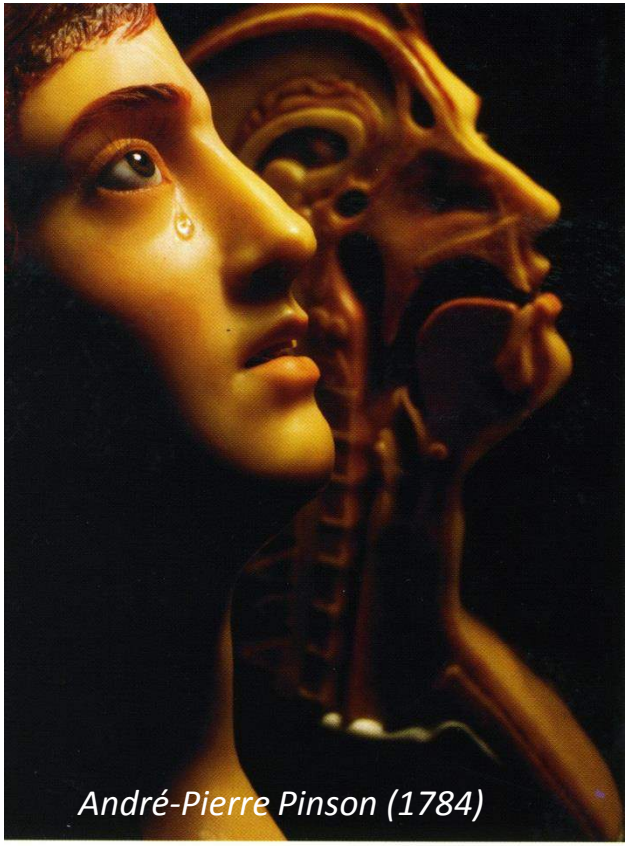
OMICS International

International Conference on
Parkinsons Disease & Movement Disorders
August 11-13, 2015 Frankfurt, Germany


André-Pierre Pinson (1784)

ADVANCED METHODOLOGY FOR THE DEVELOPMENT OF PRECLINICAL DIAGNOSTICS OF PARKINSON'S DISEASE

M.V. Ugrumov

Institute of Developmental Biology RAS, Moscow, Russia

Neurologic and mental diseases: an epidemy of the XXI century

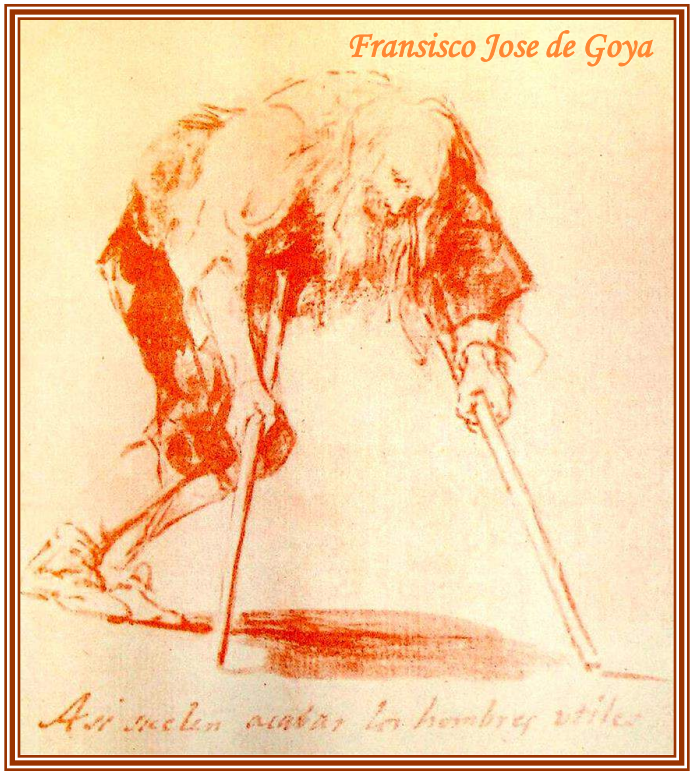
Actual prevalence of socially important diseases:

- ✓ **Cardiovascular**
- ✓ Oncological
- ✓ Neurological and mental

Predicted prevalence of socially important diseases:

- ✓ **Neurological and mental**
- ✓ Cardiovascular
- ✓ Oncological

Disease	2009	2040
Parkinson's disease	**16 MM**	**32 MM**
Alzheimer's disease	25 MM	75 MM
Depression		



$ 10 000 - 38 000
per patient per year

Every day, millions of American families experience the difficult reality of Alzheimer's disease. The physical and emotional demands of caring for a loved one with Alzheimer's can be overwhelming, but no one should face this disease alone. Doctors are now able to start treatments earlier, slowing the loss of brain cells and the progression of debilitating physical and mental impairments.

