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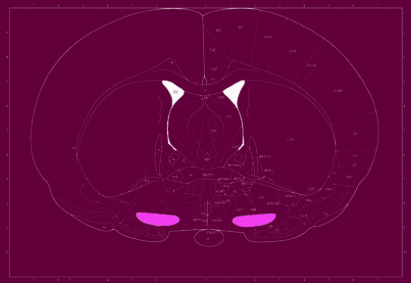
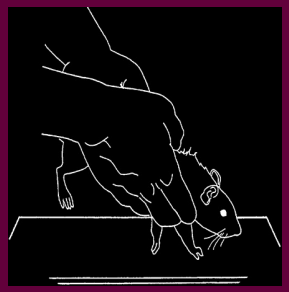
a cesp team

université PARIS-SACLAY
FACULTÉ DE PHARMACIE

Development of Drugs and Health Products
Unit 11 Pharmacology/Toxicology

Animal models of neurodegenerative diseases : The example of animal model of Parkinson Disease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Parkinson Disease : to develop a model....

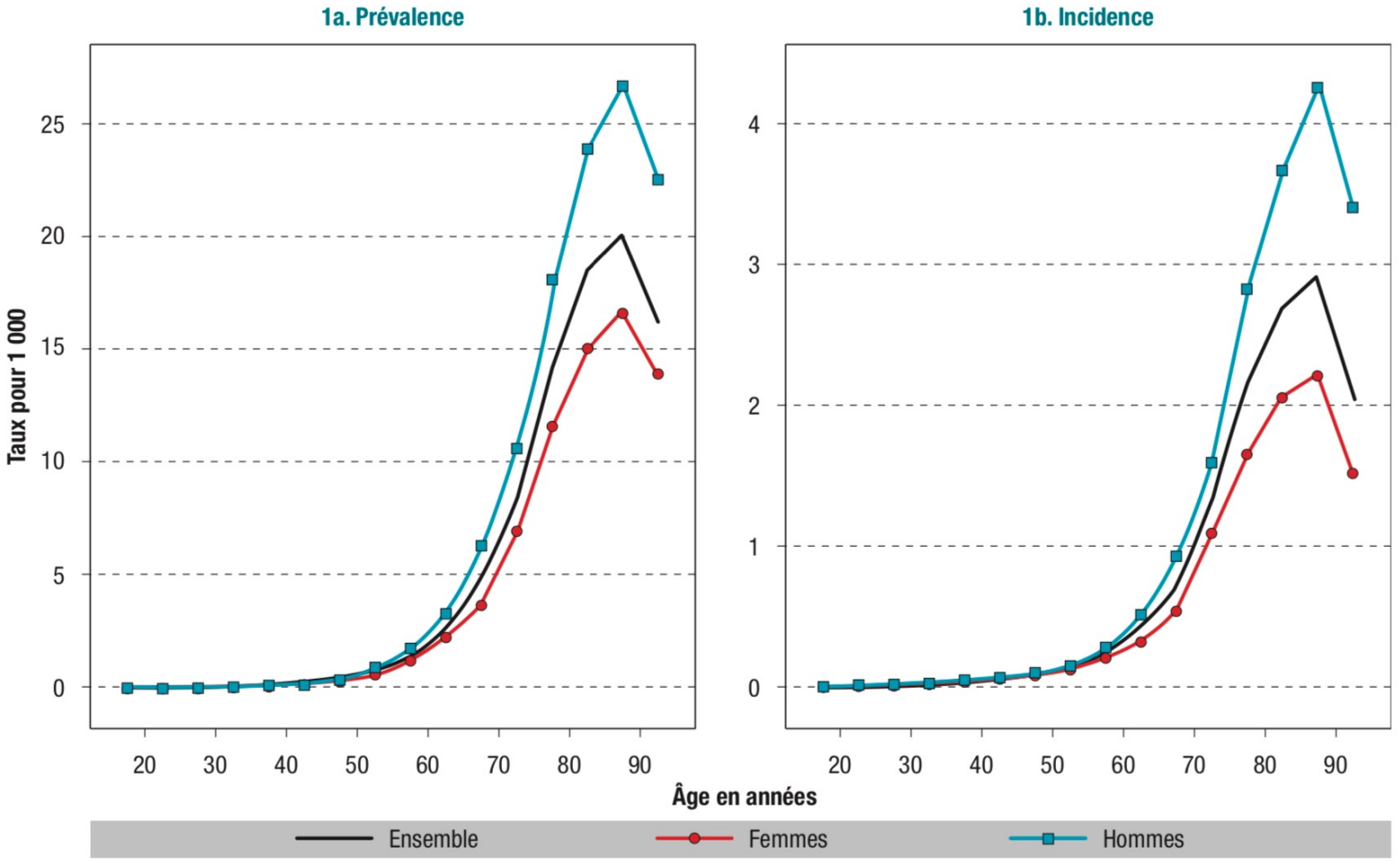
...you have to know the pathology

Parkinson Disease : Epidemiology

- Onset: Median at age 60
- Incidence: 60-140 / 100,000 in 70-79 year olds
- Ratio Male> Female: 1.5-2
- Slightly higher prevalence in Western countries (vs. Asia)
- 5% Non-sporadic, often early forms.
- Despite dopathotherapy, progression to marked physical disability and mental deterioration within 10 years

Parkinson Disease : Epidemiology

Prévalence (a) et incidence (b) de la maladie de Parkinson en France en 2015, par âge et sexe



Parkinson's disease: preclinical and prodromal phases

- ✓ Before onset of motor symptoms:
  - ✓ Preclinical phase: Asymptomatic neuronal degeneration
  - ✓ Prodromal phase:
    - ✓ Can start up to 12-14 years before the pathology
    - ✓ Pathologies of the peripheral nervous system and the olfactory bulb:
      - ✓ Hyposmia
      - ✓ Constipation
      - ✓ Altered REM phase of sleep
      - ✓ Anxiety-depressive disorders

Parkinson's disease: clinical consequences

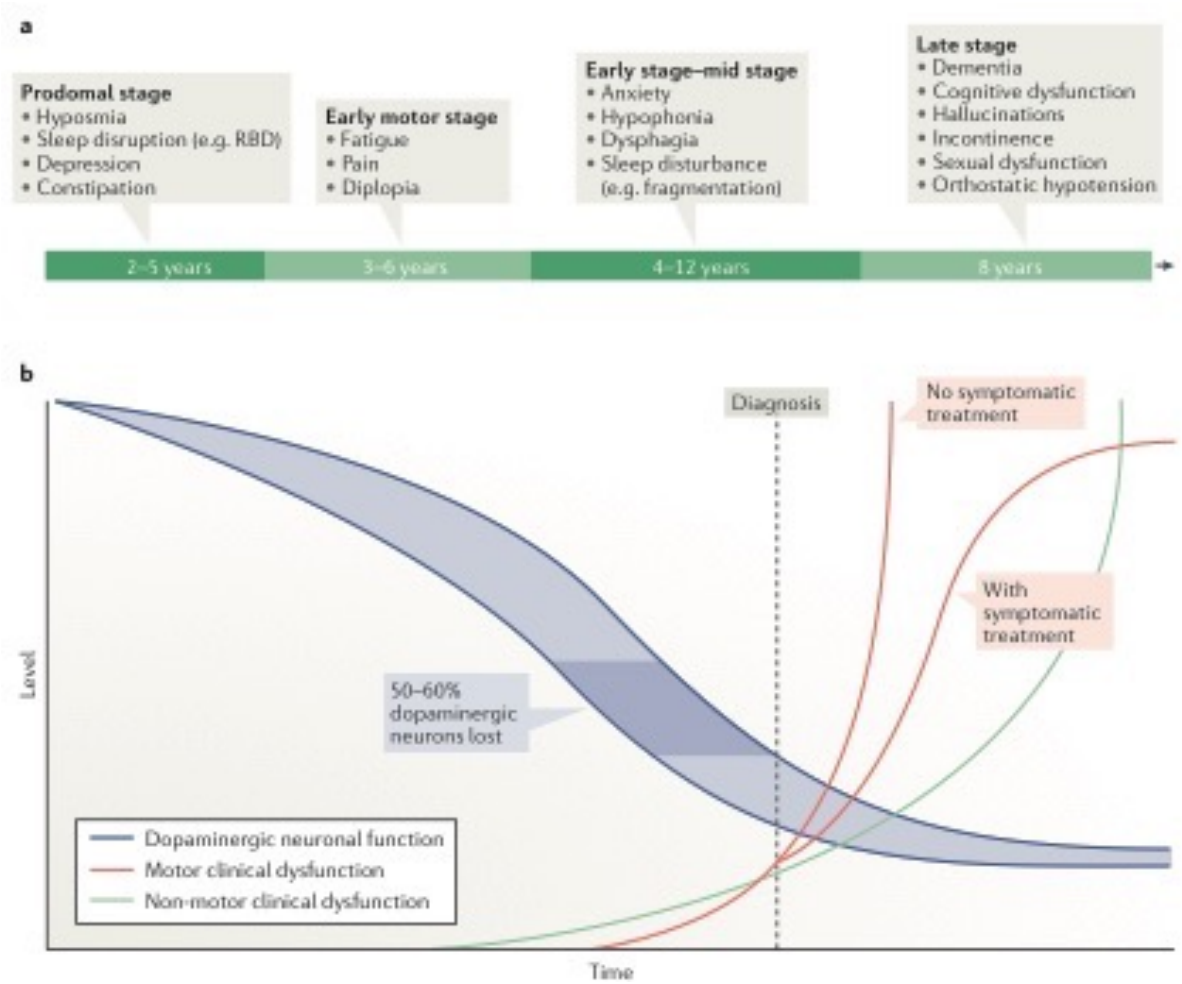
- ✓ Resting tremor
  - ➢ Resulting from the succession of phases of activation and inhibition of motor neurons from 3 to 6 contractions / sec.
- ✓ Muscle stiffness
  - ➢ Due to muscle hypertonia
- ✓ Bradykinesia / Akinesia
  - ➢ Disappearance or decrease in automatic movements, voluntary movements
- ✓ Freezing
- ✓ Severity of symptoms ↗️
- ✓ Clusters of proteins and neurofilaments characterize a classic disease (Lewy bodies) – post mortem

Parkinson's disease: clinical consequences

First symptoms:

- Asymmetric appearance
- Symptoms that disappear after dopatherapy
- Absence of the following symptoms:
  - Cerebellar involvement (disturbances in balance and walking)
  - Neurovegetative dystonia (fatigue, dizziness, vertigo, postural hypotension)
  - Cortico-basal degeneration

Parkinson's disease: Symptoms evolution



Parkinson's disease: non-motor symptoms

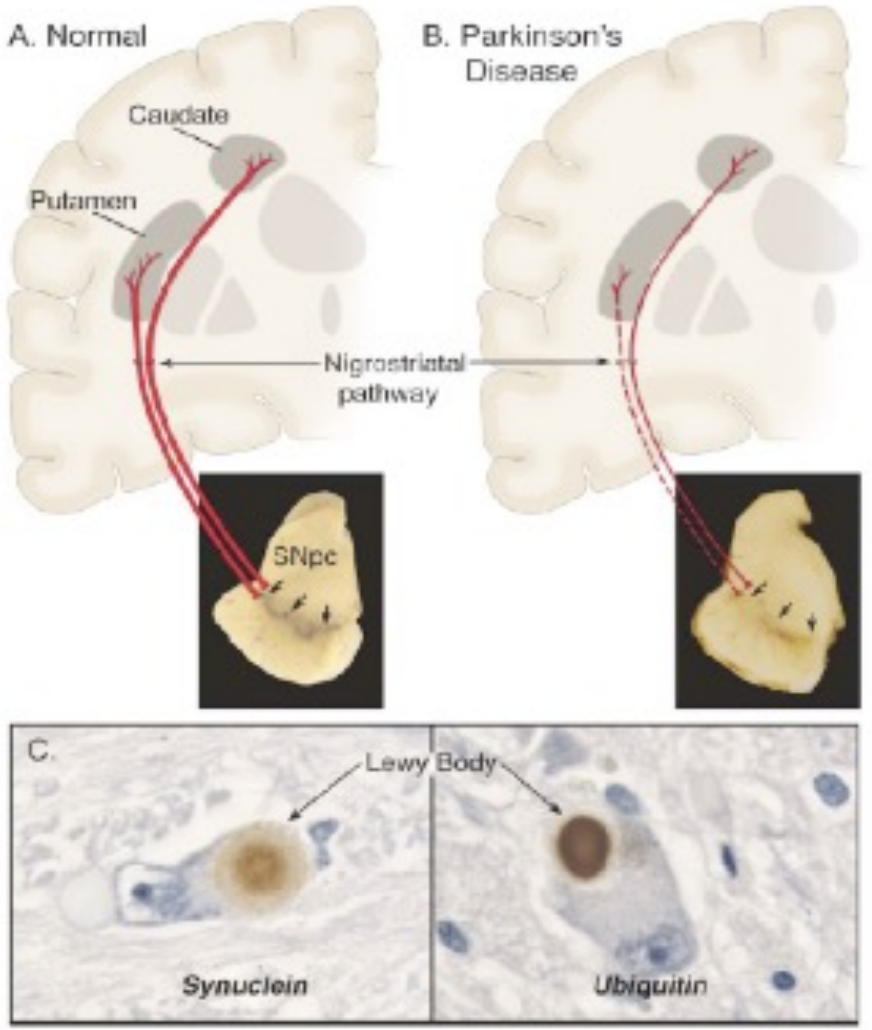
Non-motor symptom	Implicated brain region	Implicated neurotransmitter
Hyposmia	Olfactory bulb and amygdala	Substance P and acetylcholine
Impaired colour vision	Retina	Dopamine
Hallucinations	Occipital cortex	Dopamine
Pain	Basal ganglia, locus coeruleus, raphe nucleus, amygdala and thalamus	Dopamine, serotonin and noradrenaline
Anxiety	Basal ganglia	Dopamine and noradrenaline
Depression	Limbic and cortical areas	Dopamine and noradrenaline
Early cognitive dysfunction	Frontal cortex	Dopamine
Dementia	Temporal, parietal and occipital lobes	Acetylcholine
Sleep disturbance	Hypothalamus and reticular formation	Hypocretin, dopamine and serotonin
Bladder hyper-reflexia	Basal ganglia	Dopamine and acetylcholine

Parkinson's disease: Neuropathology

Loss of the pigmented part of the pars compacta of the substantia nigra:

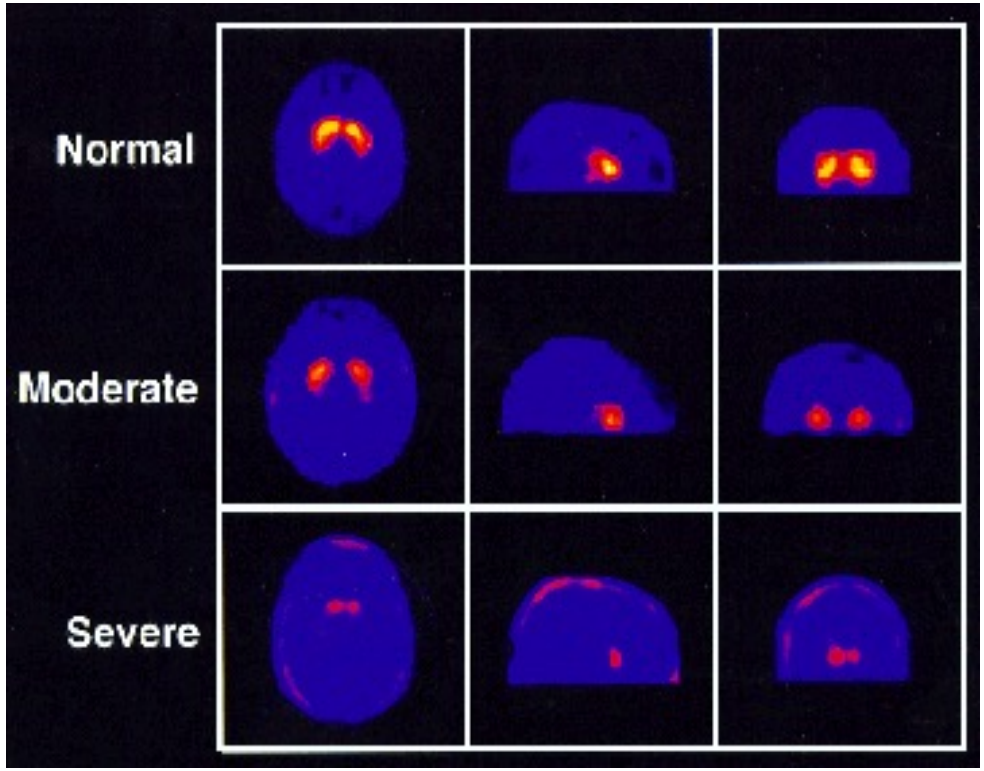
-> contains DA neurons which synthesize neuromelanin

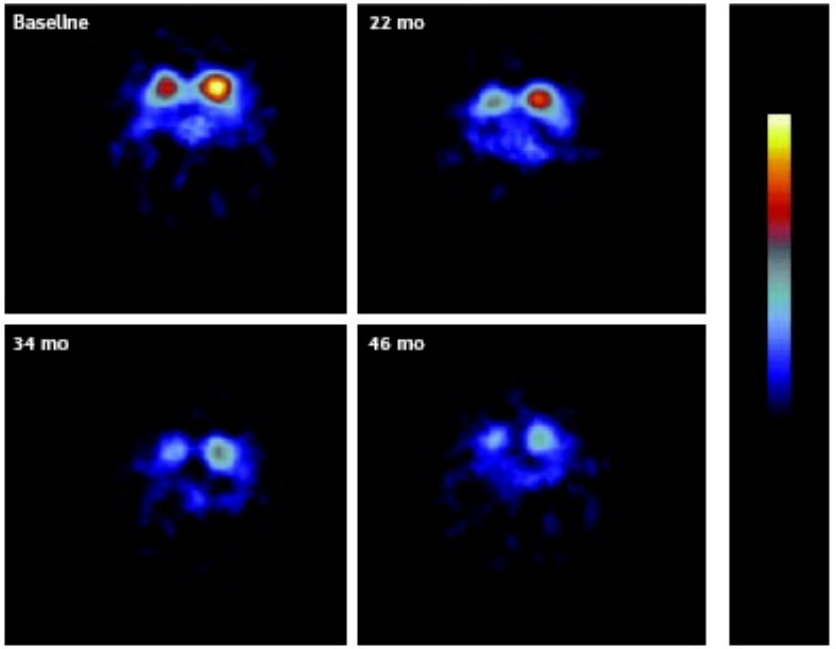
Loss of Tyrosine hydroxylase (TH-ir +) immunoreactive fibers



Parkinson's disease: Neuropathology

Asymmetric degeneration proportional to the duration and severity of the pathology.





