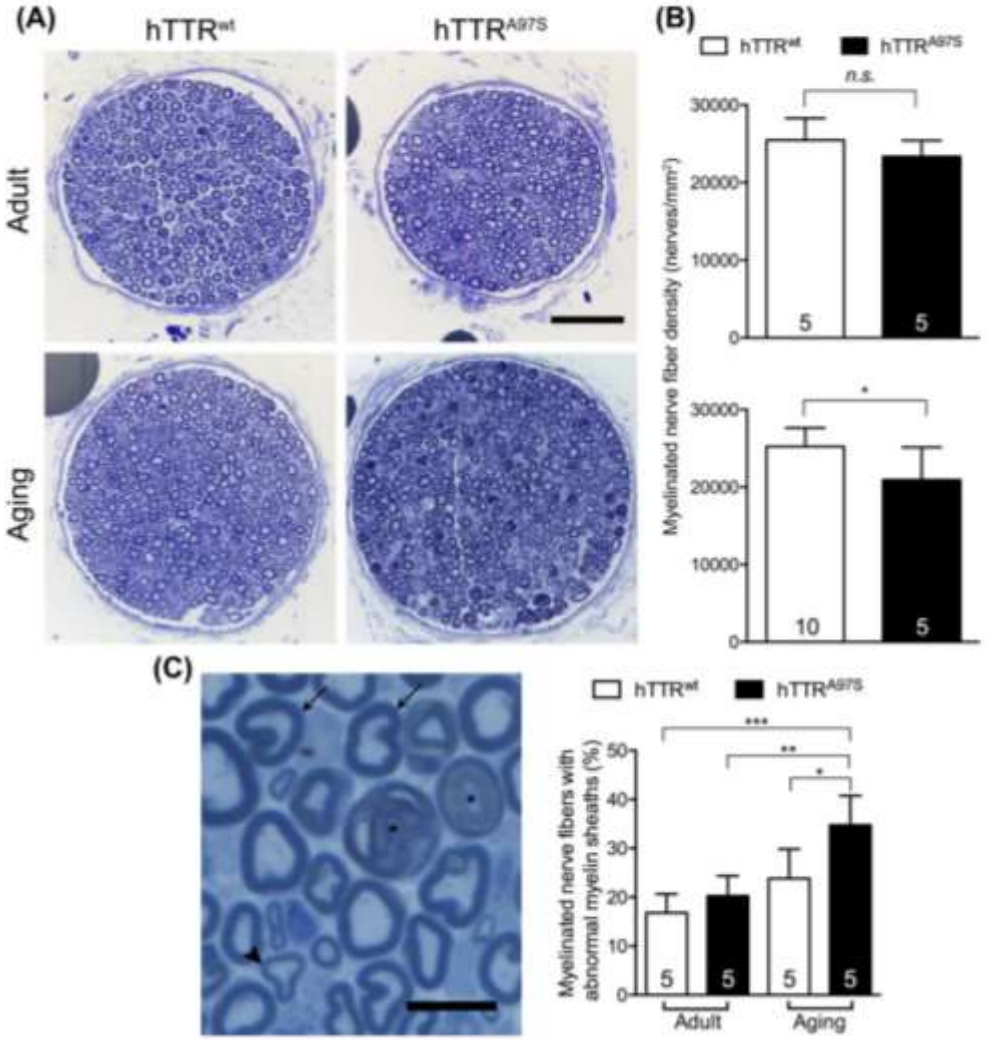
hTTRA97S model (knock-in)

- Sensory nerve degeneration and consequent abnormal sensations are the earliest and most prevalent manifestations of FAP due to amyloidogenic TTR.
- FAP is a relentlessly progressive degenerative disease of the peripheral nervous system.

✓ In the adult group, nerve profiles, neurophysiology, and behaviour were similar between hTTRwt and hTTRA97S mice.

✓ By contrast, **aging hTTRA97S mice showed small fibre neuropathy with decreased intraepidermal nerve fibre density and behavioural signs** of mechanical allodynia.

✓ Significant **reductions in sural nerve myelinated nerve fibre density and sensory nerve action potential amplitudes in these mice indicated degeneration of large sensory fibres**.



Kan HW, Chiang H, Lin WM, Yu IS, Lin SW, Hsieh ST. Sensory nerve degeneration in a mouse model mimicking early manifestations of familial amyloid polyneuropathy due to transthyretin Ala97Ser. *Neuropathol Appl Neurobiol*. 2018;44(7):673–686. doi:10.1111/nan.12477

Other models

Cellular models

Alzheimer's Disease

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

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

Huntington's Disease

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	

Other models

Other animals

Caenorhabditis elegans

Drosophila melanogaster

Neurodegeneration models in *Caenorhabditis elegans* ***(C.elegans)***

- **The $A\beta_{1-42}$ fragment** is the main neurotoxic peptide generated from APP

- Invertebrate APP-like genes **do not** include the region encoding the neurotoxic $A\beta_{1-42}$ fragment and, therefore, direct disease modelling based on endogenous APP cleavage is not possible.

✓ The most developed invertebrate model of $A\beta_{1-42}$ toxicity involves intracellular expression of a human $A\beta_{1-42}$ fragment in the ***C. elegans*** bodywall muscle, leading to adult-onset progressive paralysis and shortened lifespan

✓ In the nematode model, $A\beta_{1-42}$ forms cytoplasmic β -amyloid with some properties similar to those found in diseased human brains