Barack OBAMA

La maladie d'Alzheimer qui touche aujourd'hui en France 860 000 personnes est un drame pour notre société, un drame familial. Chaque année ce sont 225 000 nouveaux cas… . Dans les années a venir, les coûts humains et financiers de la maladie, qui sont d'ores et déjà élevés tant pour les familles que pour la société, vont encore s'alourdir ..."

Nicolas SARKOZY

Advanced methodology for the development of preclinical diagnostics of Parkinson's disease

- **Introduction. Parkinson's disease: New insight into the pathogenesis, diagnostics and treatment;**

  Methodology for the development of preclinical diagnostics of Parkinson's disease;

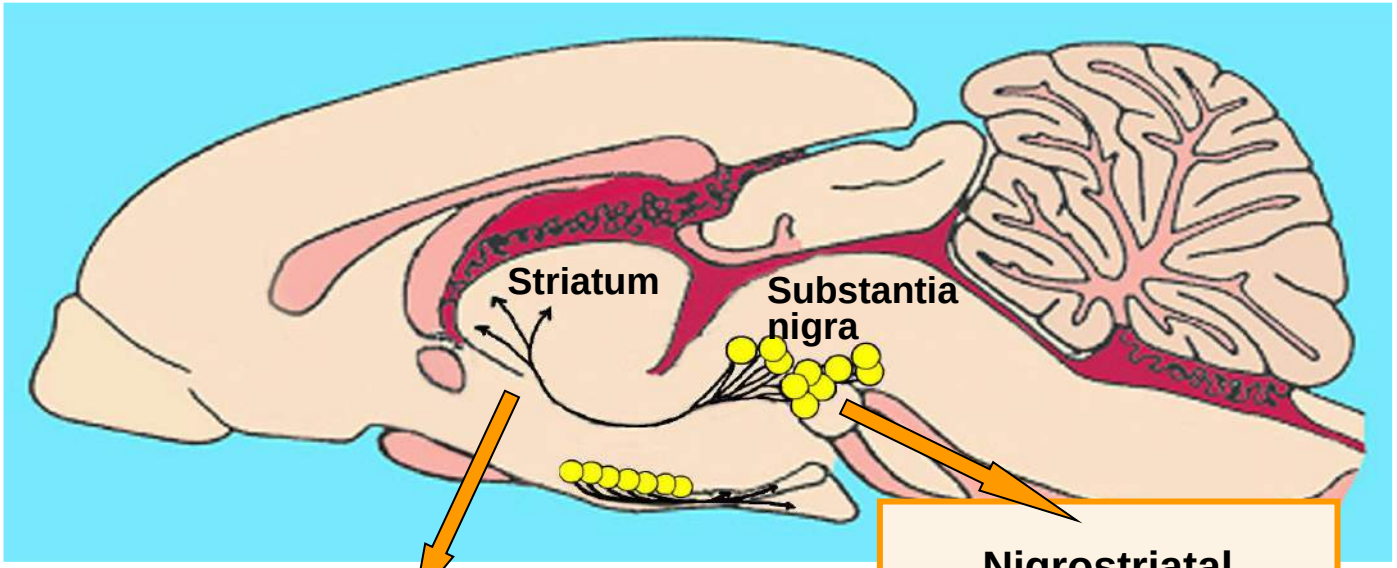
  Search for peripheral biomarkers in untreated PD patients at the early clinical stage;

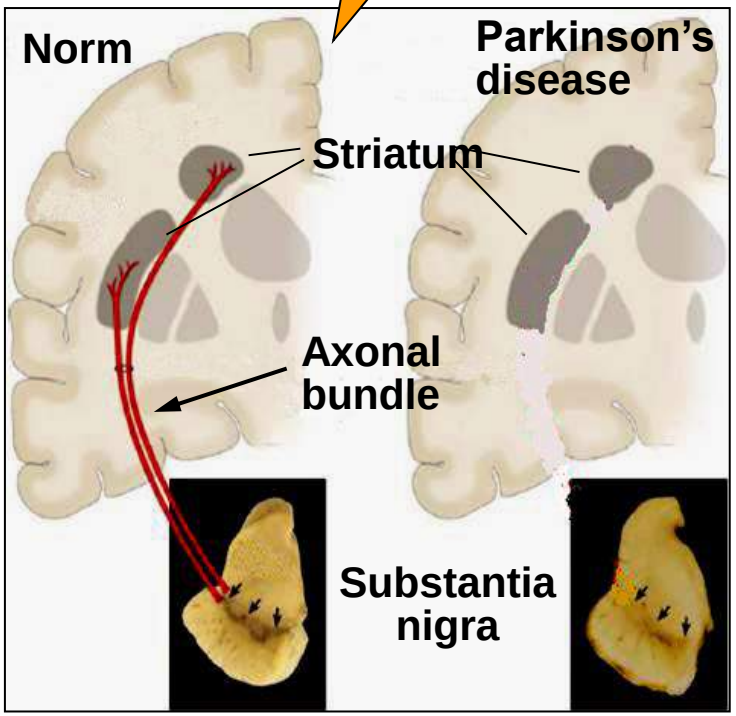
  Experimental modeling of Parkinson's disease at the preclinical stage for a search peripheral biomarkers;