From Parkinson Group Study, 2002
Schapira et al., 2006

Parkinson's disease: Lewy bodies

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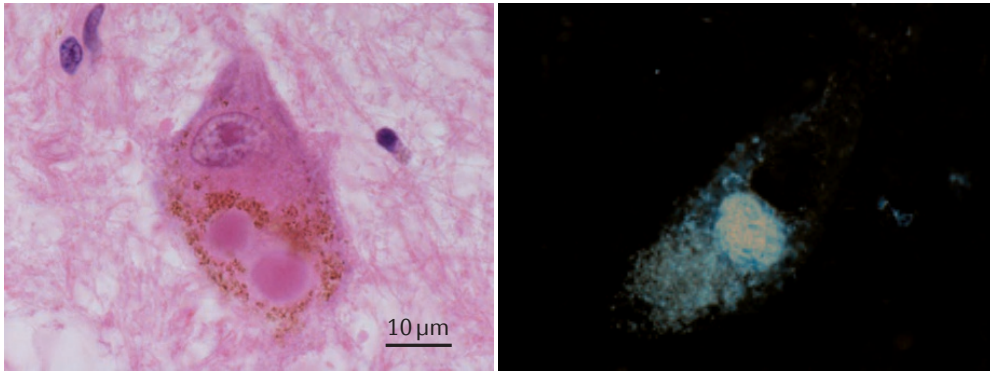

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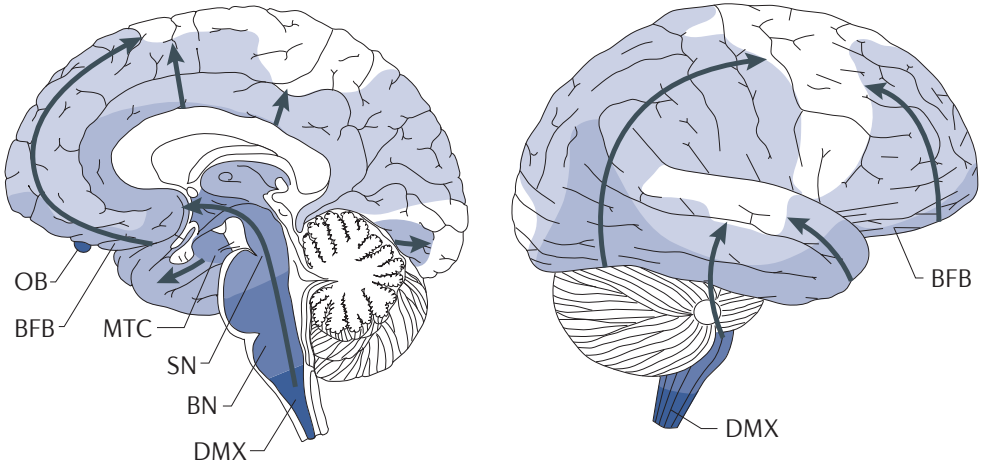
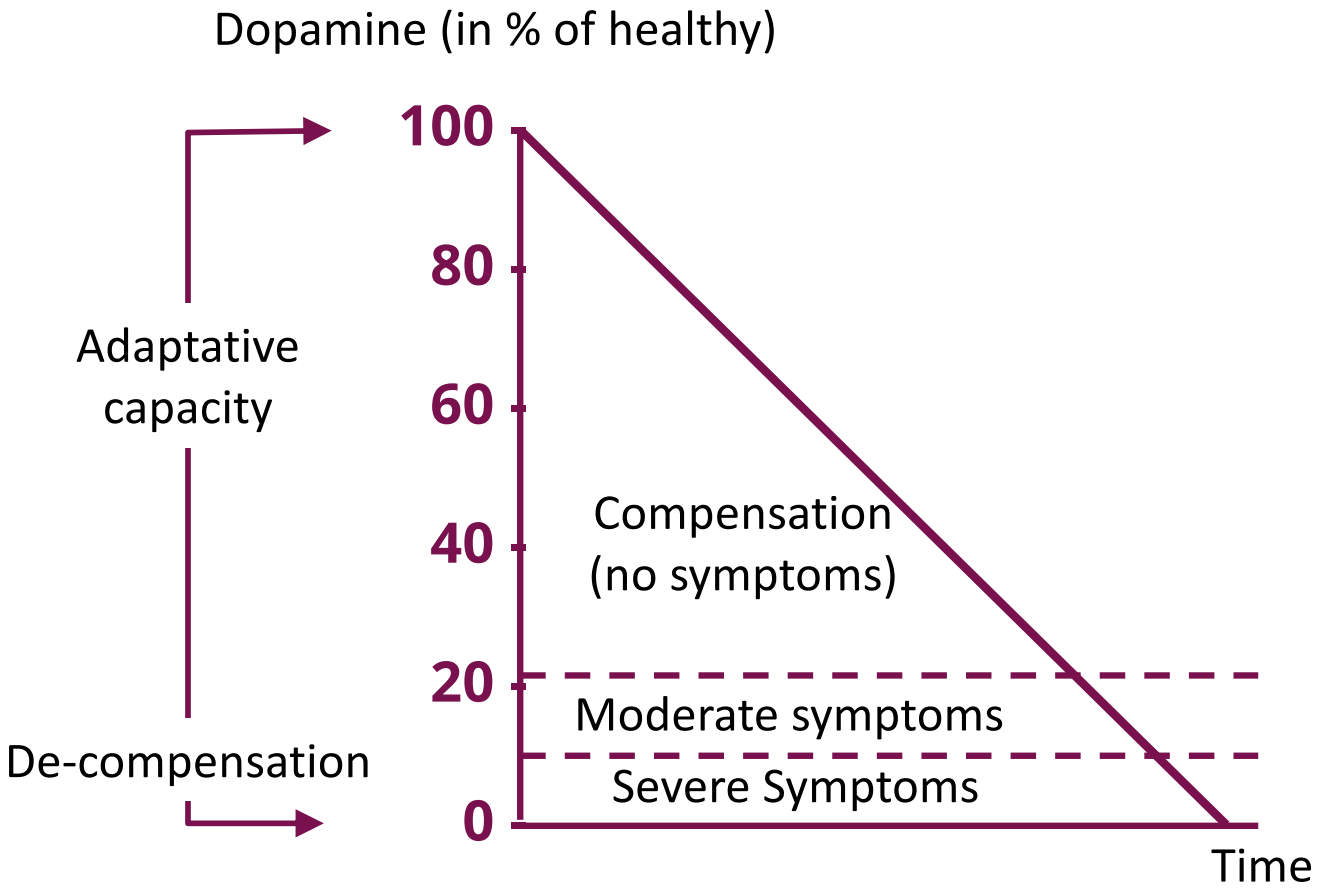
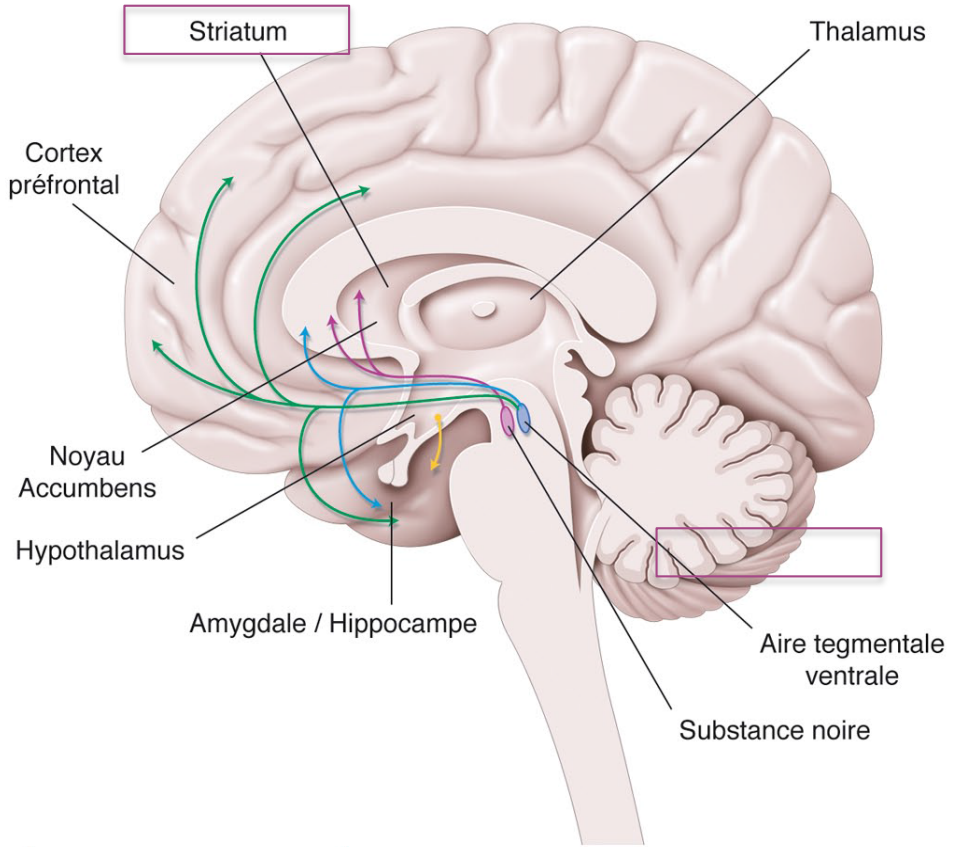


Parkinson's disease: a progressive pathology



Nigrostrial pathway:

Substancia nigra →
- **Caudate nucleus**
- **putamen**
- **globus pallidus**



Voies dopaminergiques

Physiological roles

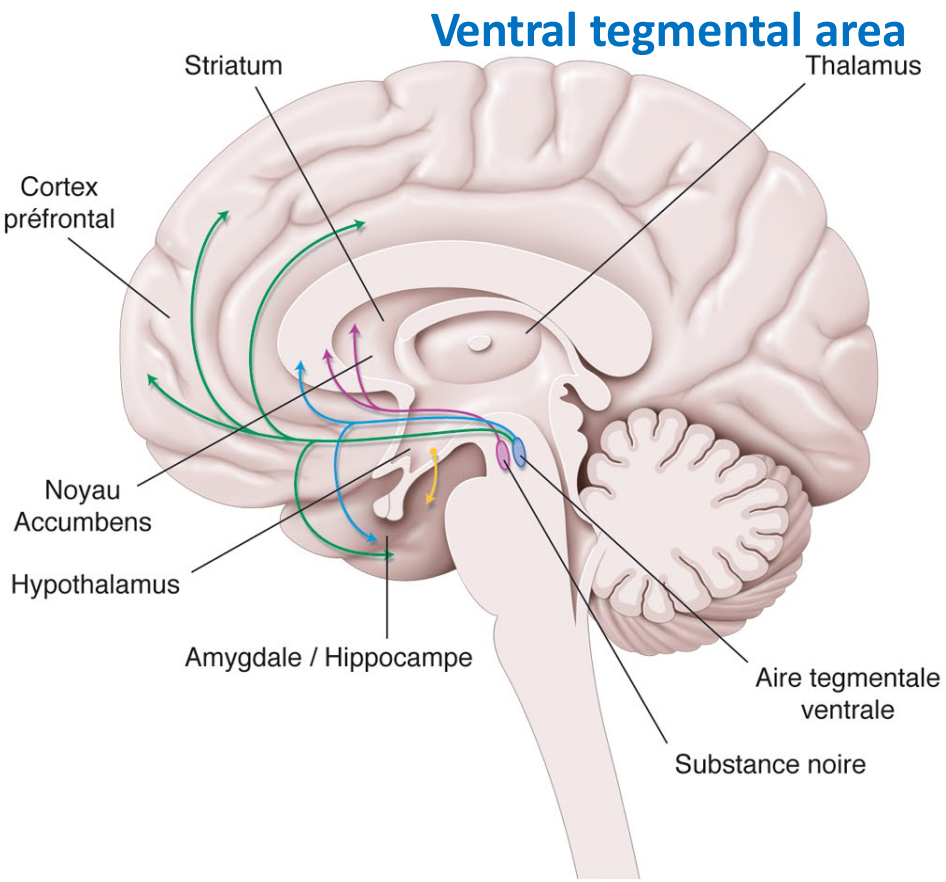
automatic motor control and logorhea

Pathophysiological roles

Hypoactivity DA: akinesia, bradykinesia, akathisia

AD hyperactivity: dyskinesia, hyperkinetic movements

Meso- Pathway



Voies dopaminergiques

- amygdala
- hippocampus
- accumbens

} - limbic

- Cortex

} - cortical

Physiological roles

Emotion, pleasure, interest, reward
Psychoaffectivity, cognition

Pathophysiological roles

Meso-limbic hyperactivity:

Auditory hallucinations, delusions, disturbances in thinking (productive disorders of schizophrenia)
Addiction

Mesocortical hypoactivity

Loss of motivation, emotional detachment (schizophrenia deficit disorders)

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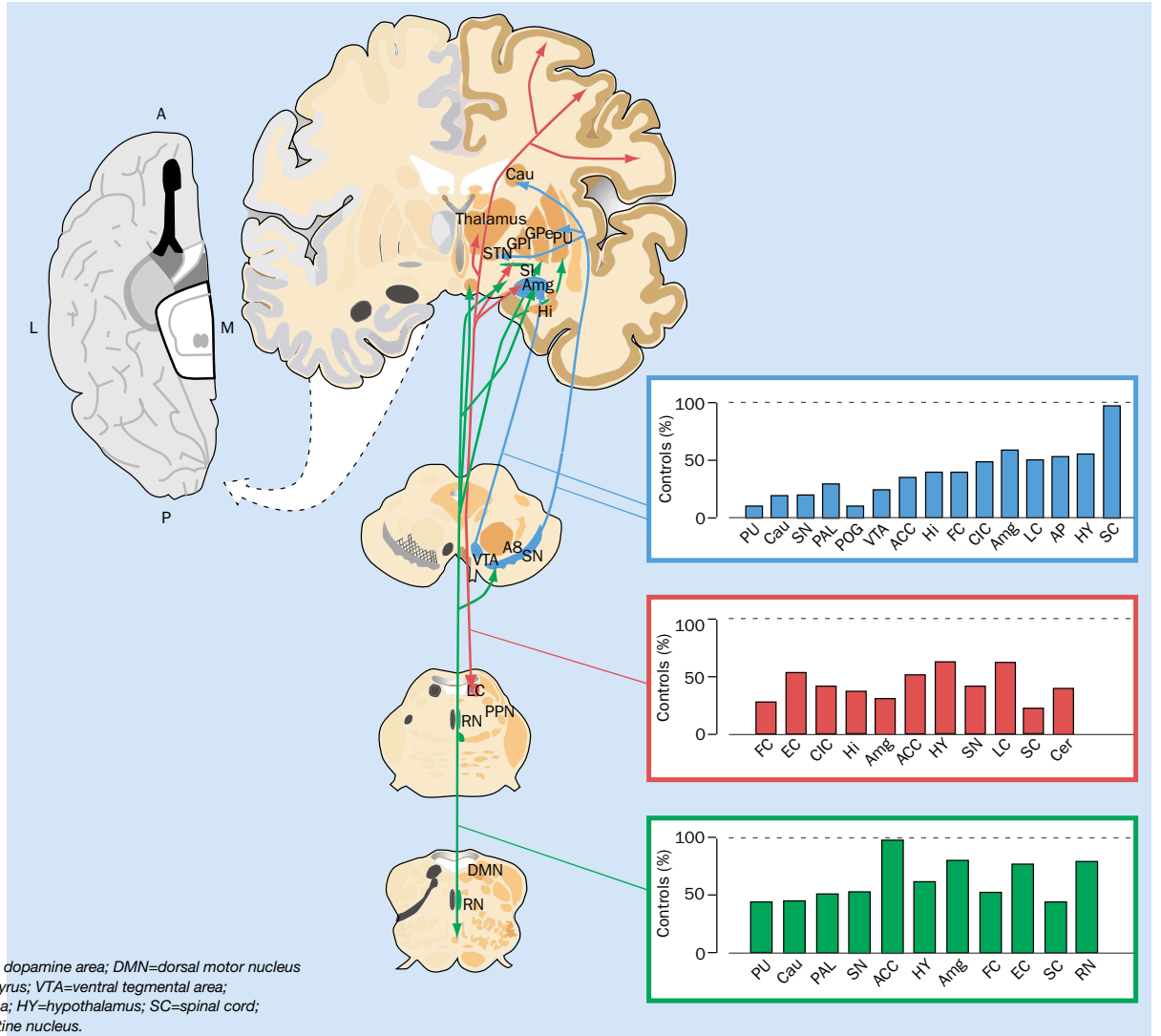
Parkinson's disease: not only a DA problematic