C. elegans – Alzheimer's

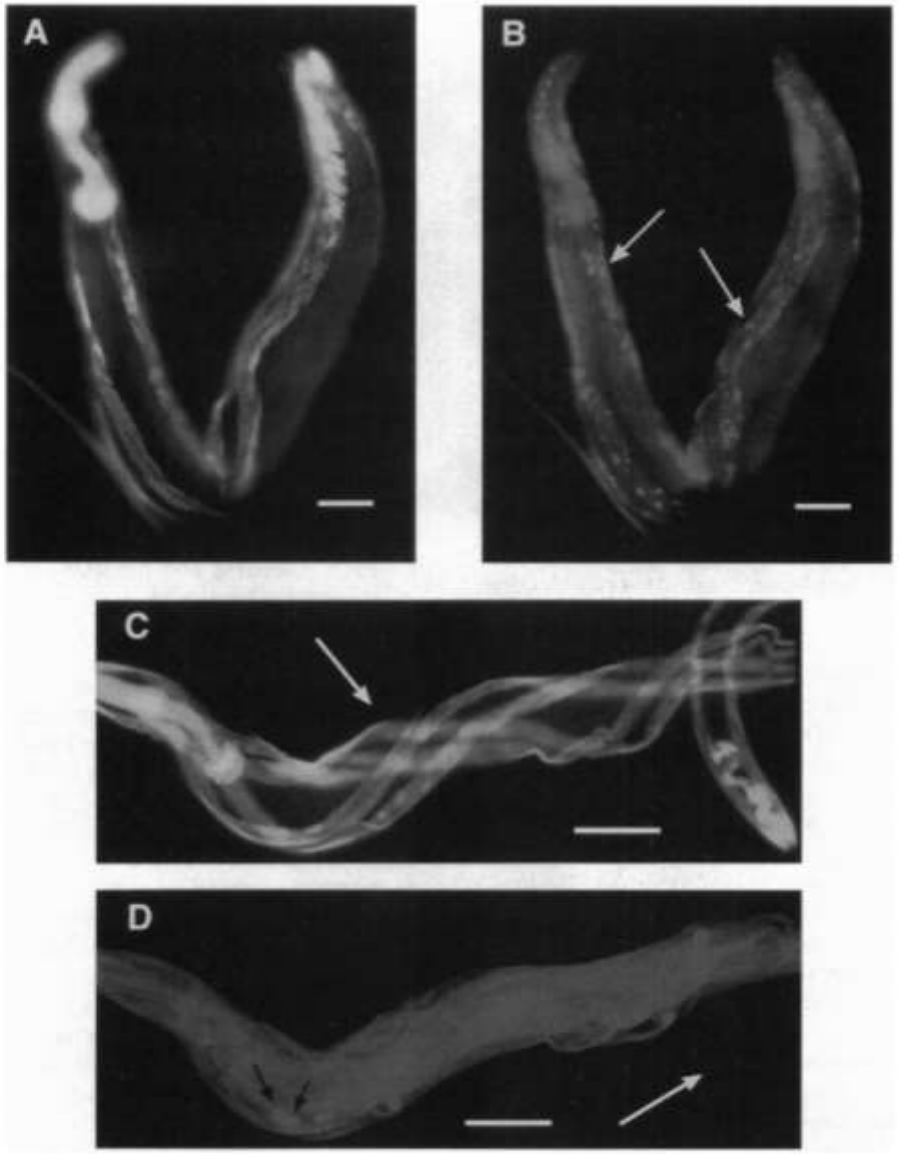


FIG. 2. Immunohistochemistry of transgenic animals. (*A*) *unc-54*/β-(1–42) transgenic male animal (from extrachromosomal line CL1019) with muscle structure visualized by Bodipy-phallacidin. (*B*) Same transgenic animal probed with anti-β-amyloid antibody. Note muscle-specific deposits. (*C*) *unc-54*/transthyretin animals (from extrachromosomal line CL1015) probed with Bodipy-phallacidin, viewed by epifluorescence microscopy. Note helical musculature of transgenic animal (arrow). (*D*) Same CL1015 animals probed with anti-transthyretin antibody. Note intense staining of coelomocytes (black arrows) and complete lack of staining in nontransgenic sibling (white arrow). The intense signal from these coelomocytes bleeds through on the Bodipy epifluorescence channel, producing faint images visible in *C*. These images were generated by digitally scanning color slides and adjusting brightness and contrast with ADOBE PHOTOSHOP (Adobe Systems). (Bars = 40 μm.)

Link, C. D. Expression of **human β-amyloid peptide** in transgenic Caenorhabditis elegans. *Proc. Natl. Acad. Sci. U. S. A.* **92**, 9368–9372 (1995)

C. elegans – Alzheimer's

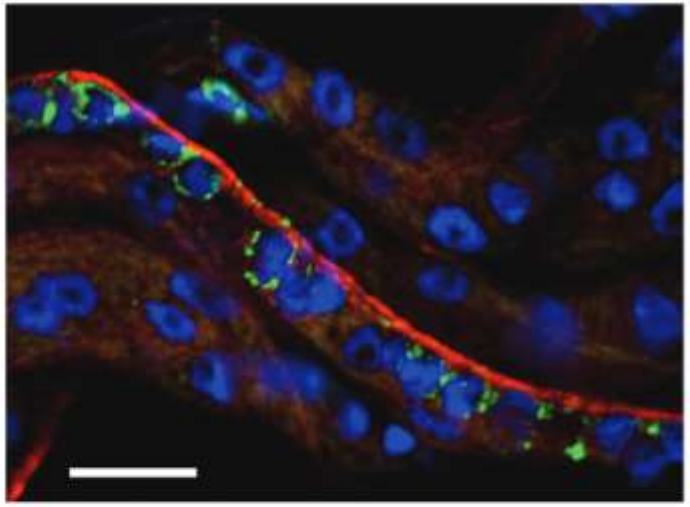
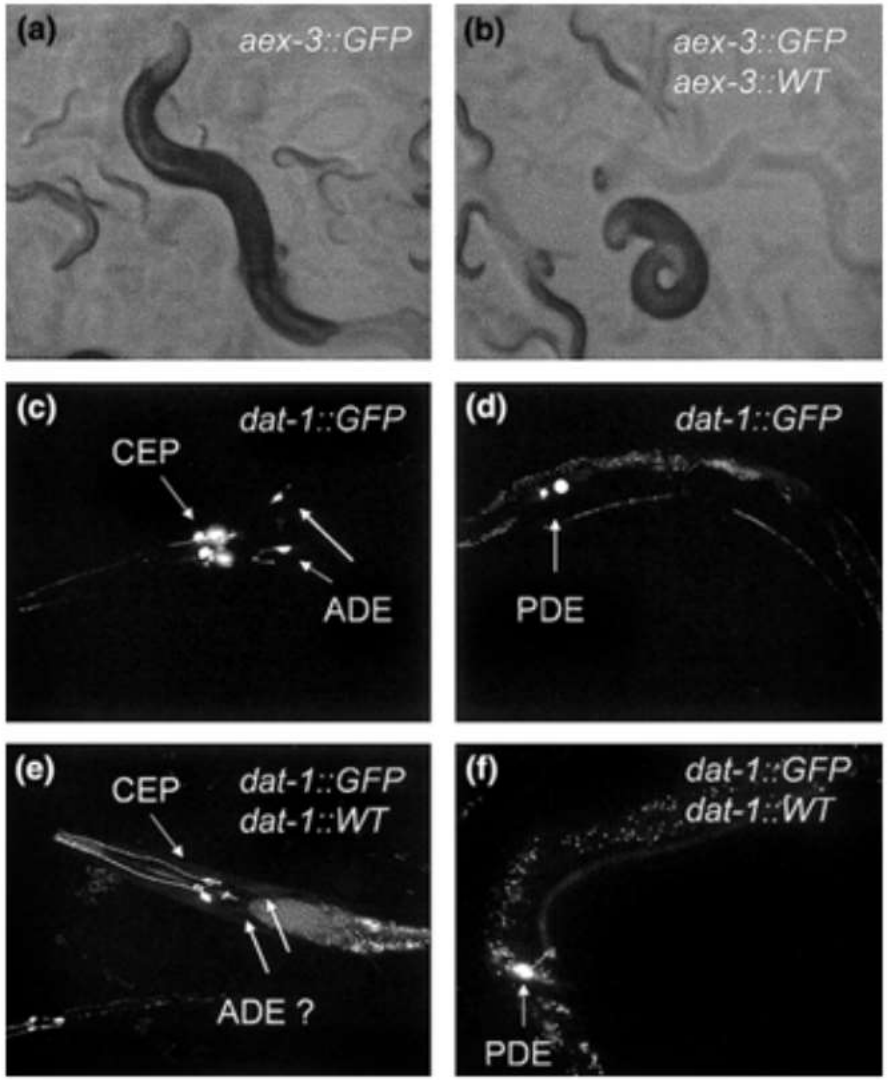


Figure 1: Intraneuronal β-amyloid peptide (Aβ) in *Caenorhabditis elegans* Alzheimer's disease (AD) model. Shown is the mid-body region of a transgenic worm with pan-neuronal expression of human Aβ 1–42, probed with anti-Aβ monoclonal antibody 4G8 (green), antisynaptotagmin polyclonal antibody (red) and DNA dye 4′,6-diamidino-2-phenylindole (DAPI) (blue). The synapse-specific synaptotagmin staining highlights the neuronal projections of the ventral cord, whereas all nuclei are stained by DAPI. Note the perinuclear immunoreactive Aβ deposits associated with ventral cord neurons. Size bar = 10 μm.