  Provocation test as a novel approach to the diagnostic of Parkinson's disease;

  Conclusions and prospect.

Nigrostriatal dopaminergic system in norm and Parkinson's disease





Nigrostriatal system

Motor behavior

↓ Neuron degeneration

Parkinson's disease

Parkinson's disease :

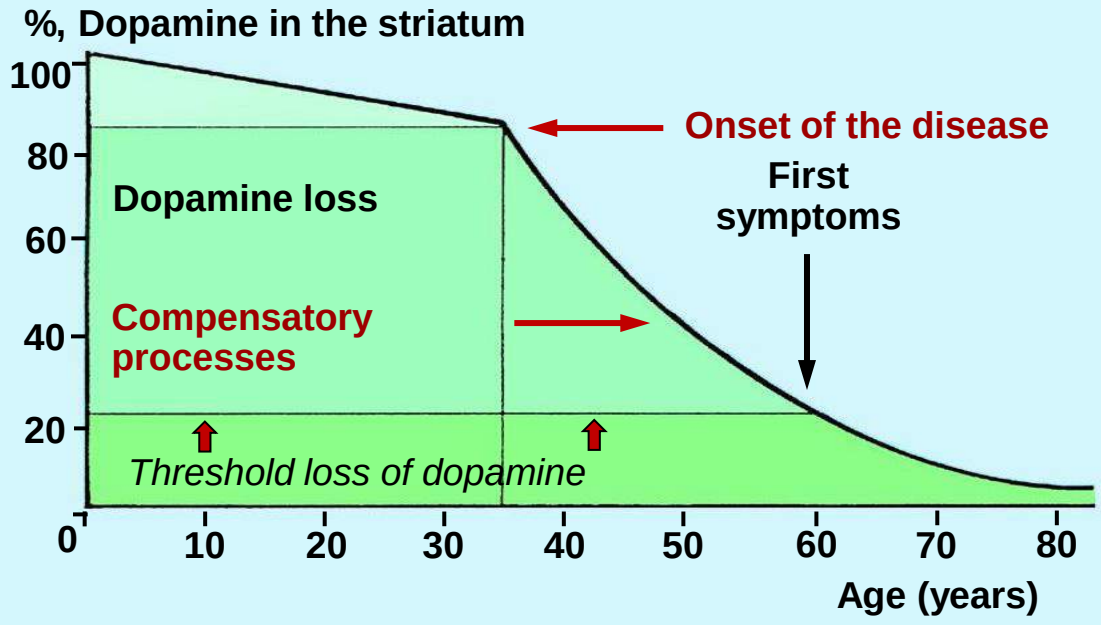
Etiology, pathogenesis, diagnostics and treatment

Risk factors
- gene mutations:
α-synuclein, parkin, POMC, etc.

- ✓ Impairment of the metabolism of functionally important proteins;
- ✓ Production of non-specific neurotoxins (*α-synuclein, tau);*
- ✓ Production of specific neurotoxins *(N-methyl(R)salsolinol)*

Pathogenesis:

- Neurodegeneration;
- Prion-like propagation;
- Neuroplasticity;
- Inflammation



Objectives are to develop:

- ✓ Preclinical diagnostics;
- ✓ Preventive treatment.