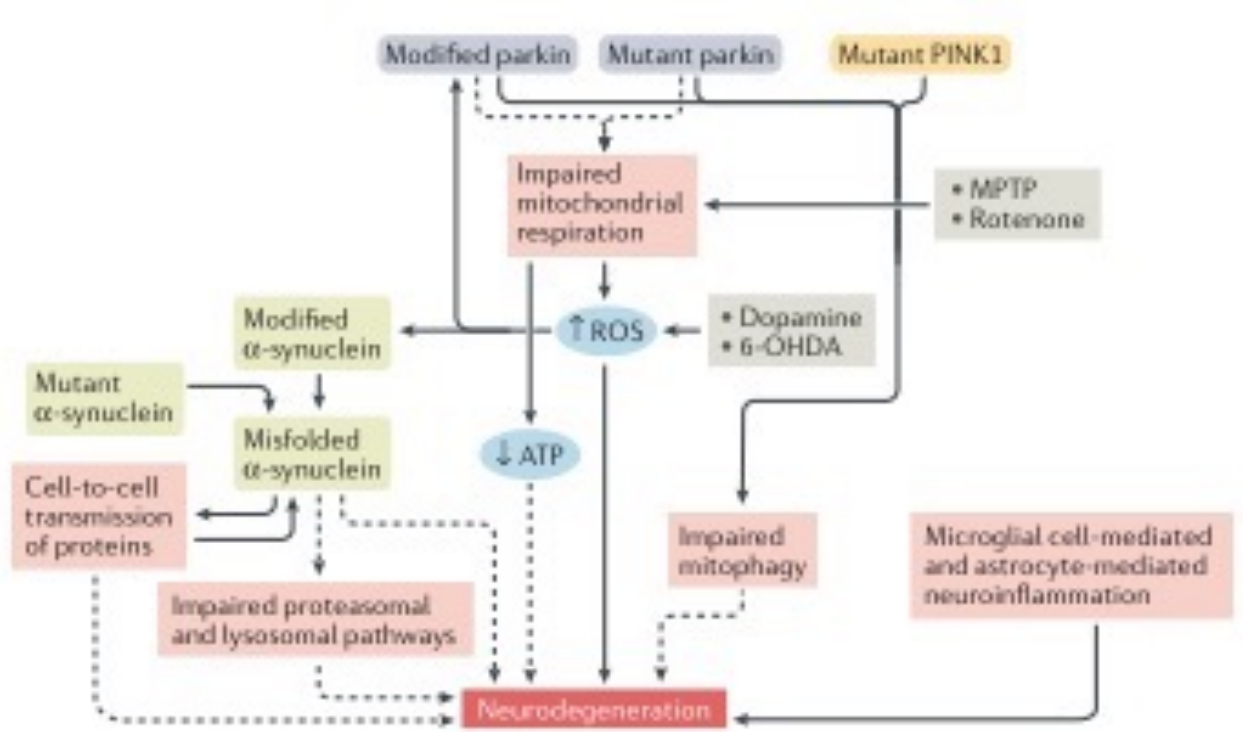
- Damage to other monoaminergic systems
- ≃50% decrease in noradrenergic neurotransmission
- ≃40% decrease in serotonergic neurotransmission
- These non-DA damage would be responsible for the co-morbidity of the pathology (depression, cognitive dysfunction, damage to the autonomic nervous system, etc.)



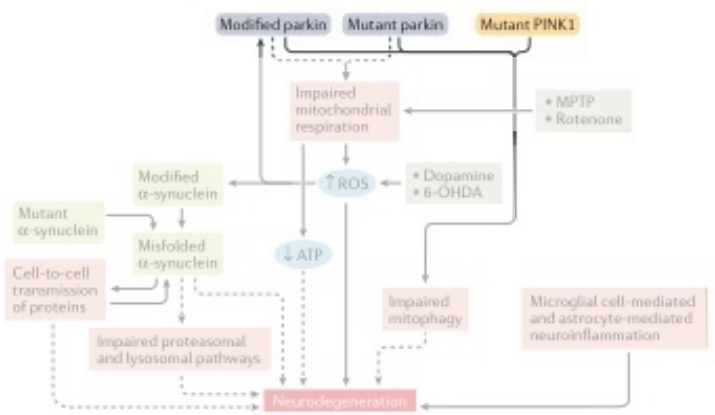
regions (adapted from reference 57). SI=substantia innominata; Amg=amygdala; Hi=hippocampus; A8=A8 dopamine area; DMN=dorsal motor nucleus of the vagus nerve; PU=putamen; Cau=caudate; SN=substantia nigra; PAL=pallidum; POG=parolfactory gyrus; VTA=ventral tegmental area; ACC=nucleus accumbens; FC=frontal cortex; CIC=cingular cortex; LC=locus coeruleus; AP=area postrema; HY=hypothalamus; SC=spinal cord; EC=entorhinal cortex; Cer=cerebellum; RN=raphe nuclei; STN=subthalamic nucleus; PPN=pedunculopontine nucleus.

Parkinson's disease: Etiology

- Several etiological causes
  - Currently no single recognized cause
  - Several avenues: genetic, environmental and toxicological



Parkinson's disease: Etiology - genetics

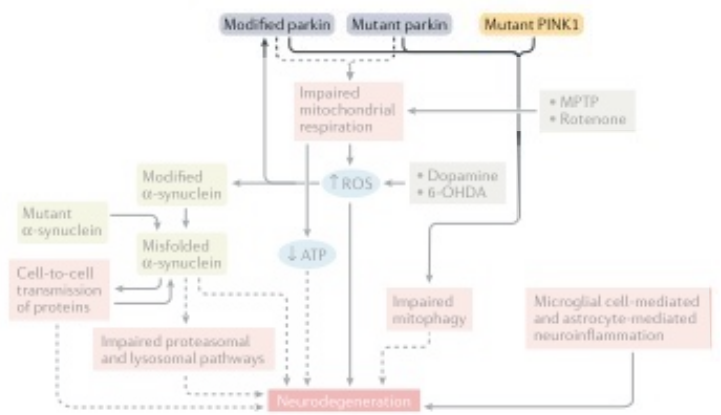


- PARK2 gene mutations in humans
  - Responsible for early forms (> 30 years old)
  - Family history with recessive inheritance

  - Parkin is an enzyme:
  - responsible for the metabolism of ROS forms
  - which indirectly suppresses factors of mitochondrial respiration
  - Participates in macro-autophagy of mitochondria with PINK1

  - Induces degeneration of DA neurons, without production of Lewy bodies

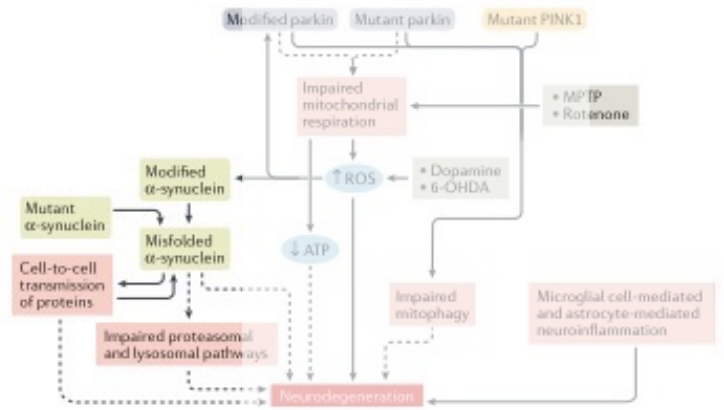
Parkinson's disease: Etiology - genetics



- PINK1 gene mutations in humans
  - Causes a parkinsonian symptom
  - Family history with recessive inheritance

  - PINK1 participates in the same metabolic pathway as PARK2
  - PINK1 participates in mitochondrial regulation
  - PINK1 mutations induce a parkinsonian phenotype in the fly, which is restored by administration of parkin which indirectly suppresses factors of mitochondrial respiration
  - Participates in macro-autophagy of mitochondria with PINK1

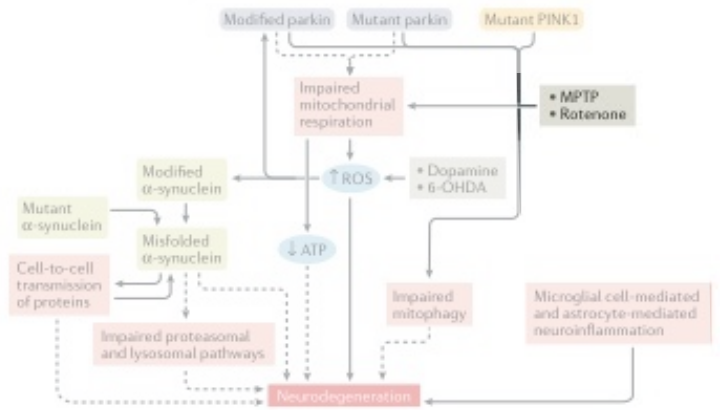
Parkinson's disease: Etiology - genetics



- Mutations in the gene coding for alpha-synucleinin humans
  - SNCA
  - Linked to dominant hereditary forms of Parkinson's

  - Alpha-synuclein present in high concentrations in Lewy bodies
  - Alpha-synuclein in a meconformation forming oligomers and protofibrils.
  - Elimination of these modified alphasynucleins altered.

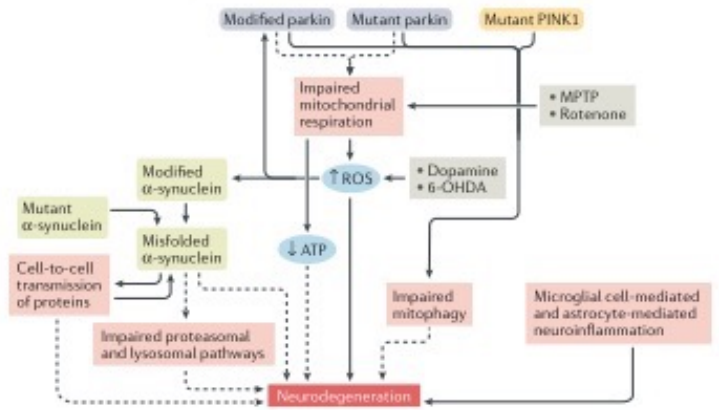
Parkinson's disease: Etiology - environmental



- Frozen Addicts (Langston et al., 1983):
  MPTP-induced parkinsonism
- Heroinomaniac patients intoxicated by MPTP presenting with pseudo-parkinsonian symptoms.
- Paraquat, Rotenone, pesticide derivatives: 10% higher risk for farmers.

Parkinson's disease: Interaction genetics x environment

- Sporadic and familial forms are similar
  - Different causes
  - ... but final mechanisms and or consequences identical



Parkinson's disease: Animal models

An animal model must meet the following criteria and reflect Parkinson's disease at the level:

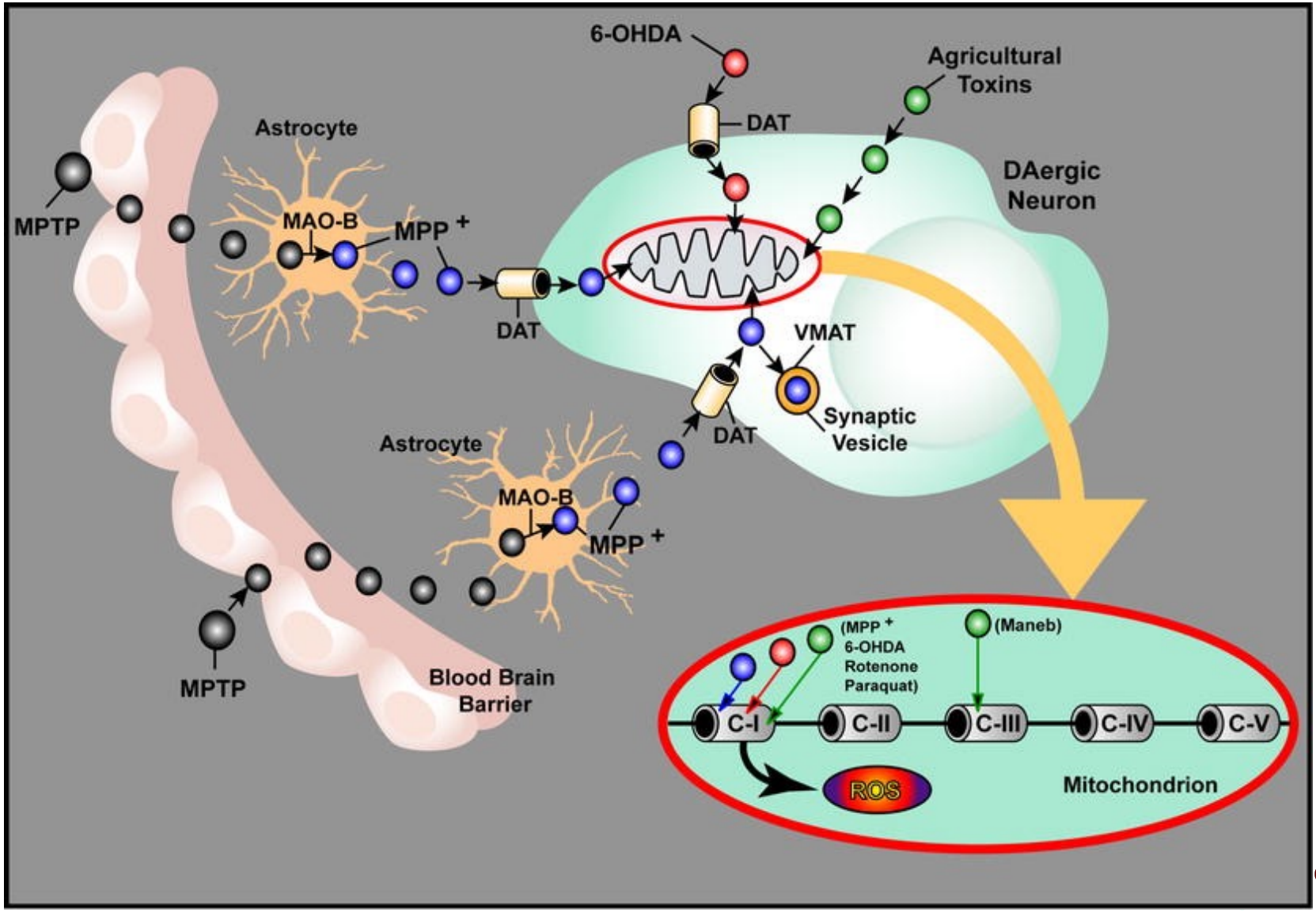
1. induced behavioral states, showing a similarity to human pathology:
   - i.e. DESCRIPTIVE VALIDITY

2. underlying neurochemical mechanisms:
   - i.e. THE THEORETICAL VALIDITY

3. response to treatment:
   - i.e. PREDICTIVE VALIDITY

NO MODEL PERFECTLY REFLECTS HUMAN PATHOLOGY.

Parkinson's disease: Toxin models



Parkinson's disease: Genetic models

Rats or mice KO - PARKIN

- Mitochondrial respiration deficit in the nigrostriate pathway
- Increase in oxidative stress markers

But...:

- no loss of DA neurons
- absence of Lewy bodies
- Moderate locomotor deficit

Animal model used to define the molecular substrates associated with Parkin and the molecular mechanisms associated with the pathology

Parkinson's disease: Genetic models

KO Rats - PINK1

- Loss of DA neurons depending on age (6-8 months)
- Motor deficit from 4 months
- Non-motor deficits: less vocalizations
- Mitochondrial respiration deficit in the nigrostriate pathway
- Aggregates of synuclein but not of Lewy body type.

Animal model used to understand the role of synuclein aggregation in the development of pathology and to test neuroprotective approaches.