Link, C. D. (2005). Invertebrate models of Alzheimer's disease.
Genes, Brain and Behavior, *4*(3), 147–156.

https://www.hsph.harvard.edu/mair-lab/c-elegans/

C. elegans – Parkinson's

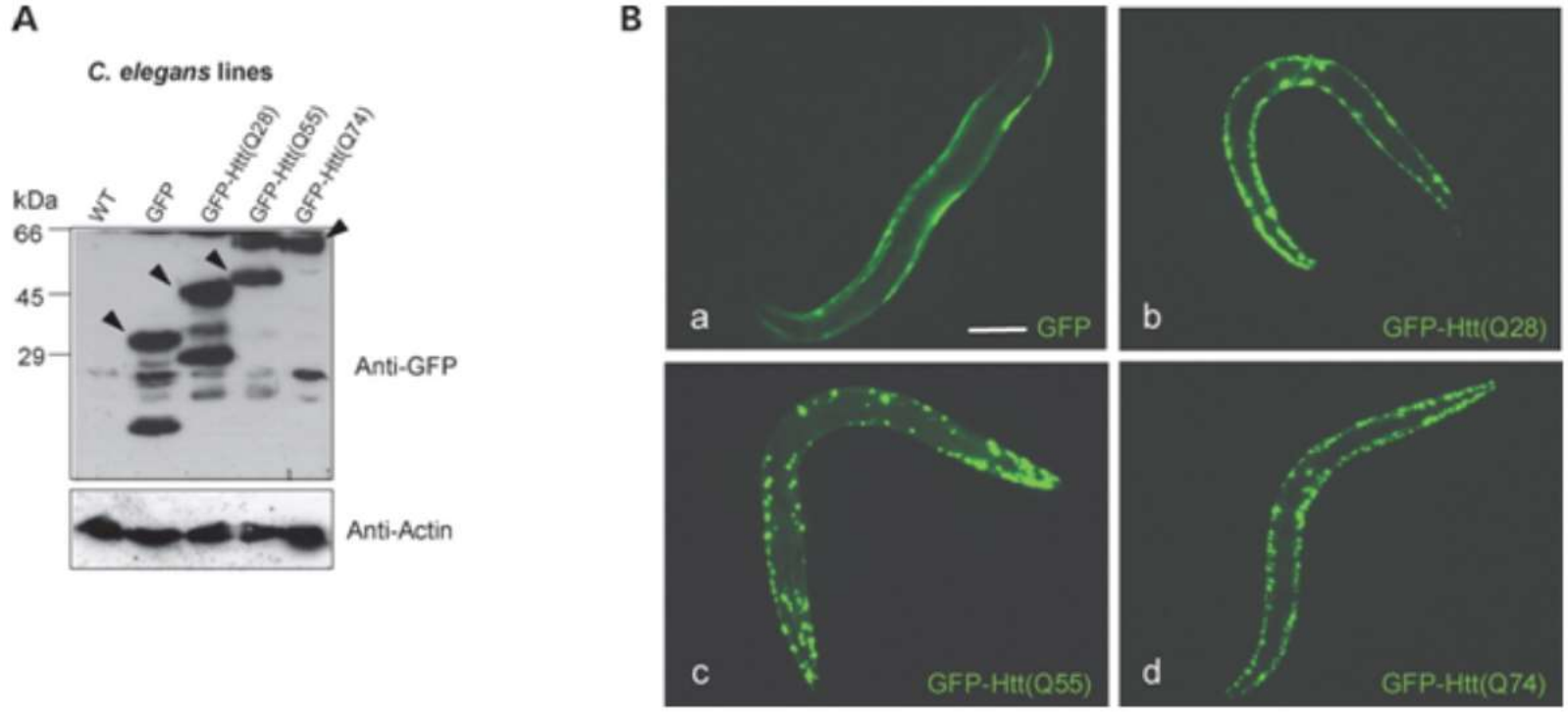


postural and movement deficits

loss of neurons

Lakso et al., 2003

C. elegans – Huntington's



A) Immunoblot showing anti-GFP immunoreaction in *C. elegans* protein extracts using 3-4-day-old animals expressing different lengths of GFP-Htt-polyQ proteins

B) GFP fluorescence micrographs of young adult (3-4 days old) *C. elegans* expressing different lengths of GFP-polyQ fusion proteins. Note that GFP fluorescence is mainly localized to the body wall muscle cells. Also, note that more compact foci form with increasing number of polyQ repeats expressed

Wang, H. et al. 2006 Hum. Mol. Genet.