None of PD patients had been cured **!**

Bezard and Gross, 1998. *Prog. Neurobiol. 55, 93-116*; *Trends Neurosci. 26, 215-221;*.
Ugrumov, M.V., 2008. In: Lajtha, A. (Ed.) *Handbook of Neurochemistry and Molecular Neurobiology, Vizi, E.S. (Vol. Ed.) Neurotransmitter Systems,* Springer, Heidelberg, pp. 21-73;

Advanced methodology for the development of preclinical diagnostics of Parkinson's disease

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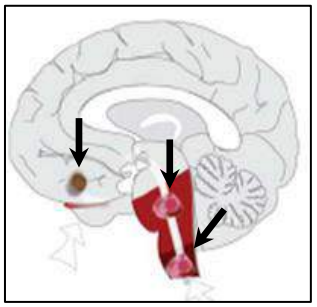
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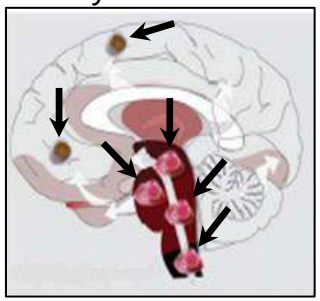
Methodology of the development of preclinical diagnostics of Parkinson's disease

Stages by Braak:

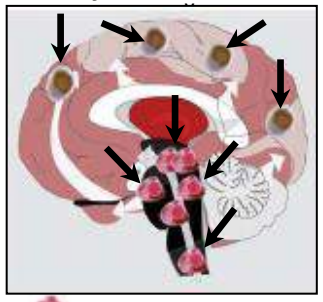
Impairment of peripheral and olfactory functions



Motor dysfunctions



Cognitive dysfunctions



Lewy bodies

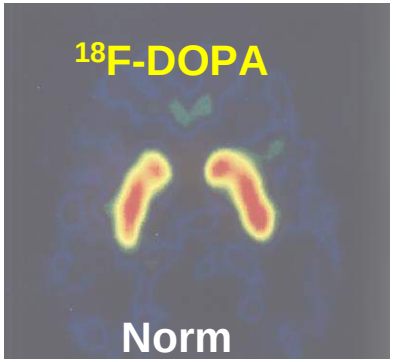
Idiopathic Parkinson's disease (syndrome) is polygenic and systemic

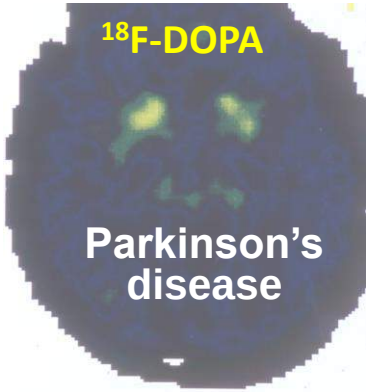
Prophylactic examination of healthy population

Risk group:
Selection of people according to preclinical peripheral biomarkers

Positron emission tomography:
Molecular markers of the failure of specific neurons

Preventive treatment





DeKosky S., Marek K., *2003. Science 302, 830-834*; Ugrumov M.V., 2008. *Handbook of Neurochemistry and Molecular Neurobiology , Neurotransmitter Systems (S. Vizi, eds.), Springer, NY, p. 21-73*.

Parkinson's disease:
Systemic neurodegeneration and non-motor manifestations

Time

Neurodegeneration in the brain

Chronology of spreading:

- Dorsal motor nucleus of vagus
- Anterior olfactory nucleus
- Laterointermediate vertebral column
- Locus coeruleus
- ***Substantia nigra***
- Basal forebrain (hypothalamus, etc.)
- Neocortex (limbic cortex, frontal and temporal cortex – multimodal association areas

Non-motor symptoms at the preclinical stage

- Deterioration of handwriting
- Depression
- Constipation
- Dysosmia
- Thermoregulation disturbance
- Decrease of sexual potency, etc.

Neurodegeneration in the periphery

Neurons innervating:

- ✓ Heart
- ✓ Gastrointestinal tract
- ✓ Salivary glands
- ✓ Adrenals, etc.

Biomarkers in body fluids (CSF, blood) at the preclinical stage

- Pathologic proteins (α-synuclein, etc.)
- Neurotransmitters and metabolites
- Hormones
- Blood cells
- Markers of oxidative stress

Goldstein et al., 2000. *Ann. Itern. Med*. 133, 338-347; Kloss et al., 2006. *Neurology* 66, 1100-1102; Braak et al., 2006. *Neurosci. Lett*. 396, 67-72; Goldstein et al., 2009. *Parkinson Relat. Disord., 15, 516-520*..

Development of preclinical diagnostics of Parkinson's disease

Current methodology:

✓ Finding of ***peripheral biomarkers*** of malfunctioning of the brain and peripheral organs as well as of compensatory processes in patients at the early clinical stage of Parkinson's disease (PD);

Problems:

✓ Inability to diagnose PD at the preclinical stage, and therefore a search in reality for peripheral biomarkers in untreated patients on the early clinical stage;

✓ *A priori* ***a lack of specific peripheral biomarkers*** of the malfunctioning of the brain and peripheral organs at PD.