Parkinson's disease: Genetic models

Rats or alpha-synuclein mice

- Models of overexpression of mutated forms of murine synuclein
- Models of overexpression of human forms of synuclein

- Overexpression by injection of AAV or genomic modification

- Degeneration of DA neurons at 18 months
- Decrease in striatal AD at 12 months + Musculoskeletal deficit
- Non-motor deficits
- Aggregates of synuclein but not of Lewy body type.
- Increased sensitivity to rotenone

Animal model useful for defining the molecular substrates associated with Parkin and the molecular mechanisms associated with the pathology

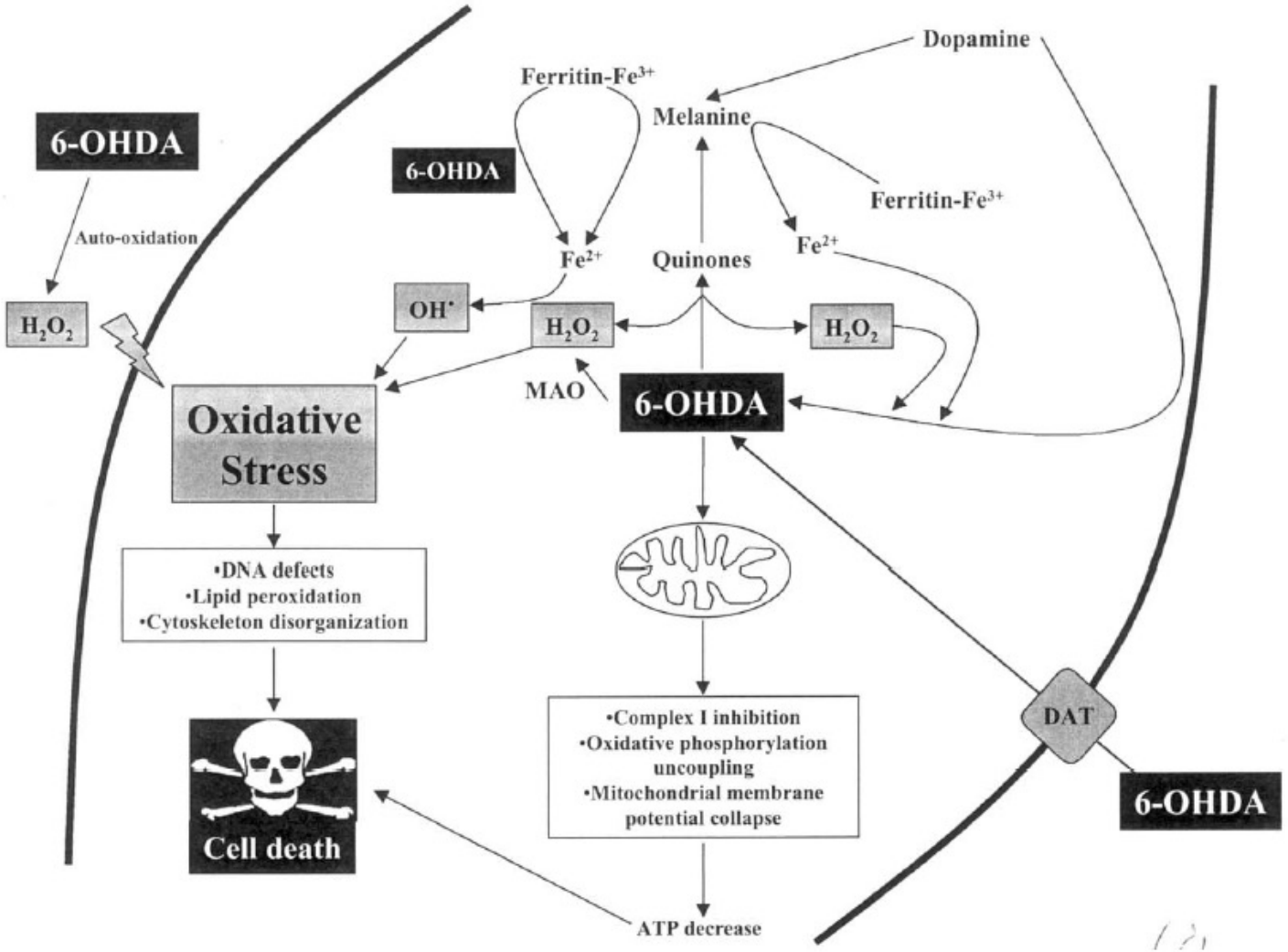
Parkinson's disease: Genetic models

Type of model	Advantages	Limitations
Transgenic models	• High construct validity: based on genetic causes of PD in humans • Some face validity: occurrence of α-syn aggregates • Potential disease-modifying therapies have shown efficacy (for example, immunotherapies targeting α-syn[102,112,115]) • Model some peripheral α-syn pathology and non-motor symptoms • Can be established in laboratories from small founder laboratories or commercial suppliers • Transgenic mouse model expressing wild-type α-syn under the *Thy1* promoter has good face validity and is well studied	• Incomplete face validity: limited and inconsistent neurodegeneration in SN • Prolonged time course to develop pathology is not optimal for evaluation of potential therapeutics • Limited reliability and utility of motor phenotypes as outcome measures • Peripheral α-syn pathology and associated non-motor symptoms may not precede brain pathology • Predictive validity: not yet demonstrated • No well-validated NHP application yet
Viral vector delivery models	• High construct validity: based on the molecular pathology of PD, both genetic and sporadic • Strong face validity: models dopaminergic neuron degeneration, accumulation of α-syn aggregates and PD-like motor deficits • Develop phenotype in weeks to months, allowing medium throughput of candidate therapeutics • Can be applied across multiple species, including NHPs • Potential therapies have shown efficacy in rodent models[117,144–147] • Several new-generation AAV2-related rodent models are widely available and represent a viable platform to evaluate novel therapeutics	• Incomplete face validity: no non-motor phenotype, thus may require application of vectors outside the nigrostriatal system • Degeneration restricted to pathways emerging from site of focal delivery, typically the nigrostriatal pathway • Predictive validity: not yet demonstrated • Resource intensive, especially NHP models
α-Syn transmission models	• Construct validity: model trans-synaptic spread of α-syn • Some face validity: progressive accumulation of α-syn aggregates and degeneration beyond site of delivery • Potential to model α-syn pathology in multiple neurochemical systems and to produce non-motor symptoms	• Construct validity: dependent on the prion hypothesis • Time to develop pathology is long • Incomplete face validity: no PD-like behavioural motor deficits • Poorly characterized with respect to robustness of model in different laboratories and ability to show efficacy of potential therapeutics

Parkinson's disease: Toxin models

- Rats 6-OHDA
- Mice :
  - MPTP
  - 6-OHDA
- Monkey MPTP

Parkinson's disease: Toxin models

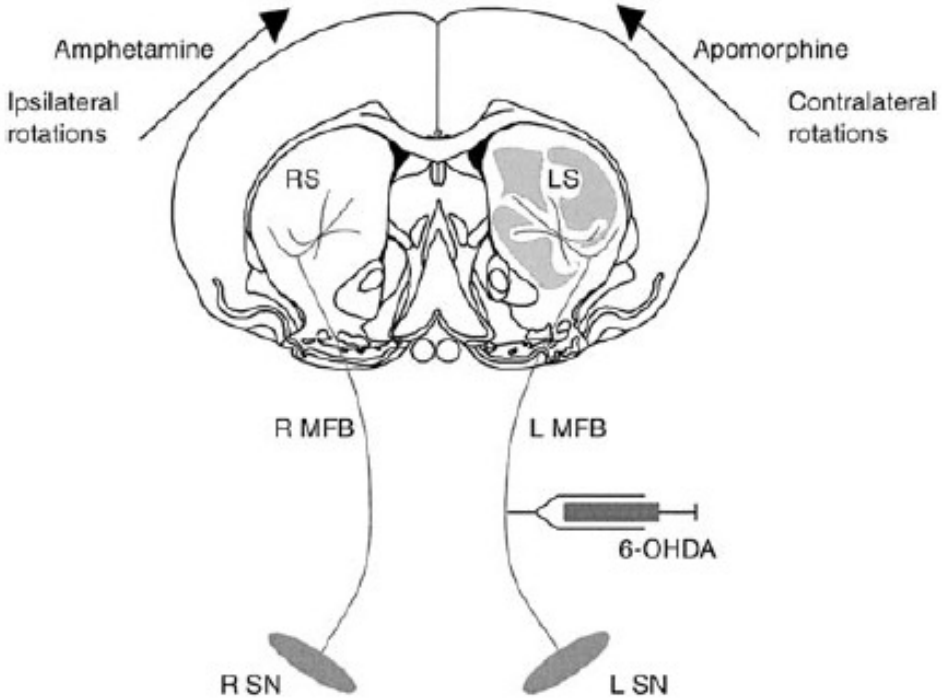


Parkinson's disease: 6-OHDA model

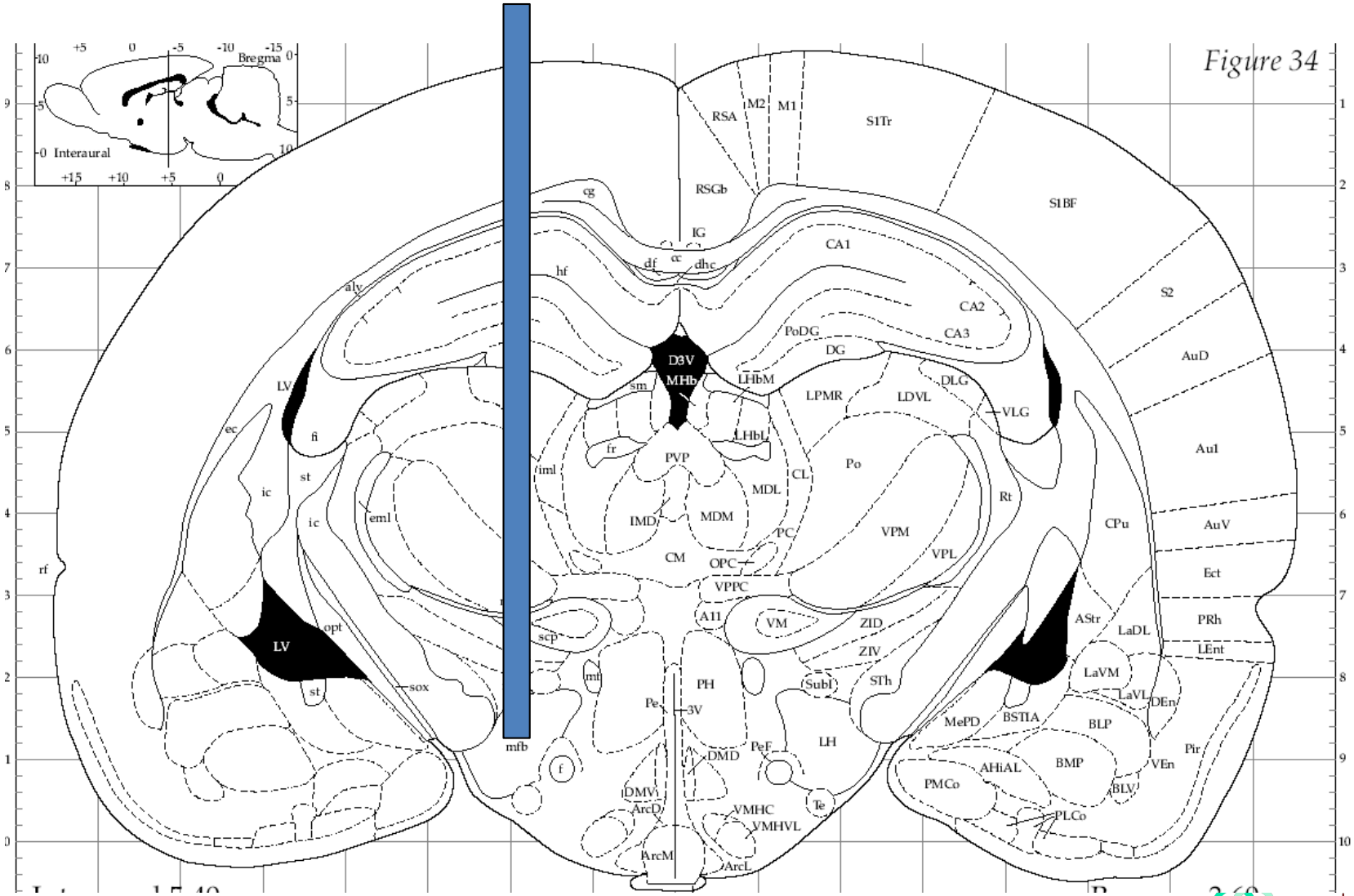
6-OHDA (6-hydroxydopamine) Rats, Ungerstedt Model, 1968

Injection of 6-OHDA into the MFB (median forebrain bundle = tract of DA fibers of the nigrostriotic pathway)

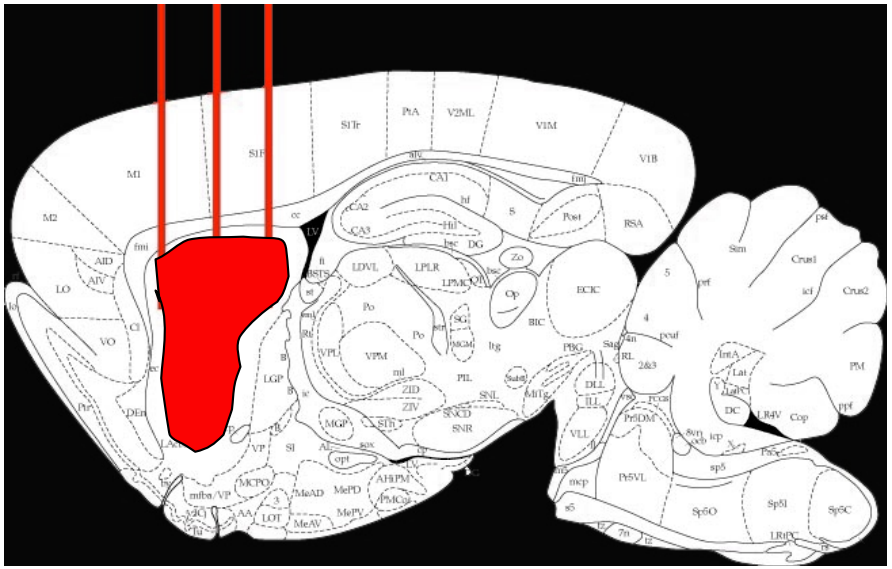
Verification of the lesion by administration of apomorphine (non-selective D1-2 agonist) which causes contralateral rotations to the lesion site.

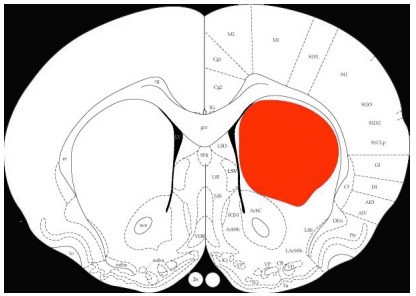


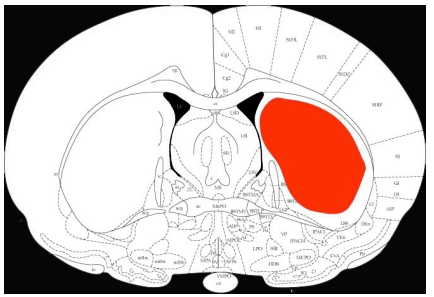
Parkinson's disease: 6-OHDA model (Substancia nigra)

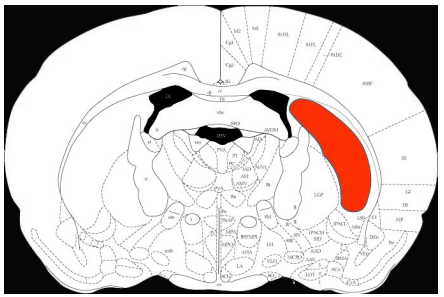


Parkinson's disease: 6-OHDA model (striatum)