Drosophila in the Study of Neurodegenerative Disease

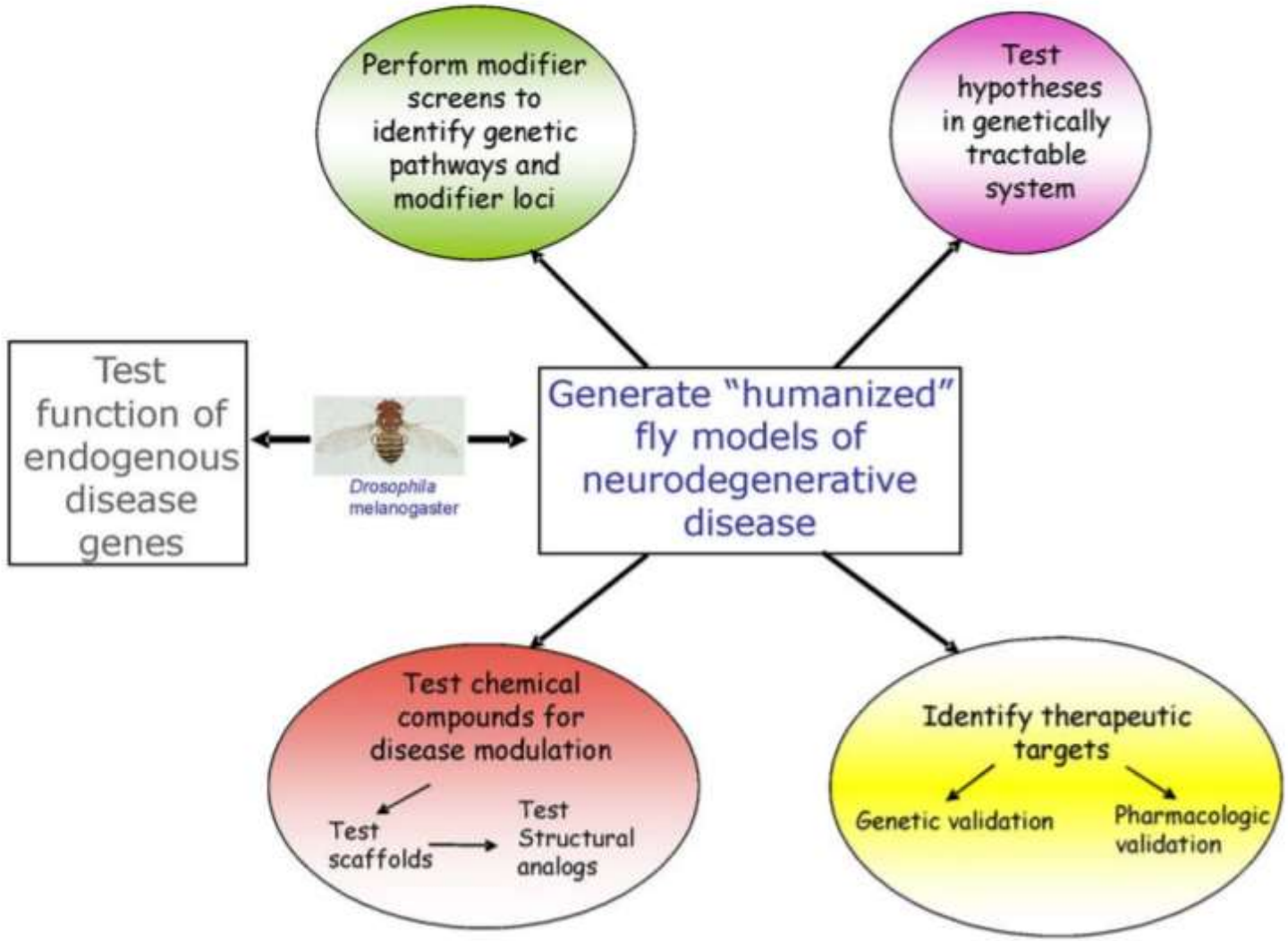


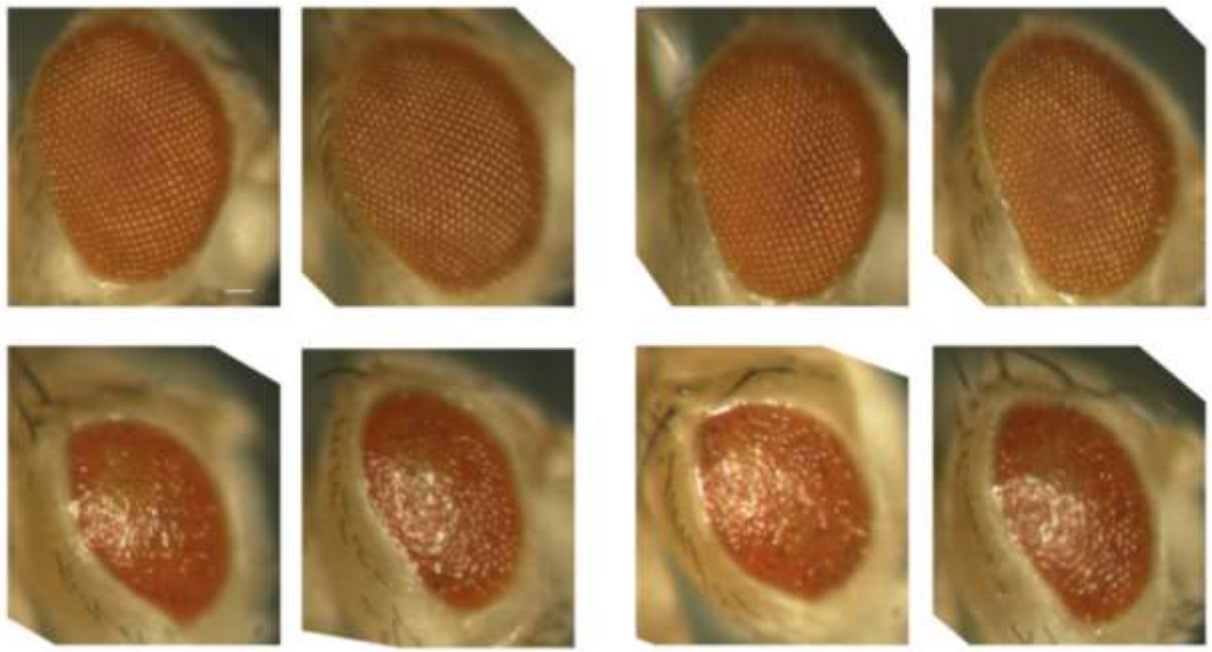
Figure 2. Schematic of Utility of *Drosophila* Models of Neurodegenerative Disease

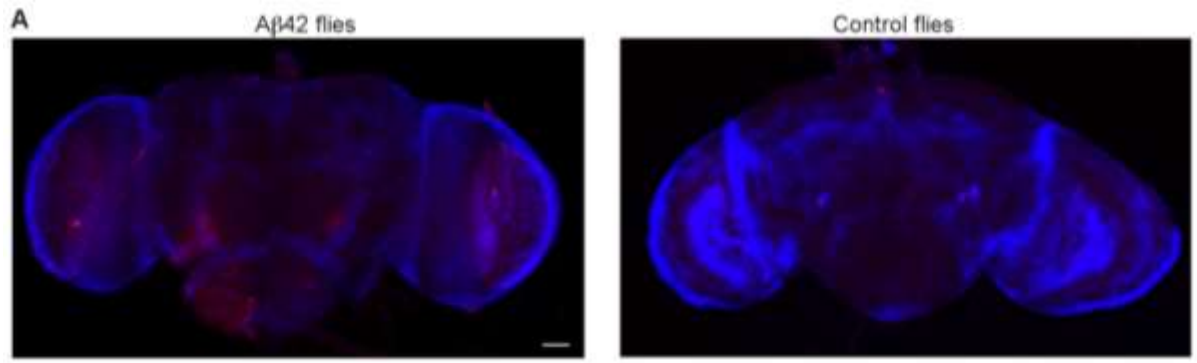
https://doi.org/10.1016/j.neuron.2006.09.025

Table 2: Summary of transgenic invertebrate Alzheimer's disease models

Organism	Transgenes expressed	Expression pattern	Salient observations	Forward genetic screen?	References
	Aβ models				
C. elegans	Aβ minigene	Muscle	Paralysis, amyloid formation	No	Link (1995)
Drosophila	Aβ minigene	Eye, pan-neuronal	Eye pathology, reduced life span	Yes	Finelli *et al.* (2004)
	tau models				
Drosophila	hAPP, hBACE, *Psn*	Eye, ubiquitous	Eye pathology, semilethality	No	Greeve *et al.* (2004)
Drosophila	human three-repeat tau	Sensory neurons	Axonal loss	No	Williams *et al.* (2000)
Drosophila	wt and R406W tau	Pan-neuronal	Reduced life span, NFTs	No	Wittmann *et al.* (2001)
Drosophila	wt human tau	Eye	Eye pathology, NFTs	No	Jackson *et al.* (2002)
C. elegans	wt, P301L and V337M tau	Pan-neuronal	Unco-ordinated movement	No	Kraemer *et al.* (2003)
Drosophila	V337M tau	Eye	Eye pathology	Yes	Shulman and Feany (2003)
Drosophila	wt human tau, PAR-1	Eye	Eye pathology	No	Nishimura *et al.* (2004)

Aβ, β-amyloid peptide; *C. elegans*, *Caenorhabditis elegans*; NFT, neurofibrillary tangle.





https://www.youtube.com/watch?v=vmR6s_WAXgc&ab_channel=CDRLab

Parkinson Disease

Non-mammalian models				
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.*

TTR

Invertebrate and cell models

Fly (*D. melanogaster*)

Expression of mutant hTTR in the nervous system led to
- Shortened lifespan
- Locomotion dysfunction

(Pokrzywa *et al.*, 2007; Berg *et al.*, 2009)

Worm (*C. elegans*)

- Amyloidogenic and non amyloidogenic TTR were expressed in body wall muscles.
- Nociceptive defect with impaired dendritic morphology seen in mutant (Madhivanan *et al.*, 2018).

iPSC

- Multisystem modelling of ATTR amyloidosis.
- Effector (hepatocytes) and peripheral target cells (neurons and cardiomyocytes) were derived from ATTR patients-specific IPSC.
- Mutant TTR produced by effector damage peripheral targets (Leung *et al.*, 2013).

Vertebrate models

Rodents

Earlier models
- Deposits found in the kidney, GIT etc. none in the peripheral nerves (Shimada et al., 1989; Yi *et al.*, 1991; Reviewed in Buxbaum, 2009.)

Later model
- Deposits found in the peripheral nerves.
- But animals lack *HSF1* (Santos *et al.*, 2010).

Most recent models
- Deposits found in the peripheral nerves. (Li *et al.*, 2018; Kan *et al.*, 2018).

Nonhuman primates

- Spontaneous development of ATTR amyloidosis (Nakamura et al., 2008; Chambers *et al.*, 2010; Ueda *et al.*, 2012).
- Amyloidogenic human TTR alleles were found in them (Ueda *et al.*, 2012).