Advanced methodology

A search for peripheral biomarkers in untreated PD patients at the early clinical stage:

- Evaluation of the medical history;
- Evaluation of functional activity of peripheral organs;
- Search for biomarkers in body fluids (CSF, plasma);
- Evaluation of gene expressions in blood cells.

The use of provocation tests, which:

- Temporarily enhance the latent failure of the nigrostriatal dopaminergic system;
- Induce the reversible appearance of motor disorders.

A search for peripheral biomarkers in experimental models of PD at preclinical stage:

- Evaluation of different types of behavior;
- Evaluation of functional activity of peripheral organs;
- Search for biomarkers in plasma;
- Evaluation of gene expression in blood cells.

Advanced methodology for the development of preclinical diagnostics of Parkinson's disease

Introduction. Parkinson's disease: New insight into the pathogenesis, diagnostics and treatment;

Methodology for the development of preclinical diagnostics of Parkinson's disease;

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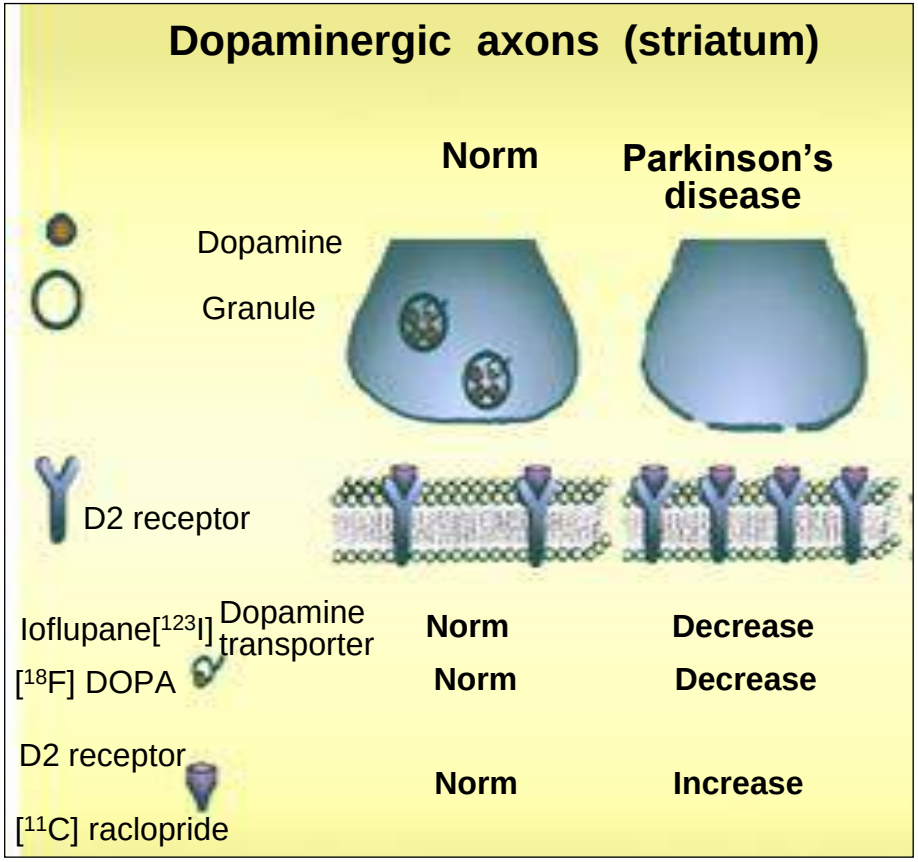
Experimental modeling of Parkinson's disease at the preclinical stage for a search peripheral biomarkers;

Provocation test as a novel approach to the diagnostic of Parkinson's disease;

Conclusions and prospect.

Biomarkers of preclinical stage of Parkinson's disease: *Body fluids*

Marker	CSF	Blood (plasma/cells)
DOPAC, L-DOPA	↓	
α-synuclein, parkin	↓	↑
Malonic dialdehyde (*oxidative stress*)		↓
Dopamine transporter		↓ Lymphocytes
MAO B		↑ Platelets
Tumor necrosis factor (*proinflammatory*)		↑



↑ Increase; ↓ Decrease

Goldstein 2012. *Brain, 135, 1900–1913;* Siderowf 2012. *Mov. Disord., 27, 608-616;* Shi et al., 2011. *Ann. Neurol., 69, 570-580.*

Untreated PD patients at the early clinical stage:
Catecholamines and hormones in blood