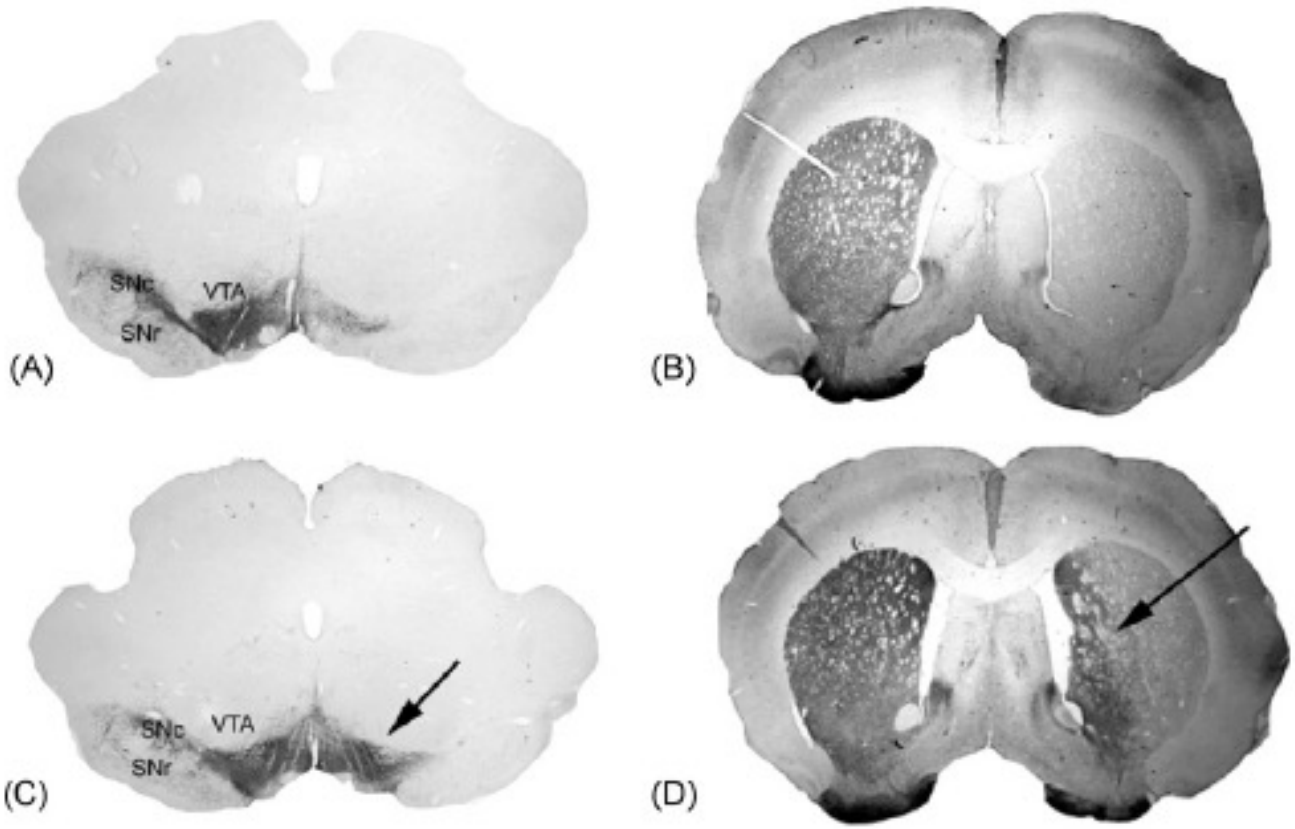


Validation de la lésion par observation des rotations induites par injection de métamphétamine (2 mg/kg, i.p.) ou d'apomorphine (0.5 mg/kg)

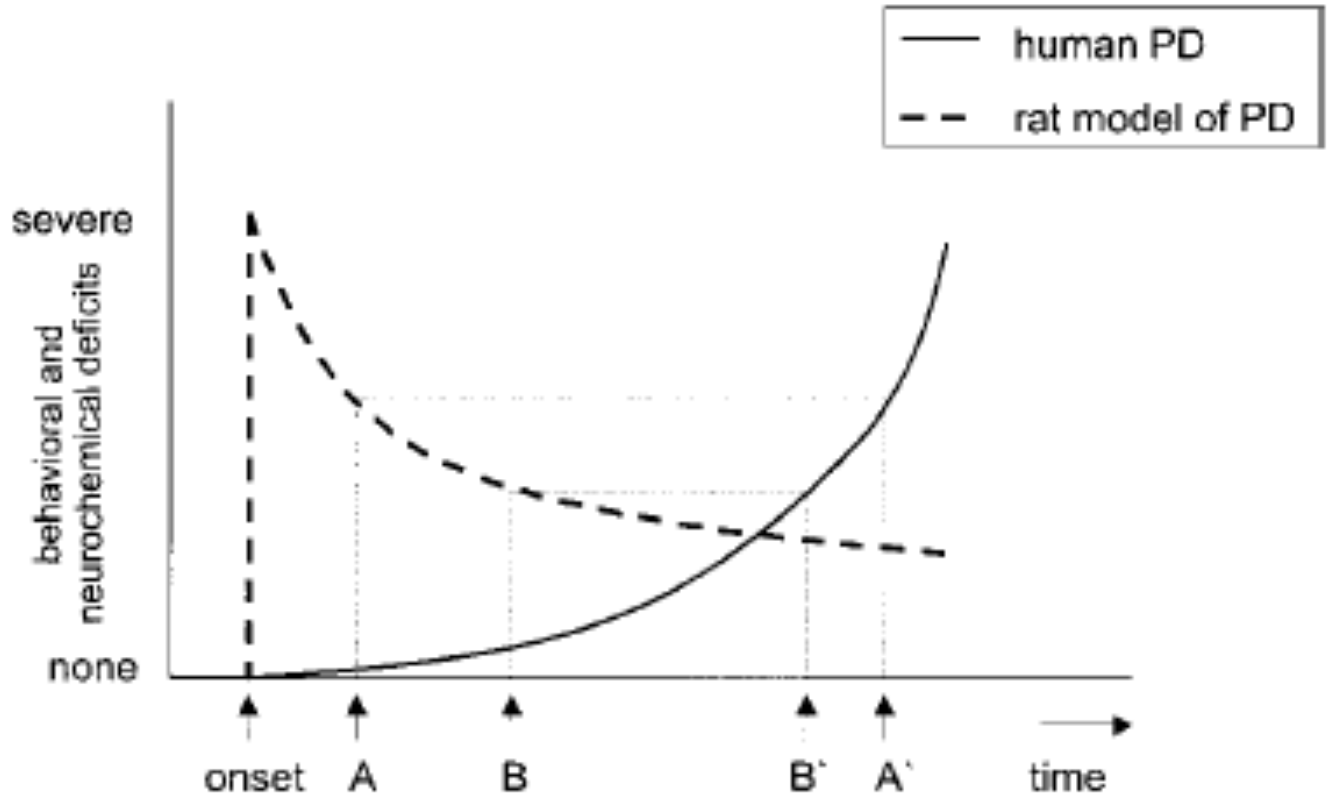
Parkinson's disease: Differences between models

MFB Injection

Striatum injection

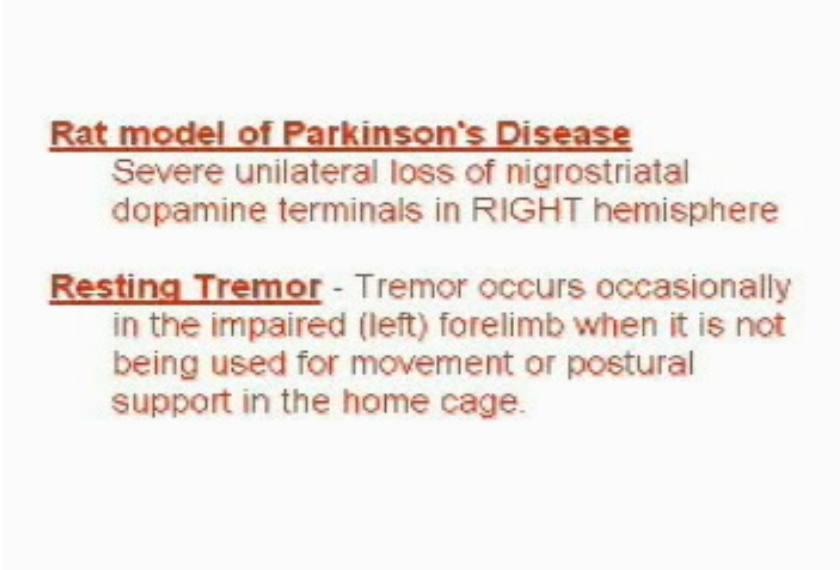


Parkinson's disease: 6-OHDA model

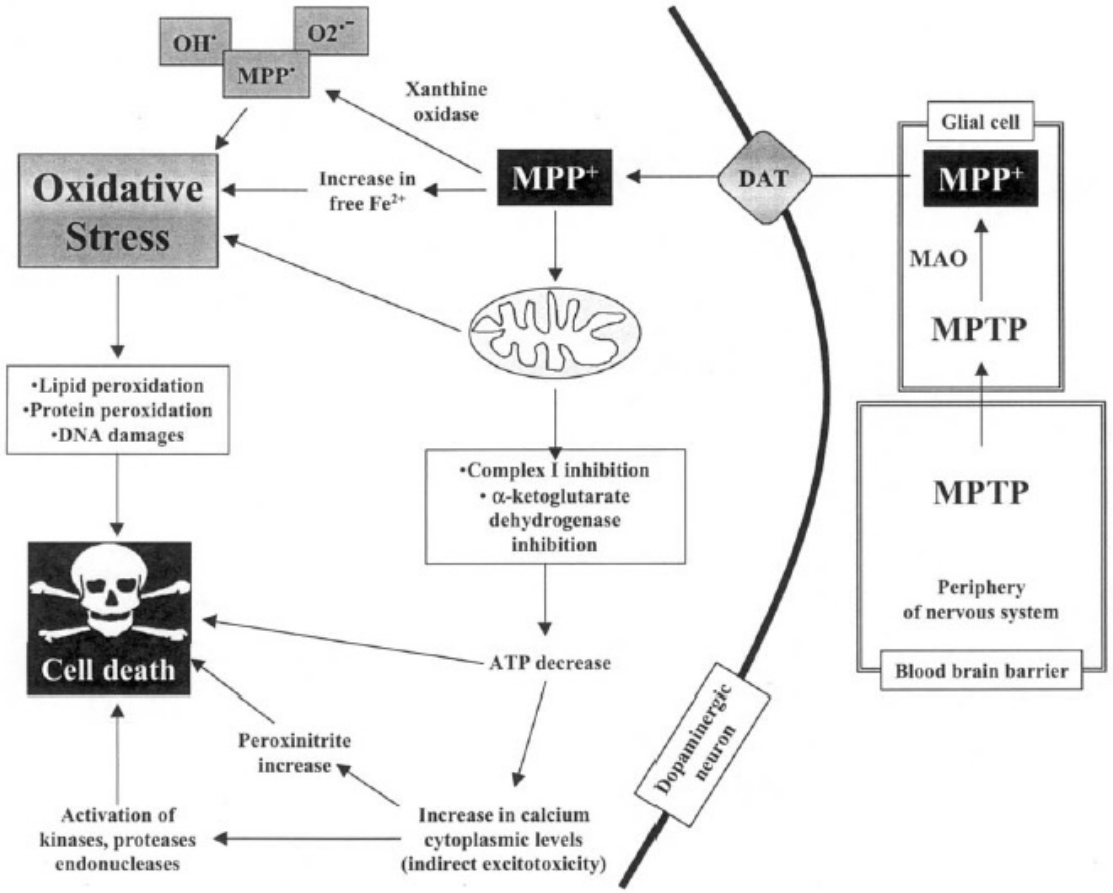


Parkinson's disease: 6-OHDA model

Model	Symptoms induced	Pathology	Favorite applications	Disadvantages
6-OHDA	Unilateral: rotation after, e.g. apomorphine treatment, bilateral: akinesia	Loss of Striatal DA-levels	**Tests of preclinical therapies, tests of new pharmacological and genetic therapeutic strategies**	**Acute damage of the DAergic system, unilateral effects, intracerebral injection**
		Striatal TH-ir fibers		
		Nigral TH-ir neurons		



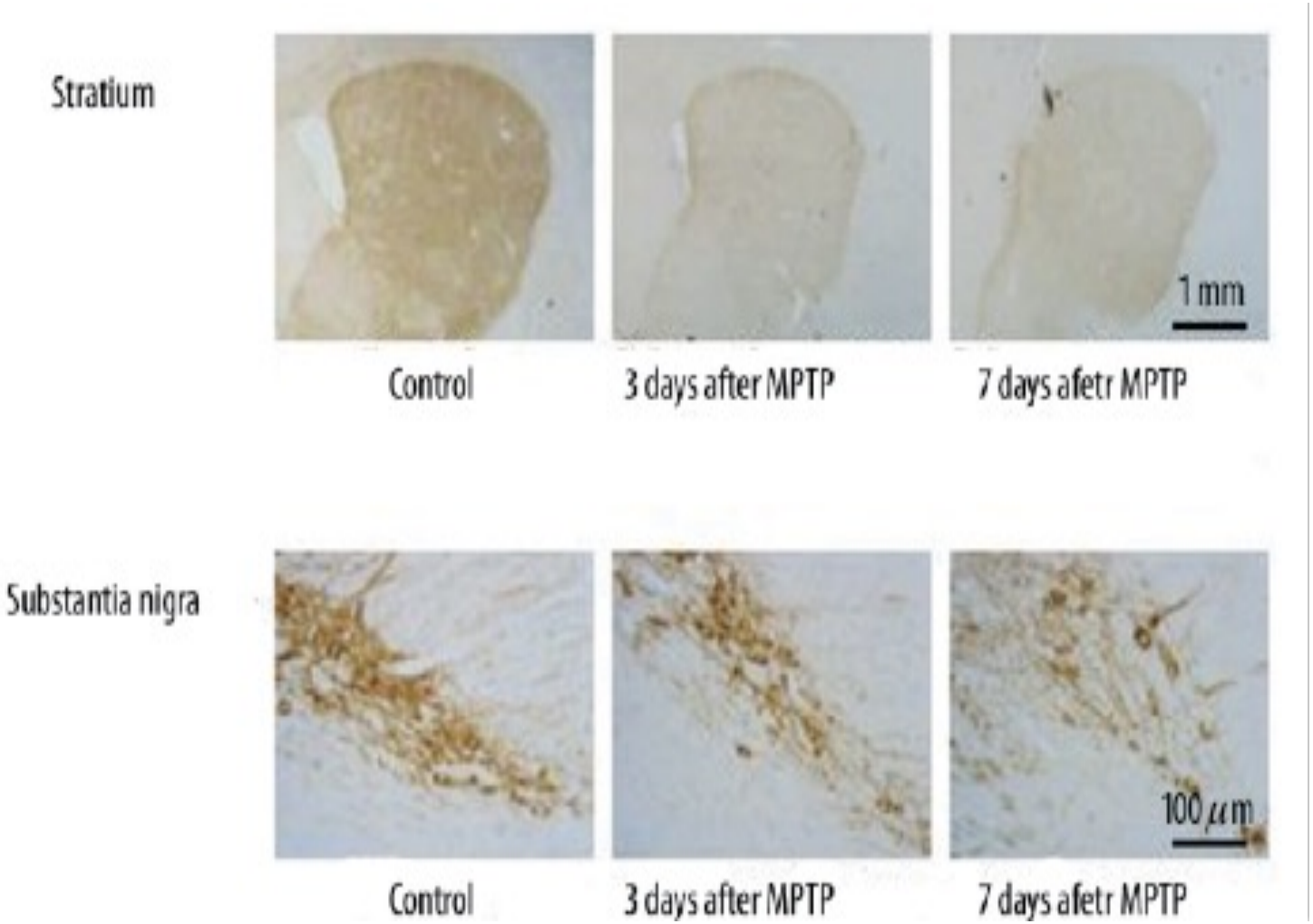
Parkinson's disease: Etiology - environmental



Parkinson's disease: MPTP Model

Model	Symptoms induced	Pathology	Favorite applications	Disadvantages
MPTP	Akinesia, rigidity and tremor (not in rodents)	Loss of Striatal DA-levels	**Tests for neuroprotective and neuro-restorative treatments**	**Acute damage of the DAergic system, non-progressive rare generation of inclusion bodies**
		Striatal TH-ir fibers		
		Nigral TH-ir neurons		
		-Synuclein aggregation (non fibrillar)		

Parkinson's disease: MPTP Model



D' après Watanabe et al., 2005

Parkinson's disease: MPTP Model

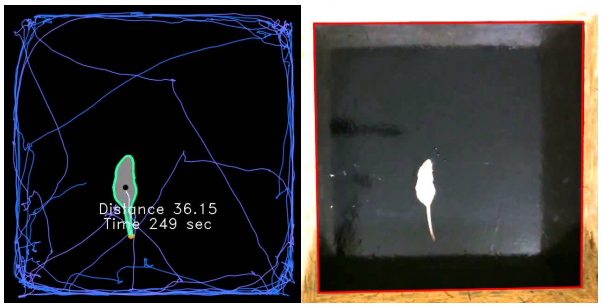
- Expensive Behavioral / Electrophysiological Experiments in Monkeys …
- Mouse: constraint effect and resistance to toxins