Ibrahim RB, Liu Y-T, Yeh S-Y and Tsai J-W (2019) Contributions of Animal Models to the Mechanisms and Therapies of Transthyretin Amyloidosis. Front. Physiol. 10:338.doi: 10.3389/fphys.2019.00338

Other models

BBB models

Models of the blood-brain barrier

Kaisar et al.

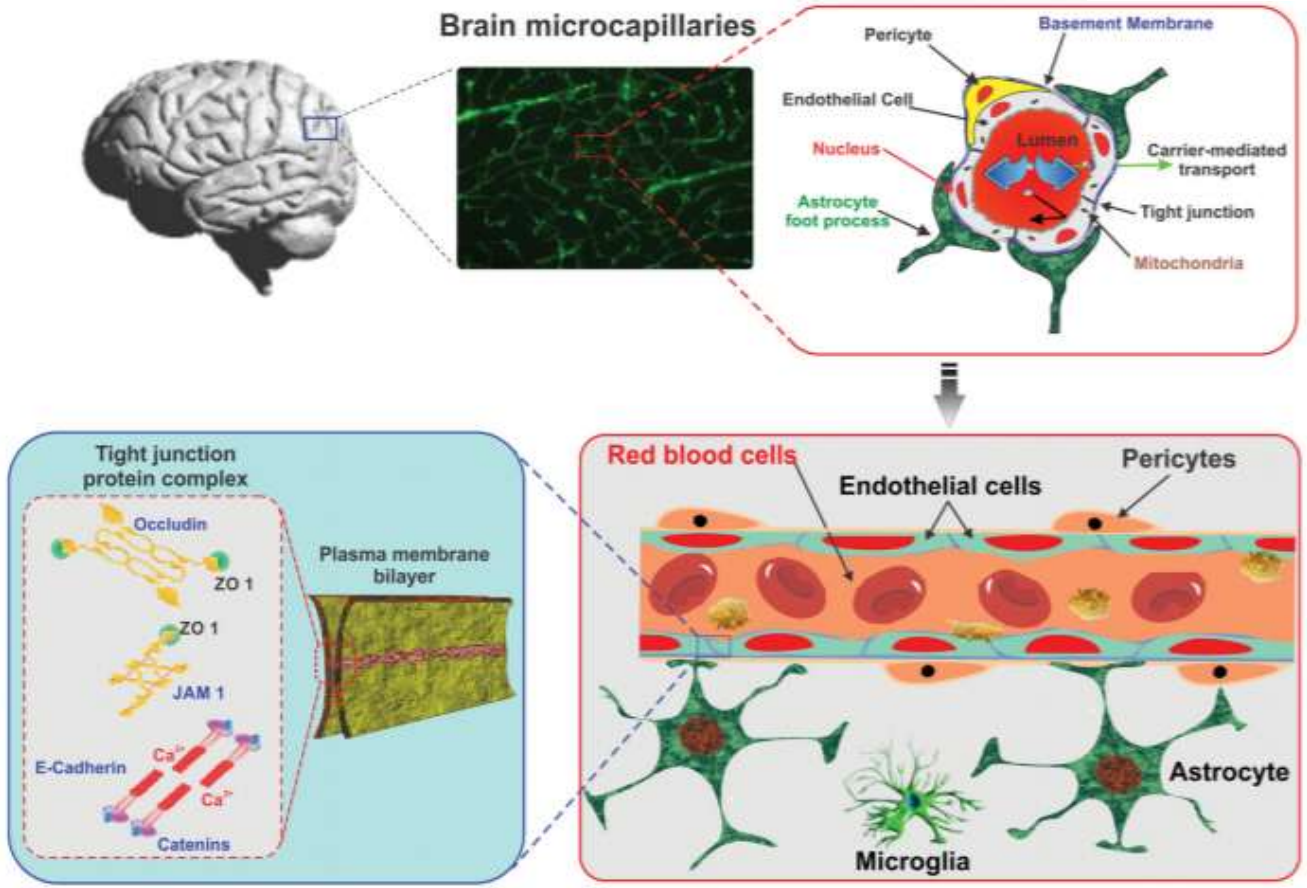
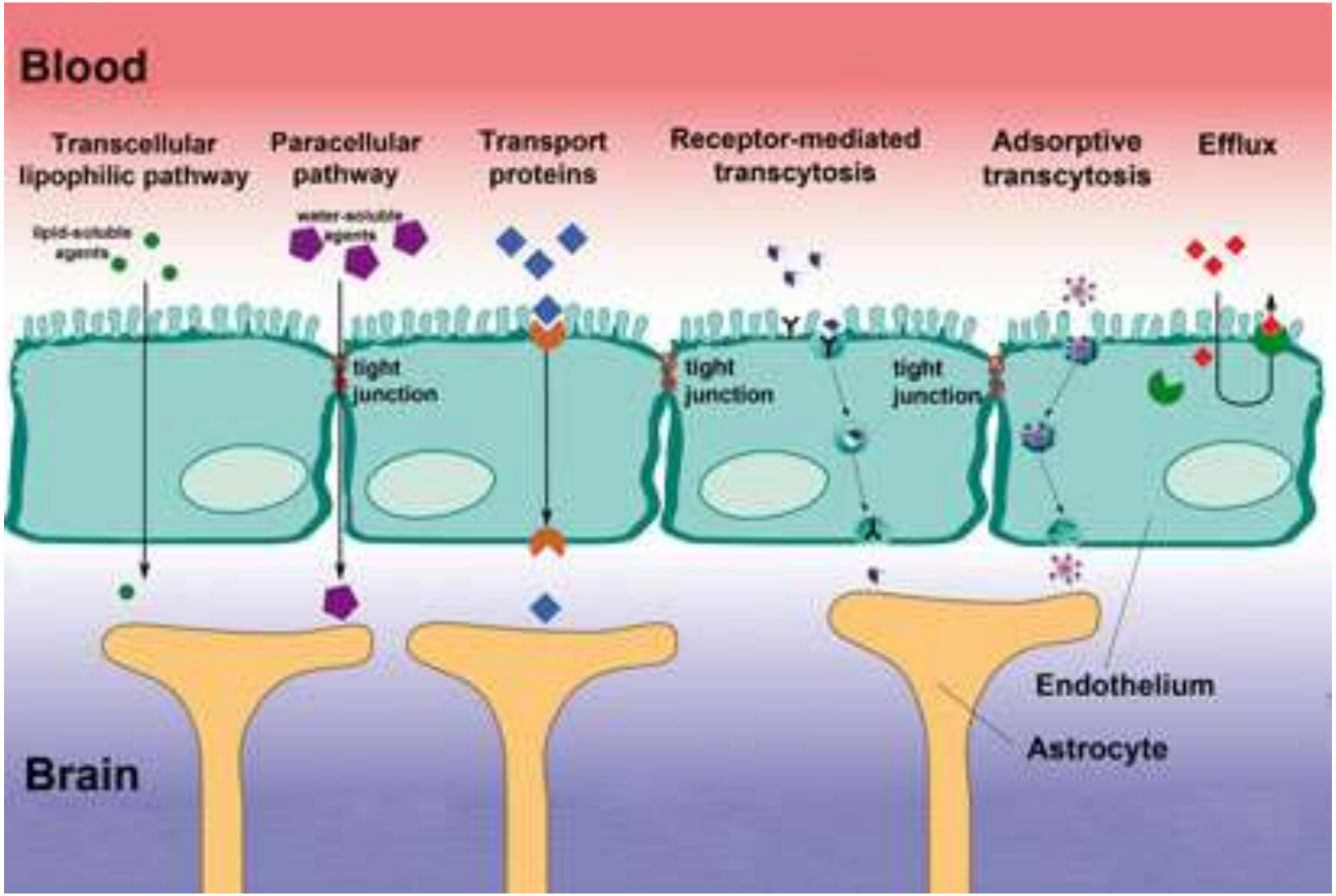