Substance	Clinical stage, tremor *(patients)*	Clinical stage, rigidity *(patients)*	Clinical stage *(patients, literature)*
Noradrenaline	↓	↓	→ Bouhaddi et al. 2004 ↓ Goldstein et al. 2003
Adrenaline	→	↓	→ Bouhaddi et al. 2004 ↓ Belikova et al. 2013
Dopamine	→	↓	→ Bouhaddi et al. 2004 ↓ Belikova et al. 2013
L-DOPA	↓	↓	No data
DOPAC	→	↓	No data
Prolactin	↓	↓	→ Murri et al. 1980; ↓ Laihinen et al. 1986
Cortisol	↑	↑	→ ↓↑ Laihinen et al. 1986; Swerdlof, 2012
Gene expression of D3 receptor	↓	↓	↓ Nagai et all., 1996

↑ Increase;
↓ Decrease;
→ No change;
— No data

Nigmatullina et al. in preparation



Is there a link between visual impairment and motor disorders

?



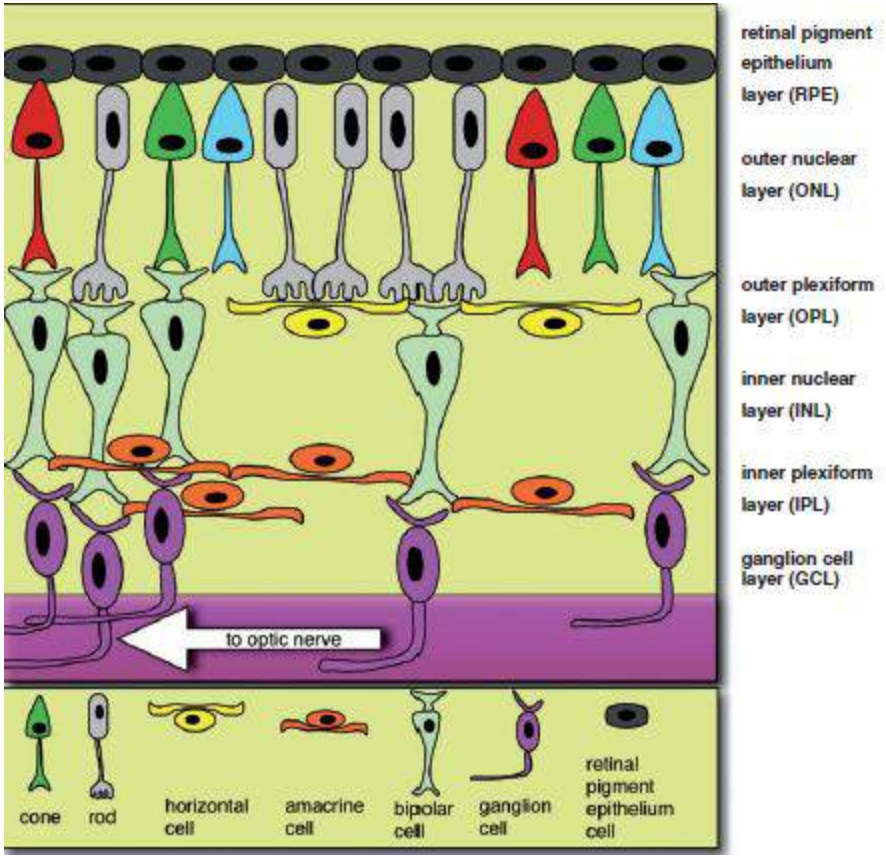
doi:10.1093/brain/awp068 Brain 2009: 132; 1128–1145

BRAIN
A JOURNAL OF NEUROLOGY

REVIEW ARTICLE

The retina in Parkinson's disease

Neil K. Archibald,[1] Michael P. Clarke,[2] Urs P. Mosimann[3] and David J. Burn[4]



Parkinson's disease

α-Synucleinopathy,
the degeneration of amacrine cells

↓

Dopamine deficiency

↓

Visual impairment

↓

Impairment of the accommodation of the eye

↑

Restoration at L-DOPA treatment

↓

Conclusions:

- Systemic nature of Parkinson's disease is manifested in the impaired vision at various levels of the visual analyzer - from the retina up to the cortex;
- Vision examination is a way for PD diagnosis at a preclinical stage.