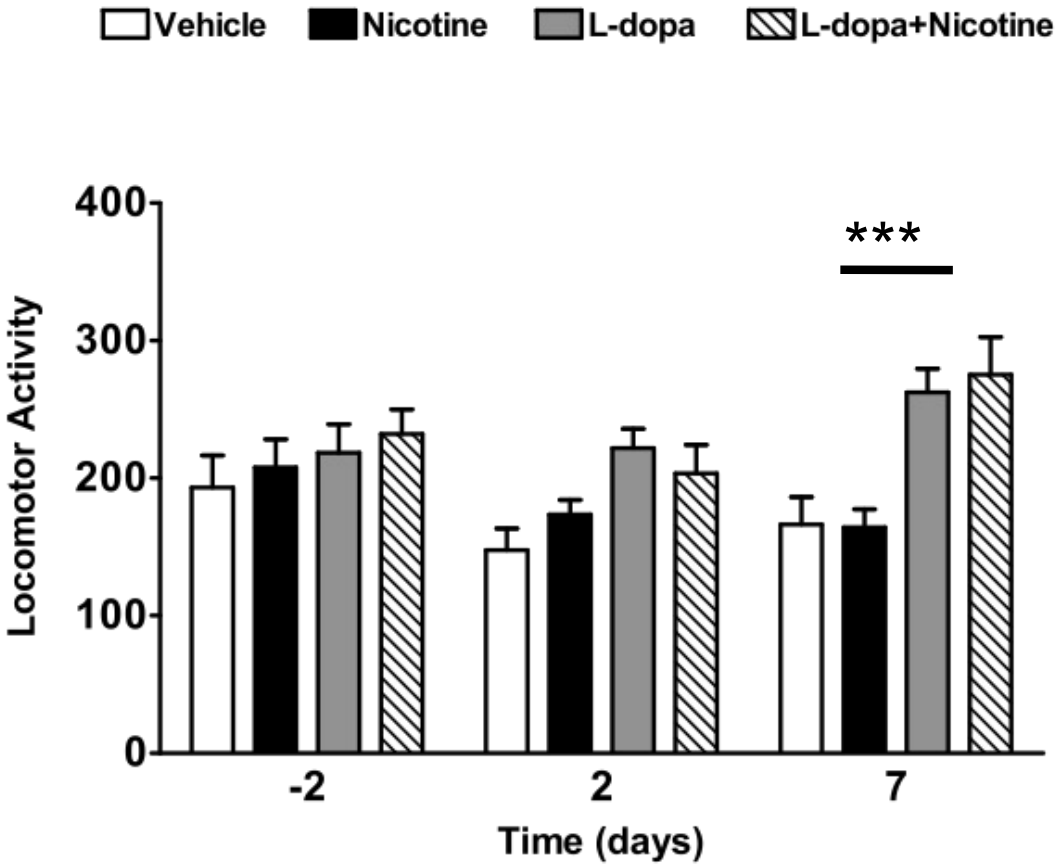
	Swiss mice		*C57 black mice*	
	Control	*MPTP*	*Control*	*MPTP*
Dopamine content (ng g^{-1} tissue)	18,253 ± 1086	17,143 ± 1406	19,242 ± 1209	9869 ± 624*
Locomotor activity (counts/5 min)	153 ± 11	142 ± 9	149 ± 10	117 ± 7*

Parkinson's disease: Behavioral analysis

- Locomotor activity
- Stepping Test
- Limb-Use Test
- Vibrissae Test
- Rotarod?
- Elevated body swing test

Parkinson's disease: Locomotor activity





Parkinson's disease: Limb Use Test in the 6-OHDA model

Rat model of Parkinson's Disease

Severe unilateral loss of nigrostriatal dopamine terminals in RIGHT hemisphere

Cylinder Test - Impaired use of LEFT (contralateral) forelimb for initiating weight-shifting movements during vertical/lateral exploration. Preferential use of right (ipsilateral) forelimb. Level of DA terminal loss is correlated with percent use of ipsilateral forelimb, relative to independent use of the contralateral forelimb and to simultaneous or alternating use of both forelimbs. Left hindlimb stepping is also impaired.

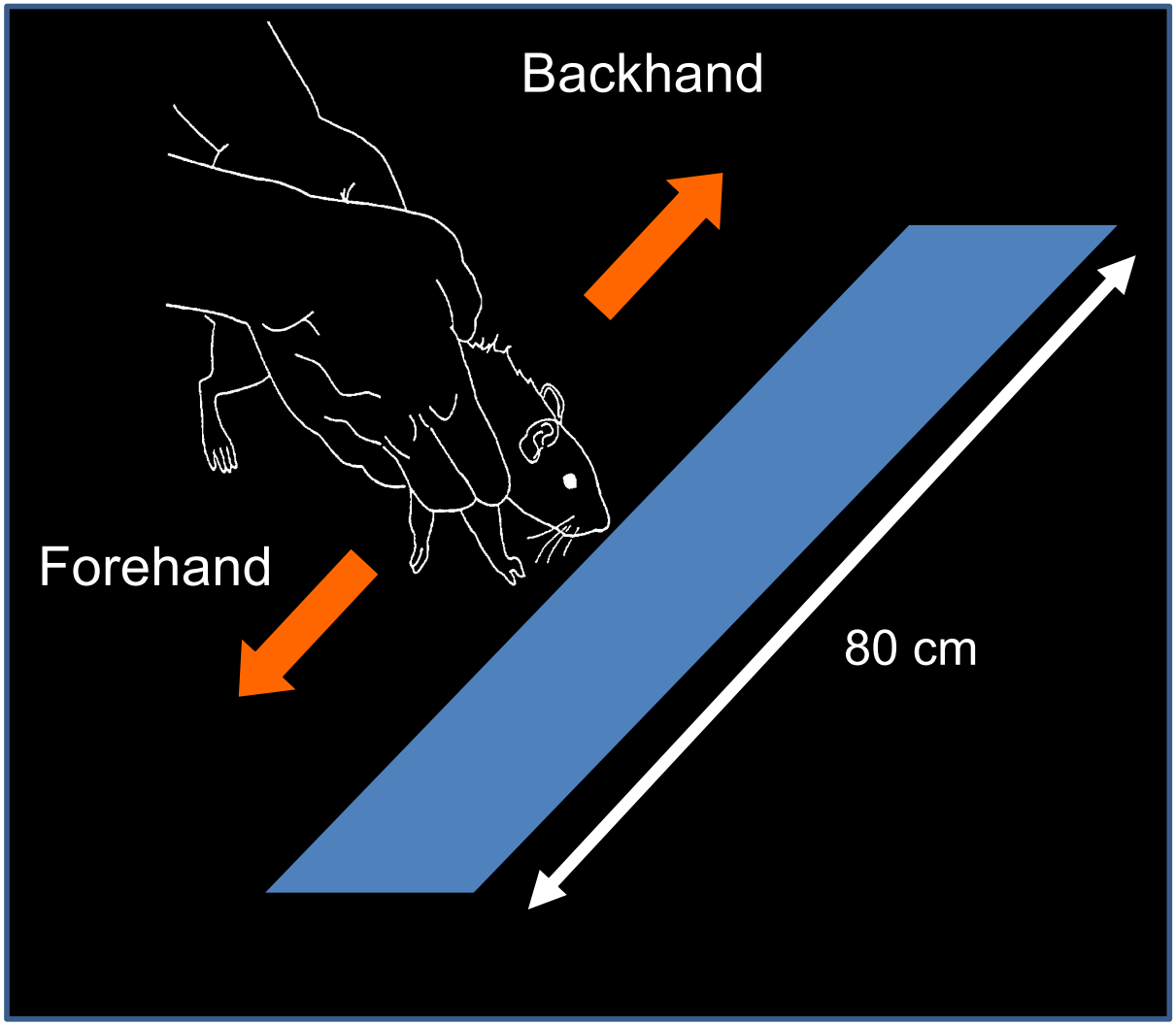
(Lundblad et al., 2002)

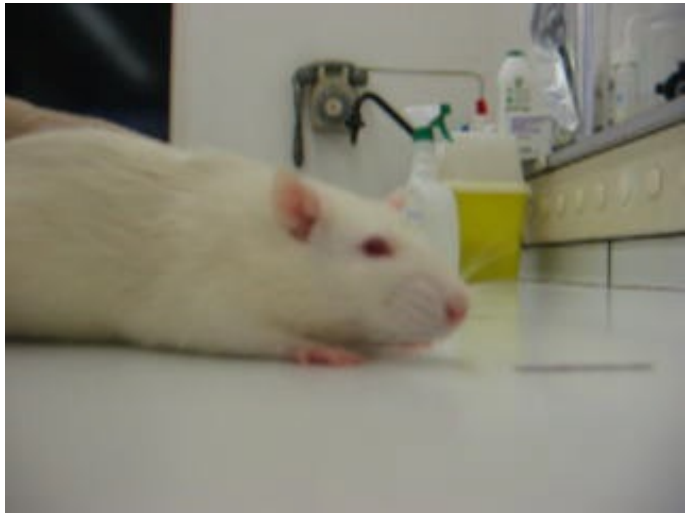
Parkinson's disease: Limb Use Test in the 6-OHDA model

Items measured

- Number of contacts of the impaired paw
- Number of contacts of the intact paw
- Number of contact of both paws
- Numbers of turns

Parkinson's disease: The Forelimb stepping Test (Olsson et al., 1995) in the 6-OHDA model





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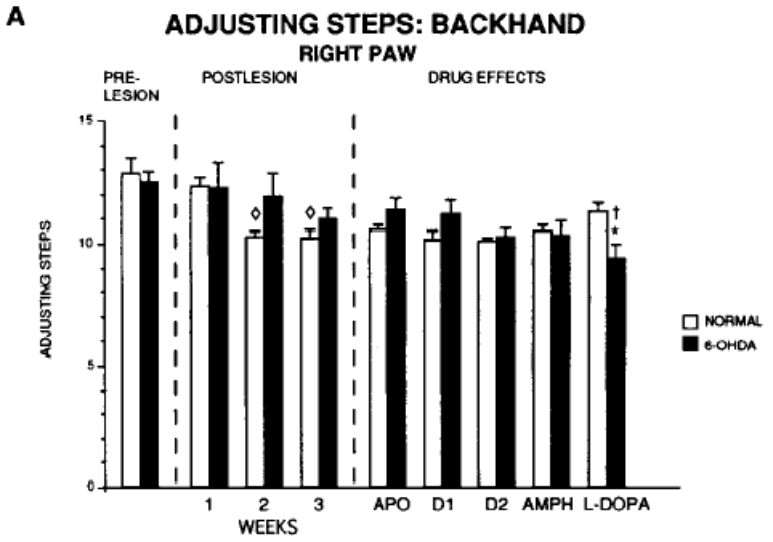
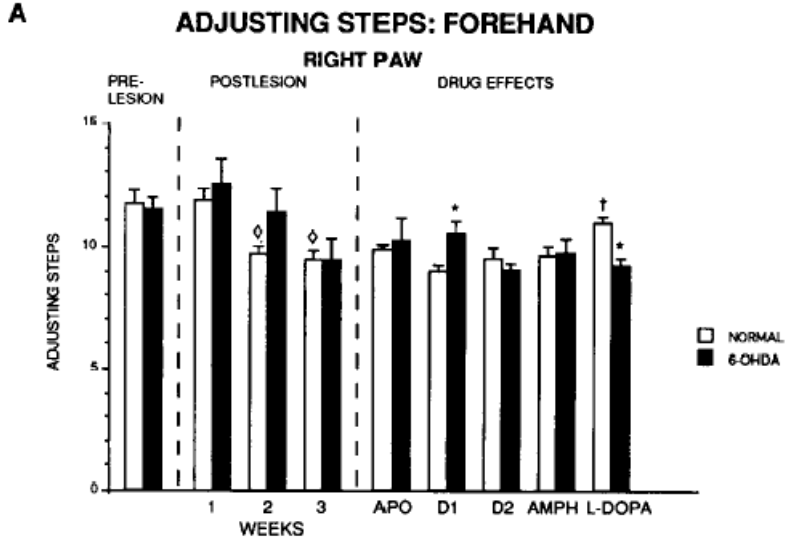
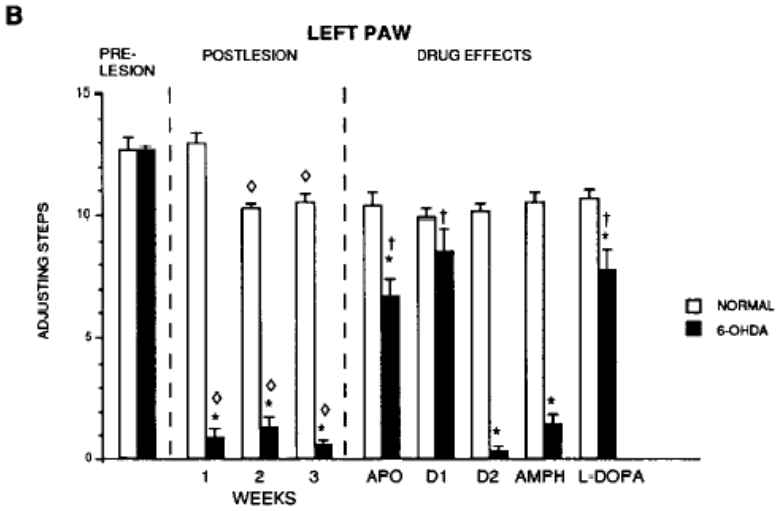
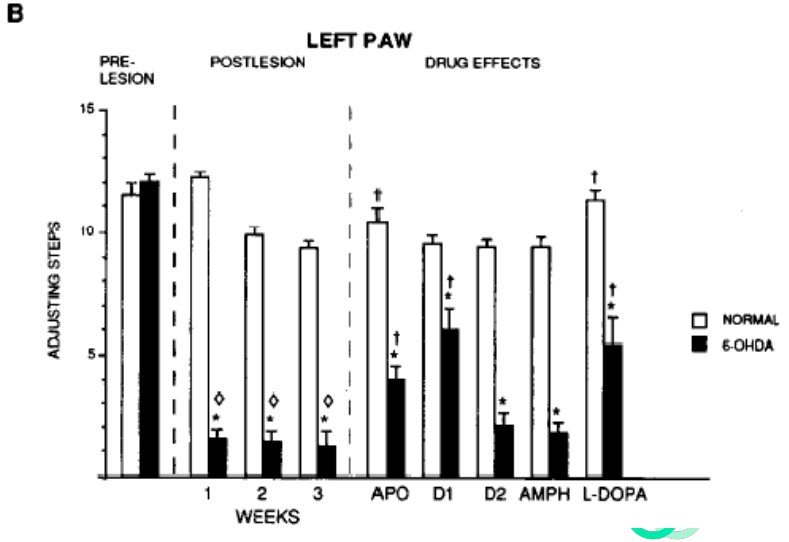
Parkinson's disease: The Forelimb stepping Test (Olsson et al. 1995) in the 6-OHDA model









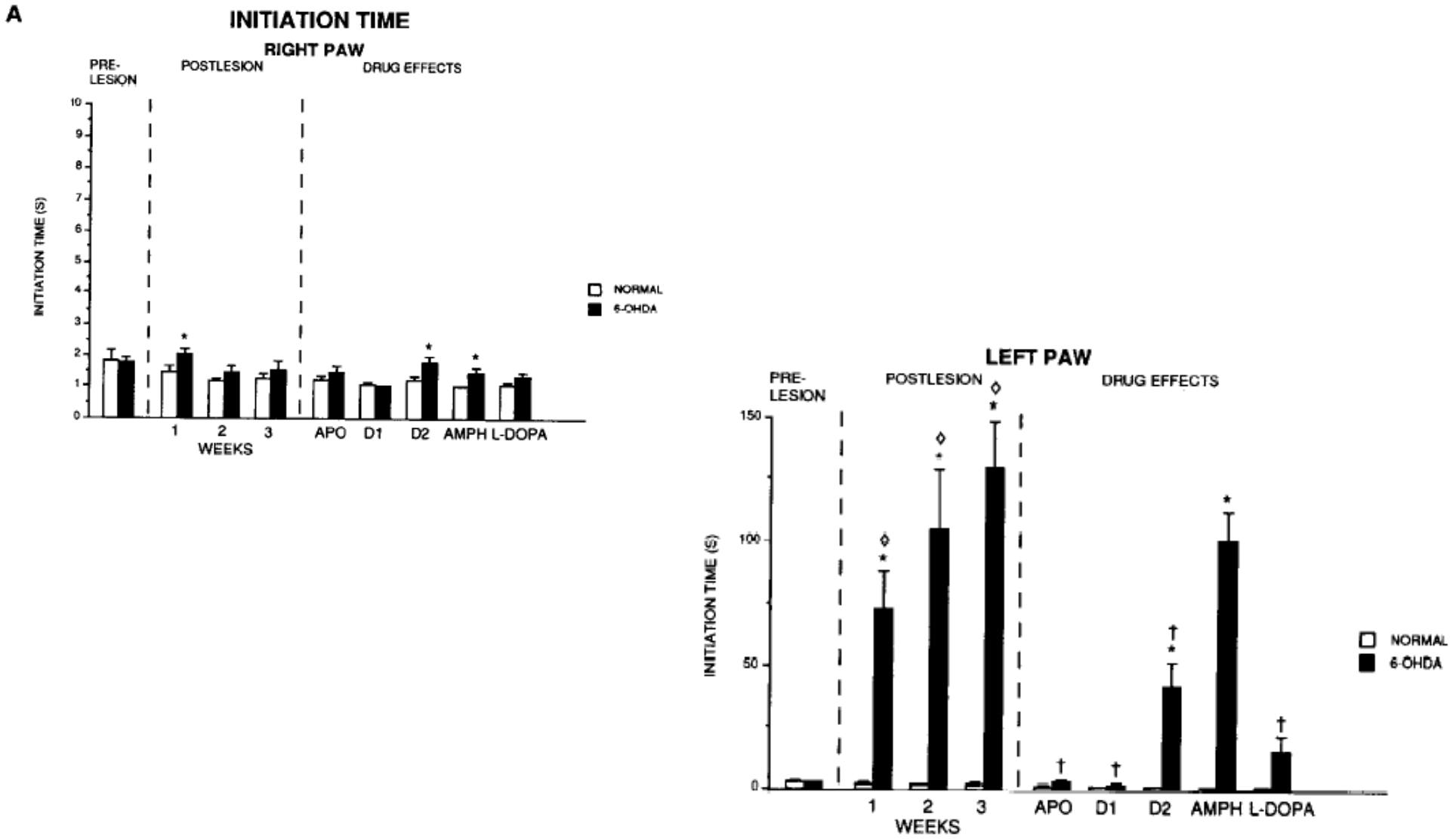
Parkinson's disease: Akinesia Test **in the 6-OHDA model**