Figure 1. Schematic illustration of BBB anatomy

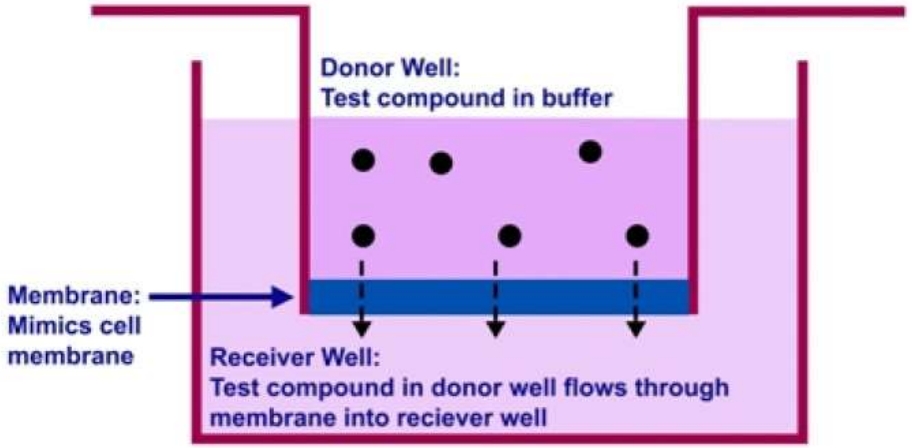
A cross-section of brain microcapillary representing luminal compartment composed of basal lamina, endothelial cells and pericytes tightly ensheathed by the astrocytic end-feet. Tight junctions (TJs), present between the cerebral endothelial cells selectively excludes paracellular trafficking of substances from entering into brain.

Brain barriers

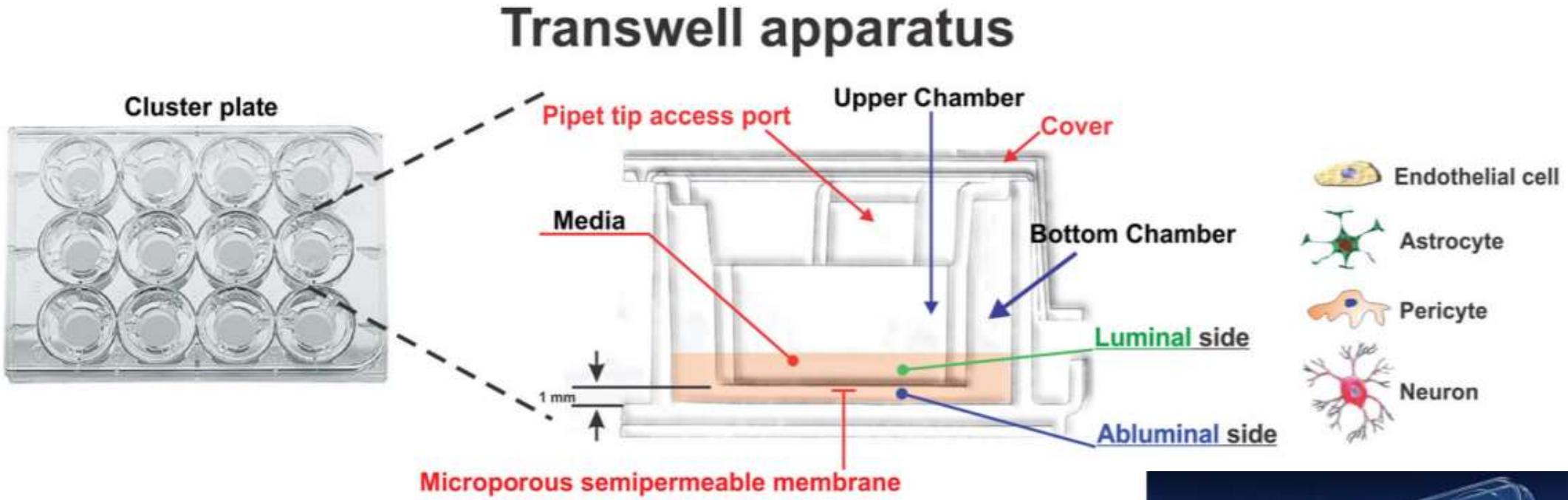


In vitro artificial models

The parallel membrane permeability assay (PAMPA-BBB) can be applied to investigate passive transcellular permeability, but does not comprise transporters that influence drug access to the CNS