Advanced methodology for the development of preclinical diagnostics of Parkinson's disease

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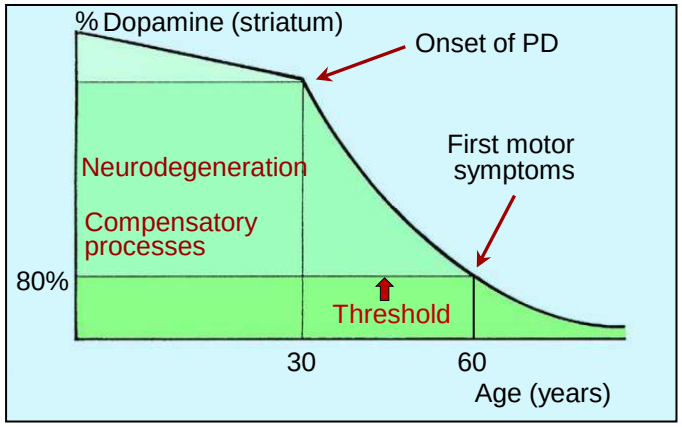
Search for peripheral biomarkers in untreated PD patients at the early clinical stage;

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Provocation test as a novel approach to the diagnostic of Parkinson's disease;

Conclusions and prospect.

Modeling of Parkinson's disease at the preclinical and early clinical stages



A threshold for appearance of motor disorders:

- Depletion of 70-80% dopamine in the striatum;
- A loss of 70-80% dopaminergic axons in the striatum;
- A loss of 50-60% dopaminergic neurons in the substantia nigra

Major requirements for modeling PD at the preclinical stage:

- A loss less than 70-80% of dopamine and dopaminergic axons in the striatum *(obligatory)*;
- A loss less than 50-60% of dopaminergic neurons (cell bodies) in the substantia nigra *(obligatory)*;
- An absence of motor disorders *(obligatory)*;
- Slow progressive degeneration of dopaminergic neurons for a long time until a threshold level of degradation of the dopaminergic system *(desirable);*
- α-Synuclein accumulation and aggregation *(not clear)*;
- Systemic degeneration of catecholaminergic neurons resulting in the impairment of central and visceral functions *(desirable)*.

Major requirements for modeling PD at the early clinical stage:

- A loss of 70-80% of dopamine and dopaminergic axons in the striatum *(obligatory)*;
- A loss of 50-60% of dopaminergic neurons (cell bodies) in the substantia nigra *(obligatory);*
- The onset of motor disorders *(obligatory)*;
- α-Synuclein accumulation and aggregation *(desirable);*
- Systemic degeneration of catecholaminergic neurons resulting in certain visceral dysfunctions *(desirable).*

Neurotoxic (MPTP) models of Parkinson's disease in mice: *Preclinical and early clinical stages*

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, pretoxin)