Rat model of Parkinson's Disease

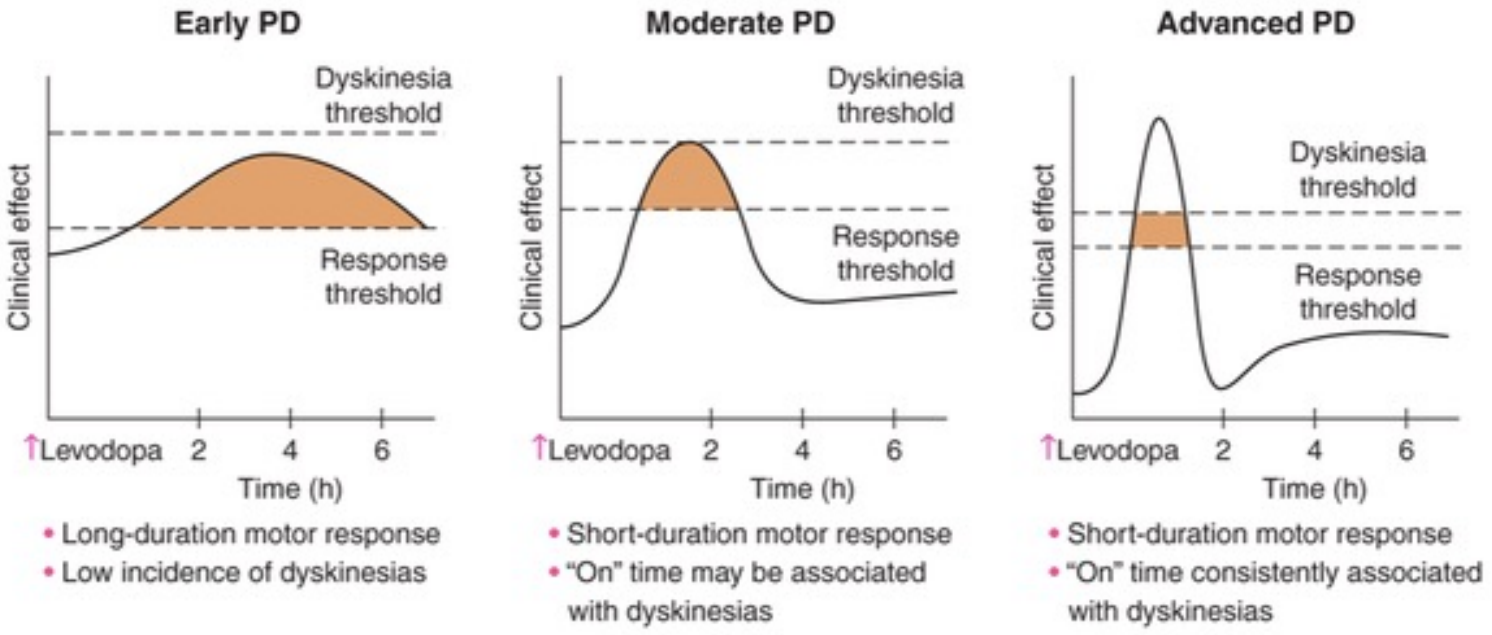
Severe unilateral loss of nigrostriatal dopamine terminals in RIGHT hemisphere

Akinesia Test - Movement initiation deficit in the LEFT (contralateral), but not the right, forelimb.
(1.6 years after right hemisphere DA depletion)

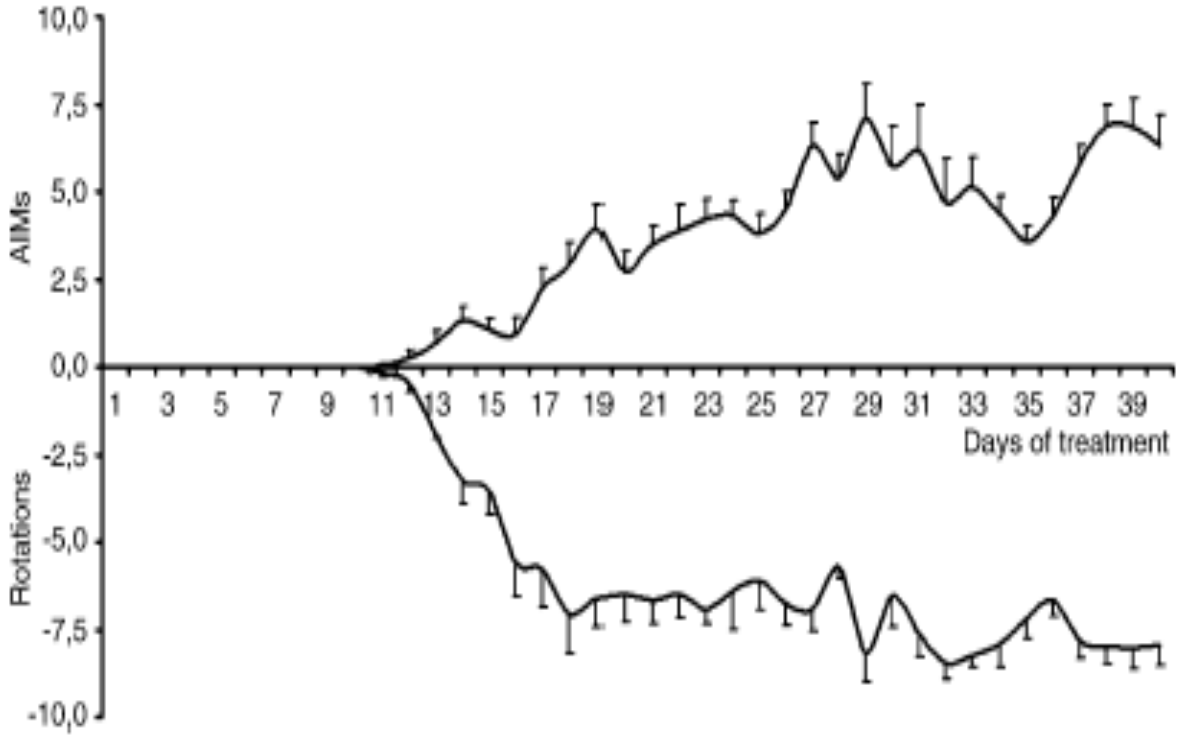
Parkinson's disease: Akinesia Test **in the 6-OHDA model**



Parkinson's disease: Levodopa induced Dyskinesia



Parkinson's disease: Levodopa induced Dyskinesia



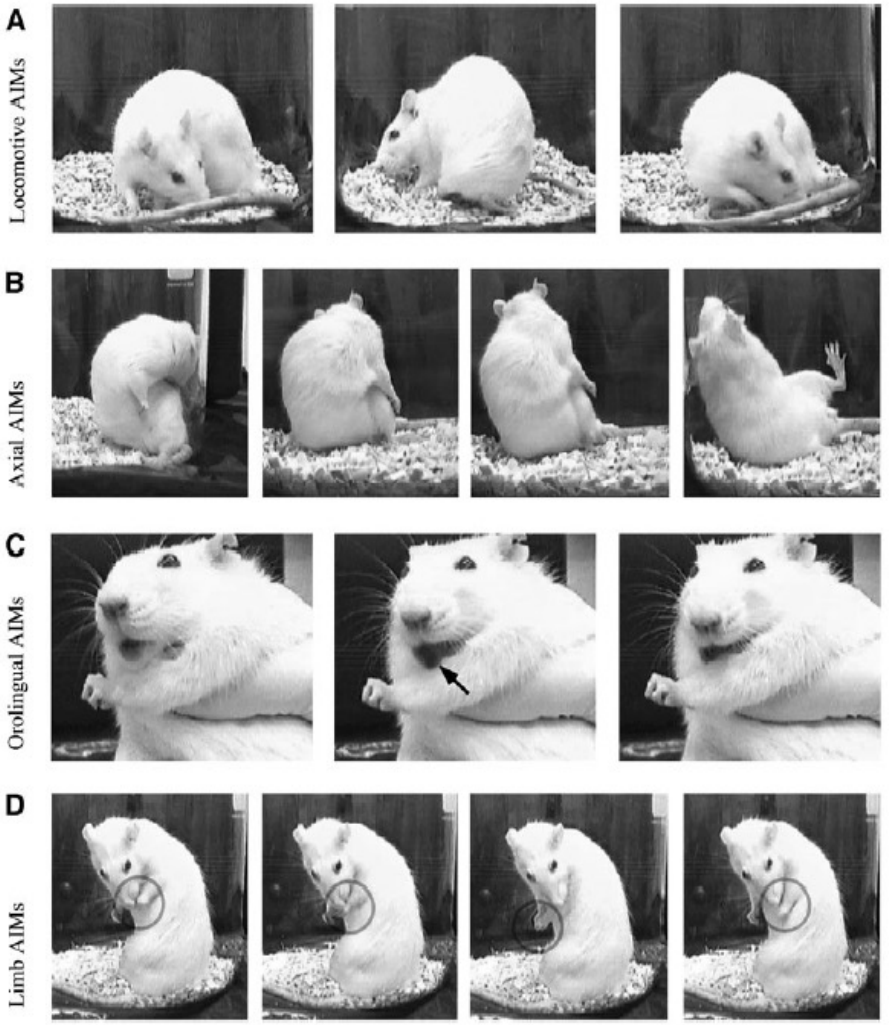
From Konitsiotis et al., 2006

Parkinson's disease: Levodopa-induced dyskinesia in the 6-OHDA model

One of the main uses of the 6-OHDA model is to be able to work on dyskinesias ("Abnormal Involuntary Movements") induced by levodopa.

In Rats

- Locomotive AIMs (rotations++)
- Axial AIMs (posture)
- Orolonguals AIMs (self-licking)
- Limb AIMs



Parkinson's disease: Levodopa-induced dyskinesia in the 6-OHDA model

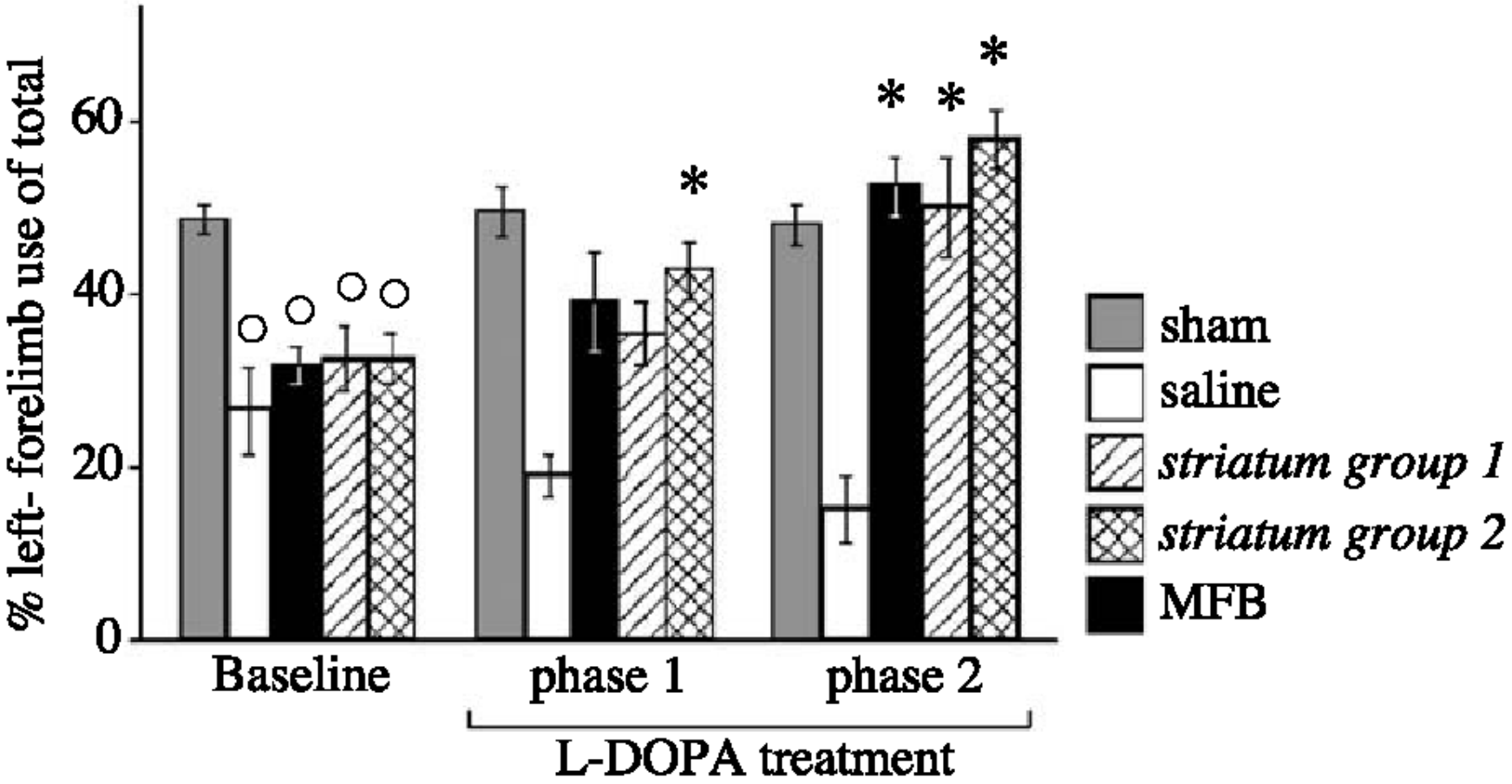
Key elements

- There is no « single » or « best » model for Parkinson Disease

- The selection of the model depends on :
  - Your question,
  - Your hypothesis,
  - The validity of the model
  - Your methodology

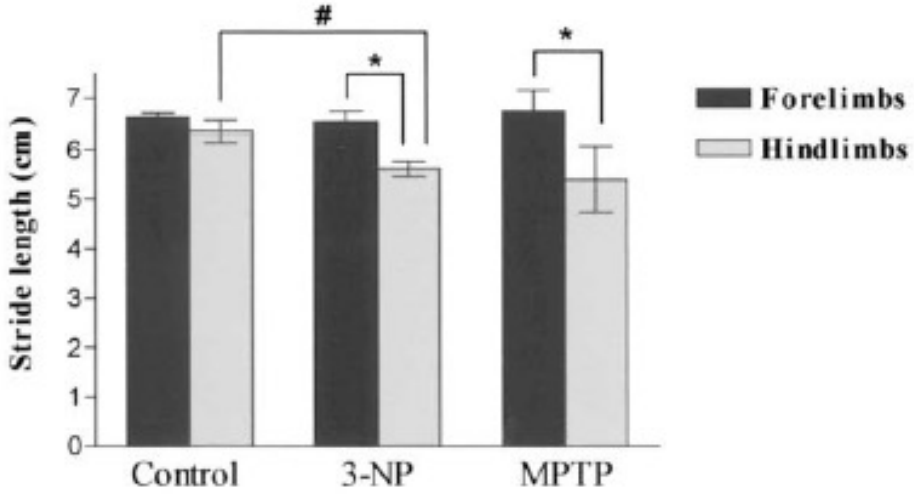
Parkinson's disease: 6-OHDA Model in mice

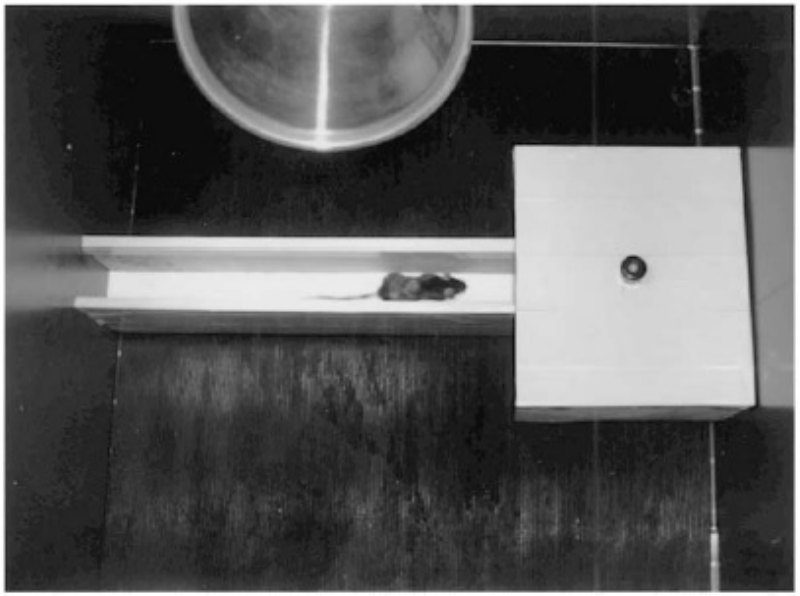
Parkinson's disease: 6-OHDA Model in mice