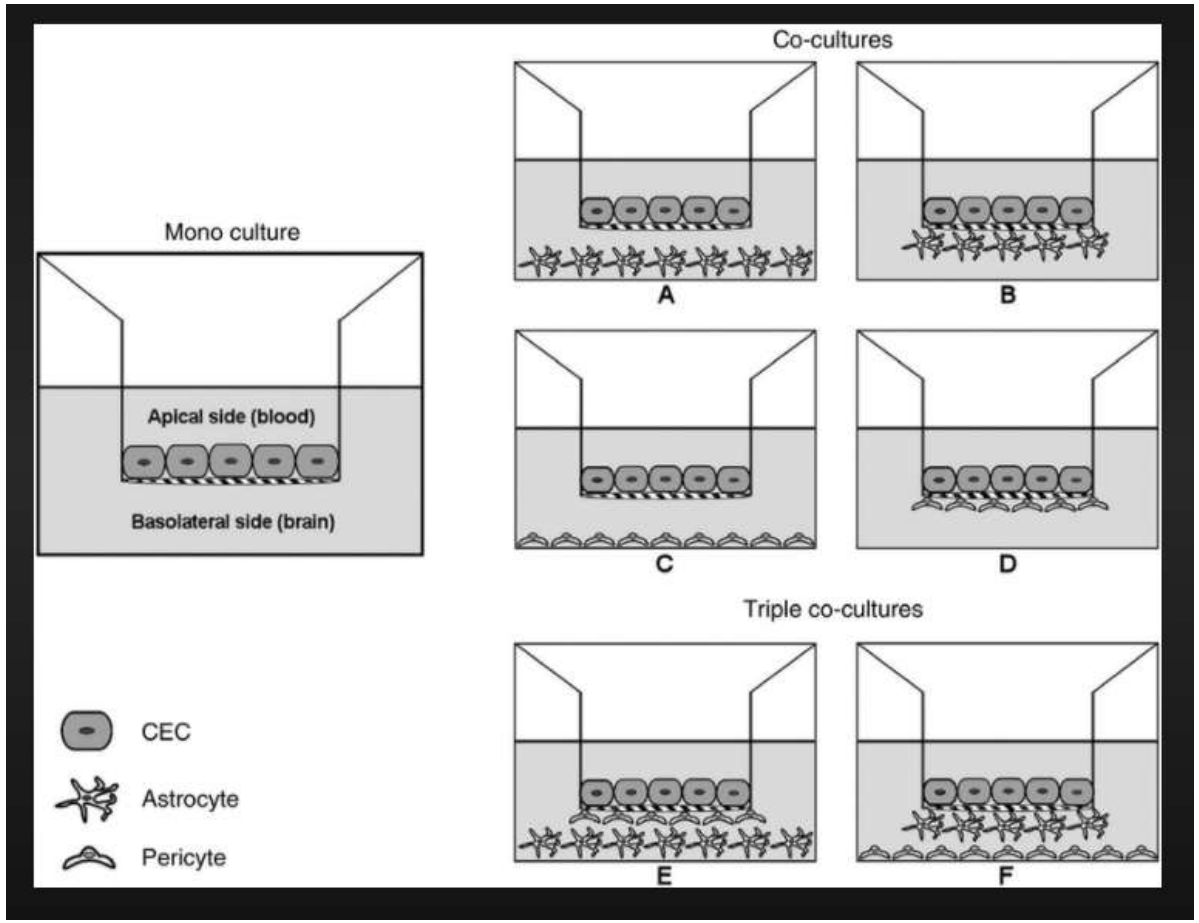


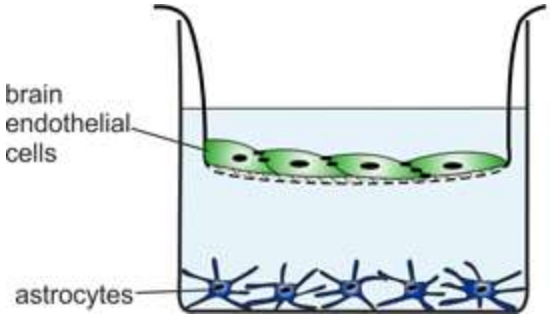
In vitro cell-based models

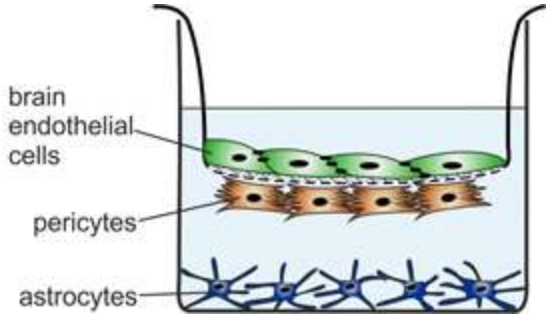


Expert Opin Drug Discov. 2017 January ; 12(1): 89–103.
doi:10.1080/17460441.2017.1253676.

In vitro cell-based models





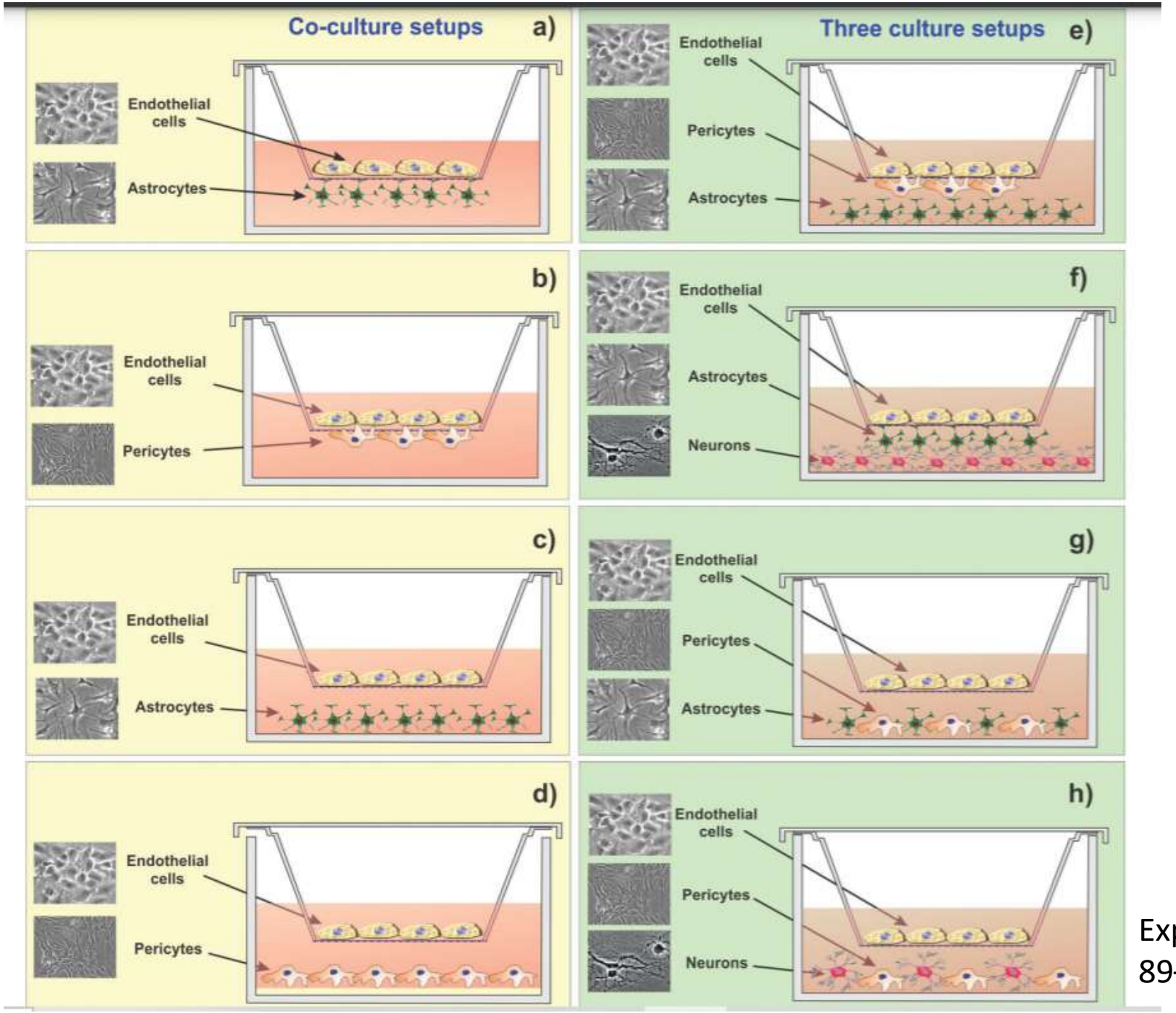


Mono, Co- and Triple culture platforms

Primary cultures / Immortalized endothelial cell lines

TEER

Coating



- Culturas primárias.
- Linhas: bend (mouse)
  hCMEC/D3 (human)

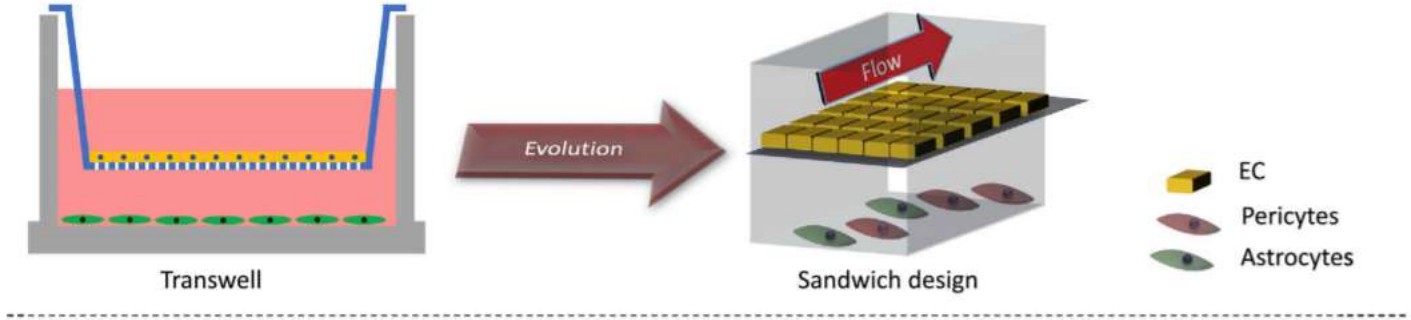
Expert Opin Drug Discov. 2017 January ; 12(1): 89–103. doi:10.1080/17460441.2017.1253676.

Static versus dynamic

Blood flow across the apical surfaces of endothelial cells, is required for the proper differentiation of brain vascular endothelium and to maintain a BBB phenotype, modulating not only morphology, but also cellular function and physiological responses.

In standard *in vitro* experiments, cells are cultured without flow (static conditions), so shear stress-dependent cellular changes are not taken into account;

in contrast, *in vitro* cell culture models under flow (dynamic conditions) simulates this mechanical stimulus and induces a more physiological, *in vivo*-like behavior, leading to increased barrier features.



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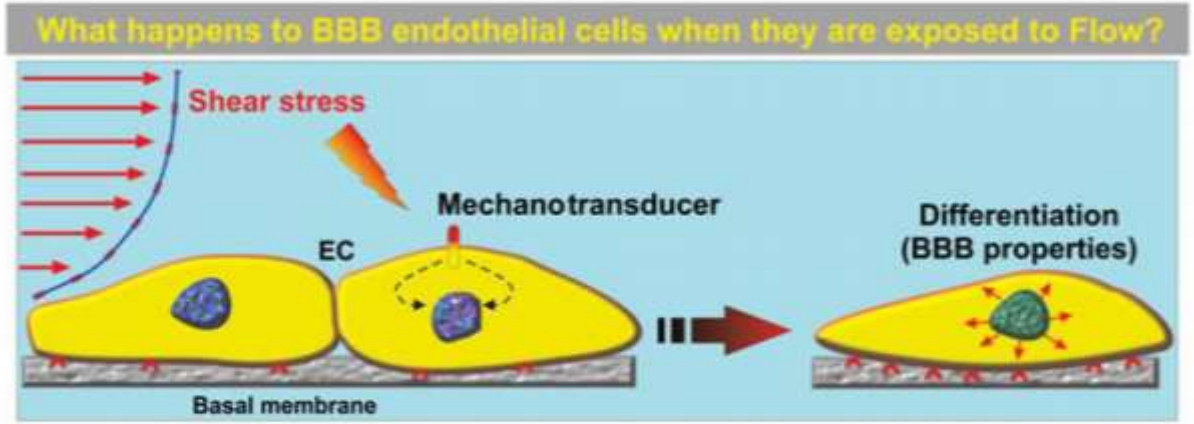
https://doi.org/10.1016/j.tibtech.2019.04.006



- Cell orientation changes with flow direction, distribution of cell cytoskeleton
- Production of vasoactive substances and improved cell adhesion
- Mitotic arrest
- Cell survival
- Glucose metabolism switch toward a more anaerobic pathway
- Tight junction formation
- The expression of asymmetrically localized enzymes and carrier-mediated transport systems that engender a truly "polarized" BBB endothelium phenotype

Figure 2. Effect of shear stress forces on BBB endothelial cells
Shear stress (SS) is a major pleiotropic modulator of the endothelial cell physiology by controlling gene involved in the regulation of cell division, differentiation, migration, and apoptosis. For example, the exposure to physiological SS promoted mitotic arrest by contact and the endothelium assumed the typical monolayer appearance observed *in vivo*.

J Pharm Sci. 2012 April ; 101(4): 1337–1354. doi:10.1002/jps.23022.

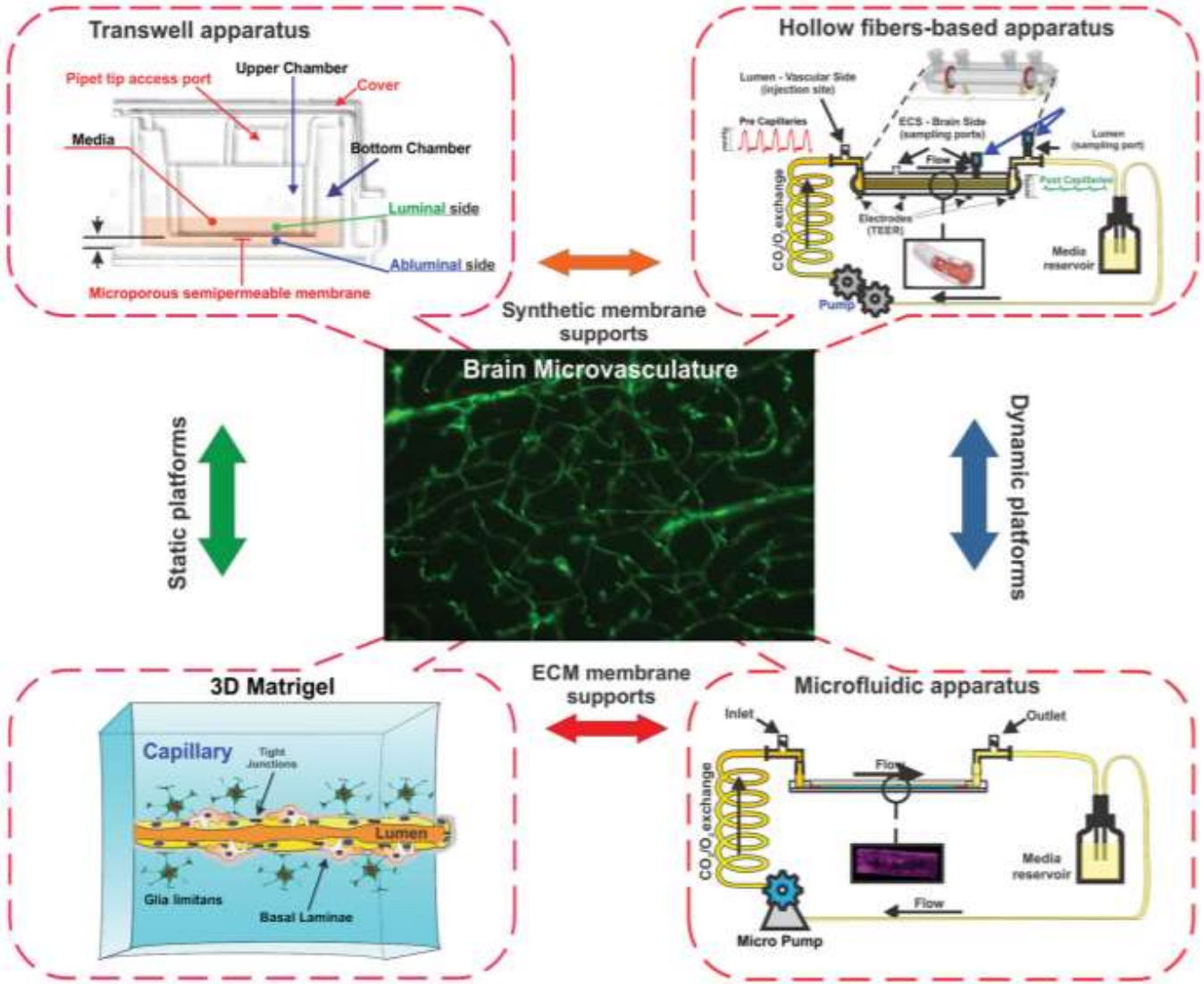


Figure 2. Schematic diagram of currently available *in vitro* BBB models simulating *in vivo* NVU milieu based on two distinct principles- static vs dynamic culture

Static models include transwell and 3D ECM platform while dynamic models utilize hollow fiber based apparatus or micro fluidic devices.

Shear stress and cell differentiation

Humans: the ultimate animal models

Reilly, M. M., & Rossor, A. M. (2020). Humans: the ultimate animal models. *Journal of neurology, neurosurgery, and psychiatry*, *91*(11), 1132–1136. https://doi.org/10.1136/jnnp-2020-323016