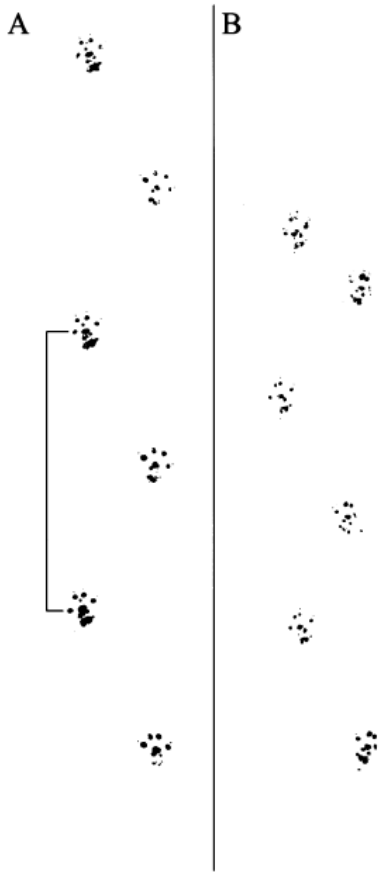


From Lundblad et al., 2002

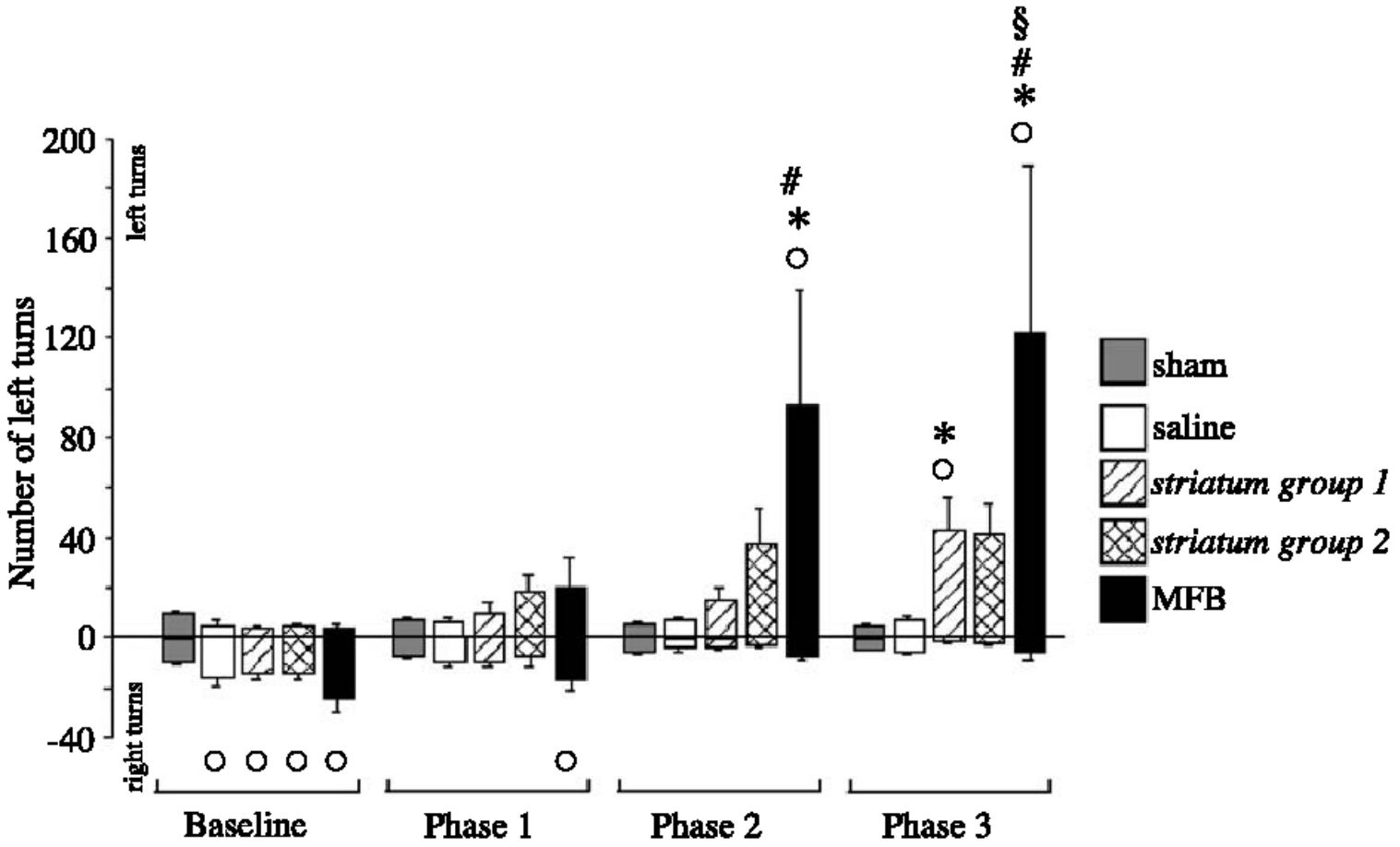
Parkinson's disease: 6-OHDA Model in mice**(Fernagut et al., 2002)**



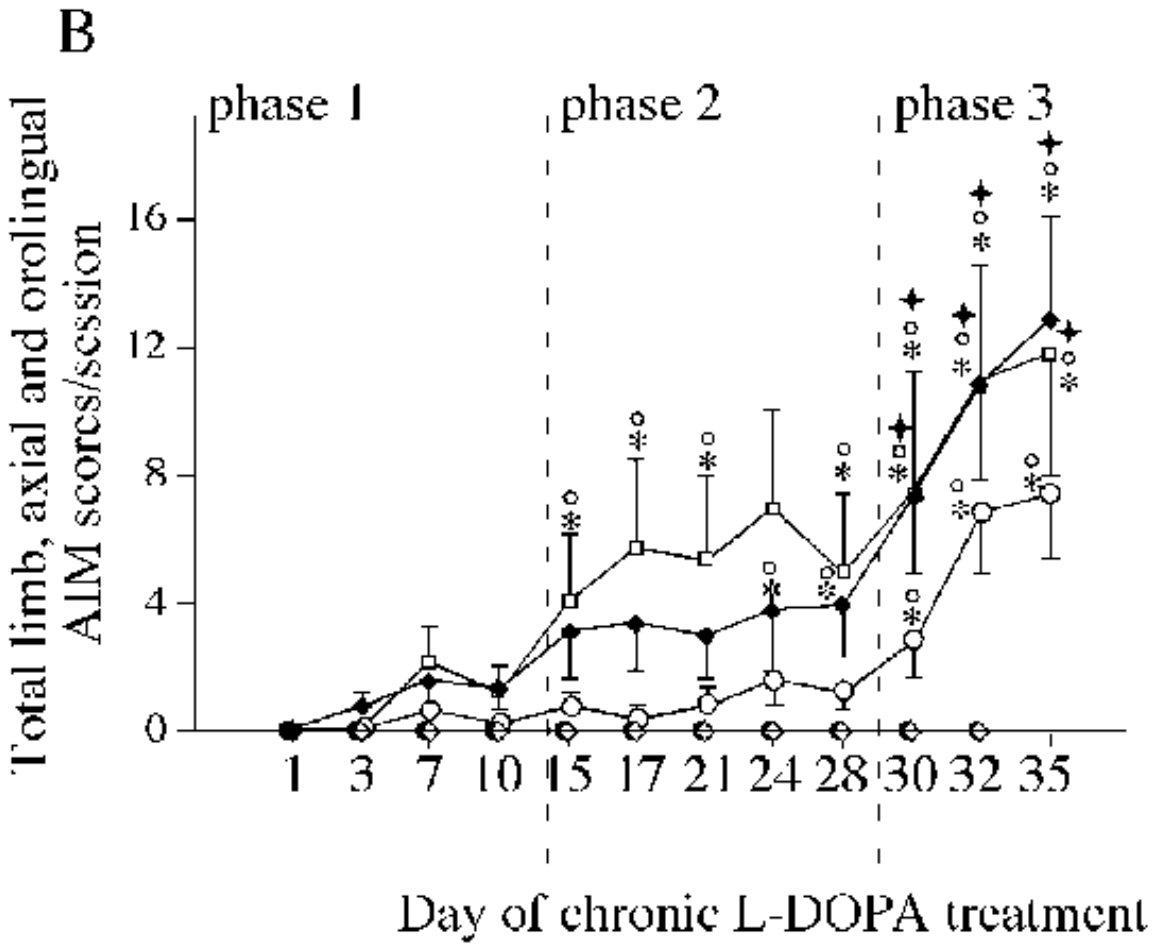




Parkinson's disease: 6-OHDA model – LID



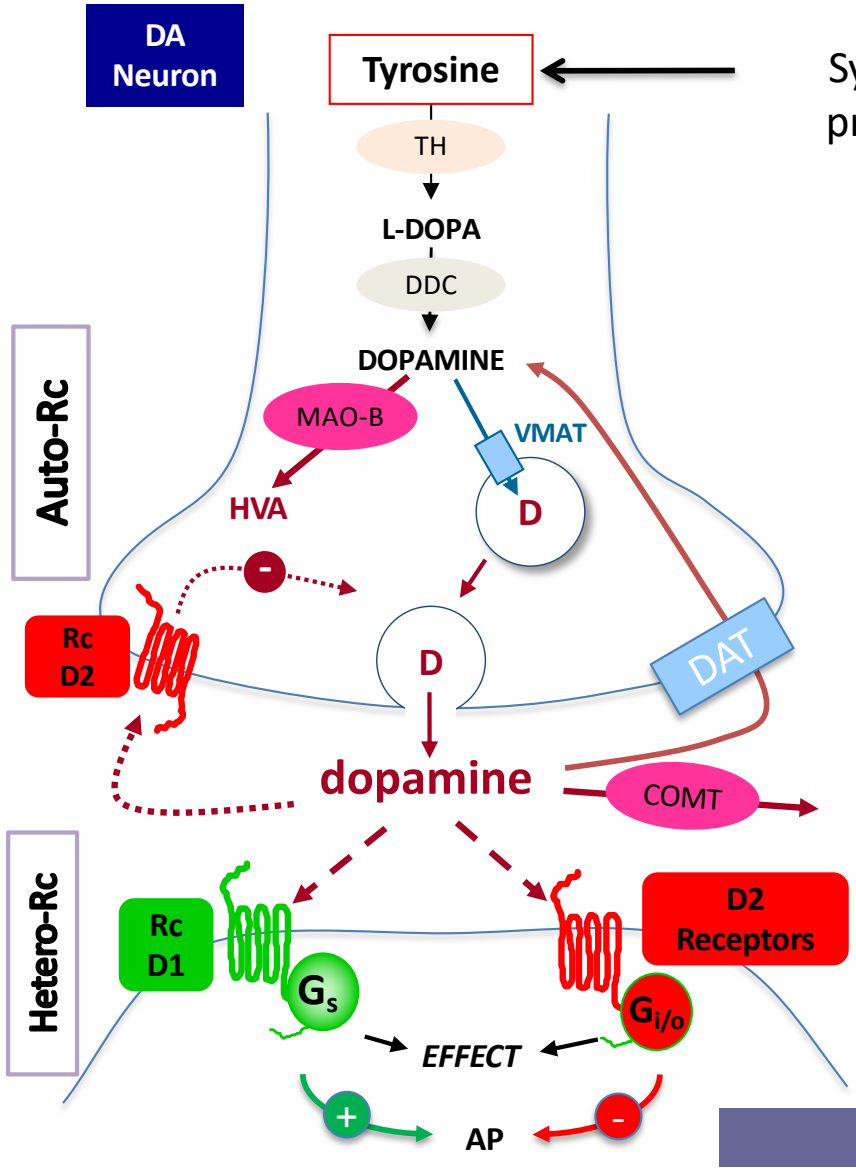
Parkinson's disease: 6-OHDA model – LID



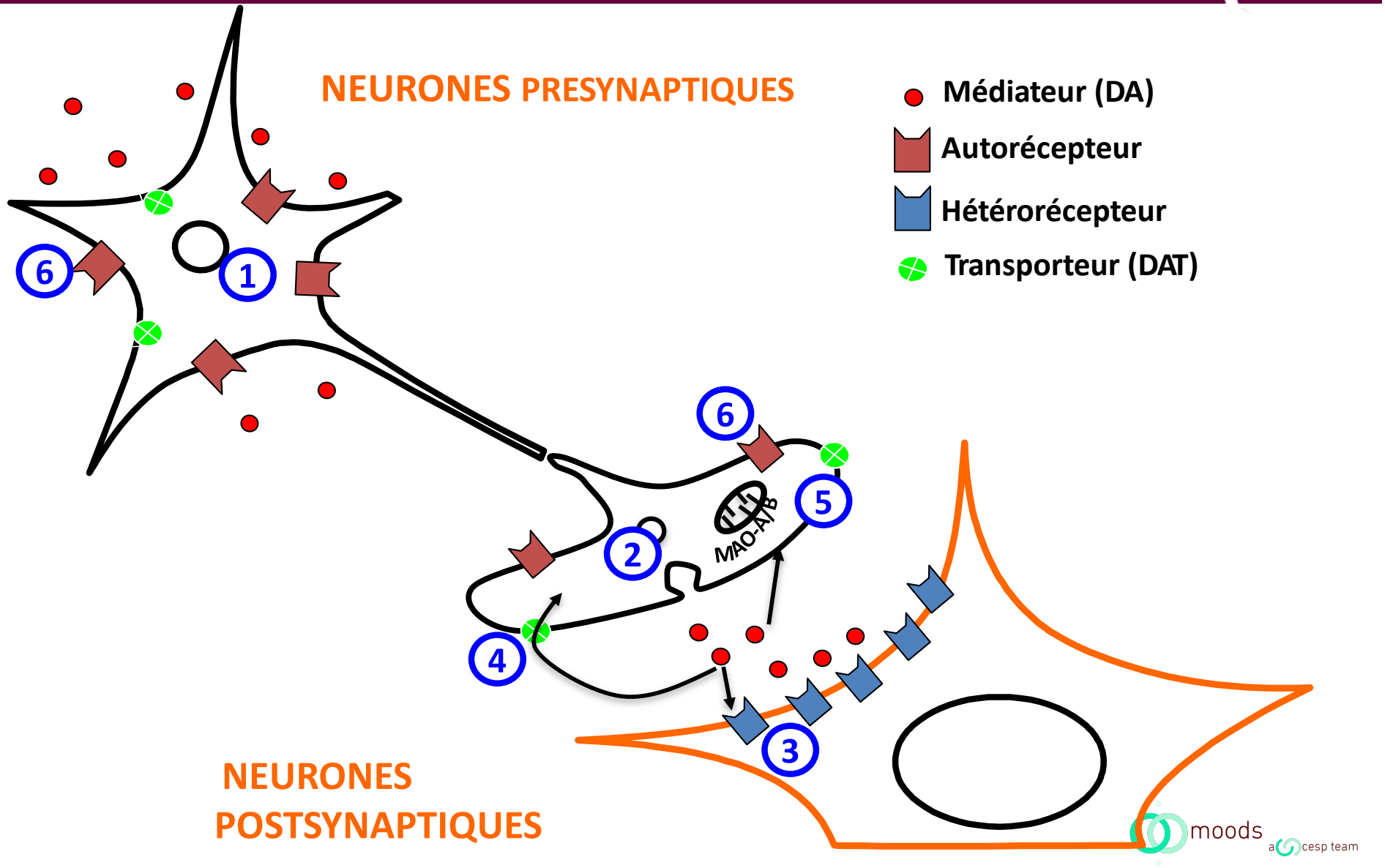
Maladie de Parkinson : le modèle MPTP

- Rotations:
  - Induites par apomorphine
  - Induites par amphétamines
- Activité Locomotrice
- Stepping Test
- Limb-Use Test
- Vibrissae Test
- Rotarod?
- Elevated body swing test
- Stride Length Test

Dopamin Synapse



Stratégies de modulation de la neurotransmission DA



Parkinson's disease: Treatments

Molecule	Mechanism of action
L-dopa (+carbidopa et benzerazide)	1)Increase in synthesis
Bromocriptine	3) DA Receptor stimulation
Ropinirole	
Rotigotine	
Pramipexole	
Bupropion	5) Reuptake inhibitor (DAT)
Selegiline	5) Enzyme inhibition (IMAO-B)
Safinamide	5) Enzyme inhibition (IMAO-B)
Capones	5) Enzyme inhibition (COMT)

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Parkinson's disease: L-Dopa

✓Treatment of reference (300-1200mg/d)

✓Short plasmatic half-life (1,5 - 3h)

✓ The surviving neurons that dampen this kinetics

✓ By their capacity for endogenous storage and secretion of DA

✓ When neuronal loss becomes critical, dopathotherapy alone can no longer ensure stable DA stimulation

✓ Motor state of the patient then tends to follow the evolution of the plasma concentration of L-DOPA, and new motor disorders appear (motor fluctuation, dyskinesia)

Parkinson's disease: L-Dopa