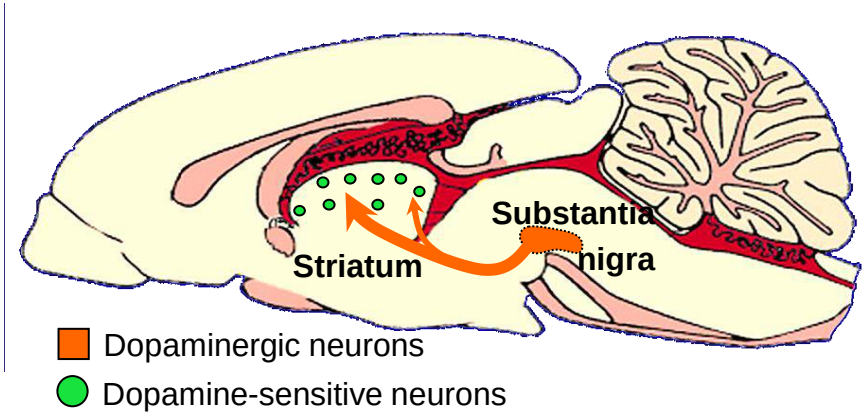


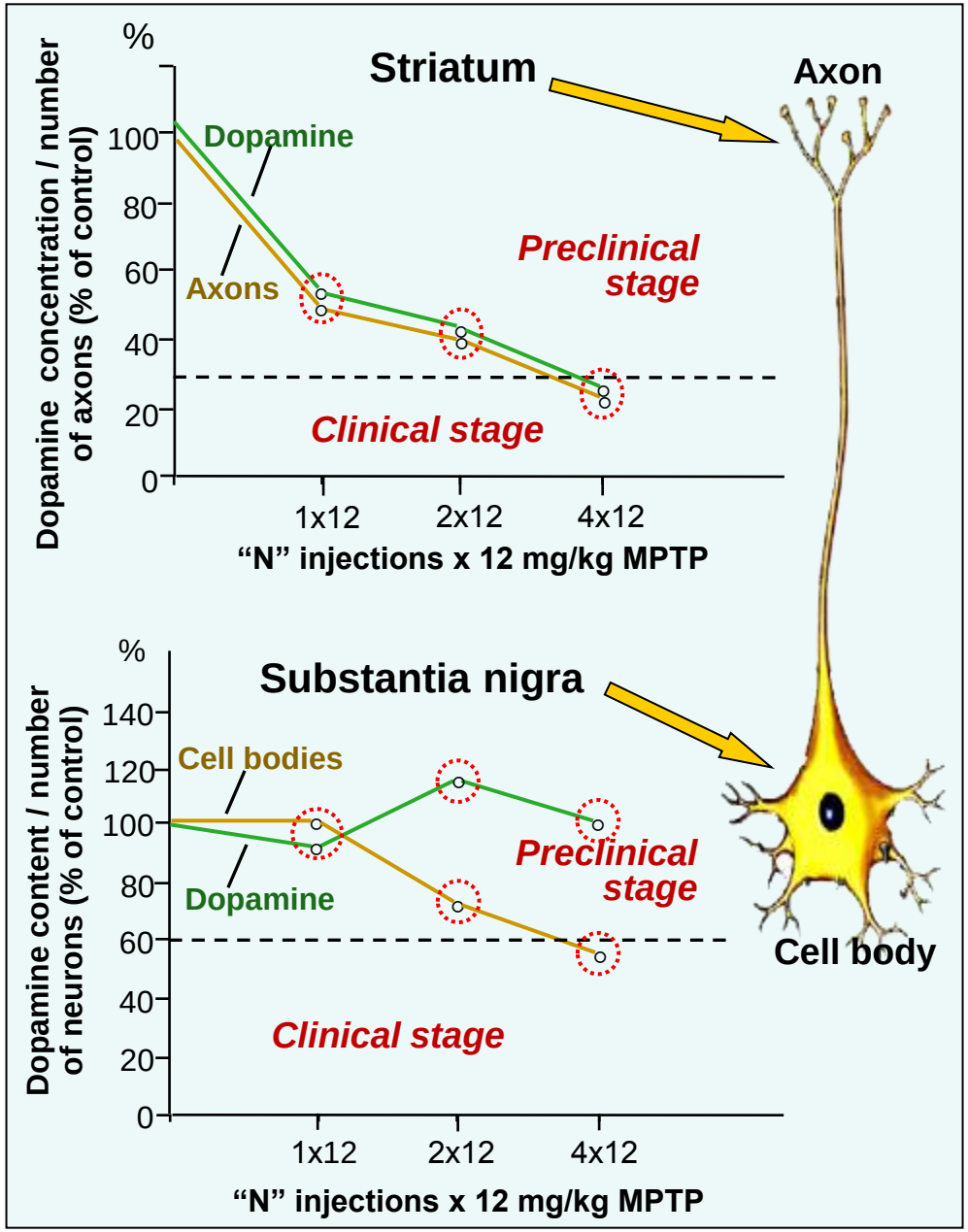
(glial cells)

1-methyl-4-pyridine (MPP^{+}, toxin)

Injections of MPTP: 1, 2 or 4 times at the individual dose of 12 mg/kg with 2-hours intervals between the injections.

Decapitation: 2 weeks after the treatment





Ugrumov M. et al. (2011) *Neuroscience 181, 175-188;*
Kozina et al. (2014) *J Neurol. Sci. 340:198-207.*

MPTP models of PD at the preclinical and early clinical stages & untreated patients at the clinical stage:

Gene expressions in the substantia nigra

Gene of protein *(functional role)*	Preclinical stage *(mice)*	Clinical stage *(mice)*	Clinical stage *(patients)*
α-Synuclein: oligomer *(neurotoxin)*	↑	↓	↓ (Bossers et al., 2008)
Syntaxin-1a: exocytosis *(SNARE complex)*	↑	↓	↓ (Mufson et al., 2006)
Synaptotagmin-1: exocytosis *(calcium sensor)*	→	↓	↓ (Simunovic et al., 2009)
Dynein: *(retrograde axonal transport)*	→	↓	↓ (Simunovic et al., 2009)
Adaptor protein complex AP-1, sigma 1: *(endocytosis)*	→	↑	↑ (Simunovic et al., 2009)
Sorting nexin	→	↓	↓ (Moran et al. 2006)

↑ Increase; → No change;
↓ Decrease; — No data

Slominskii et al., in preparation

MPTP models in mice of PD at the preclinical and early clinical stages: *Prospect of the use*

- Evaluation of the molecular ***mechanisms of neurodegeneration*** in the central and peripheral nervous system;
- Evaluation of the ***mechanisms of neuroplasticity*** in the central and peripheral nervous system;
- Search for ***mechanisms triggering a transition from*** preclinical stage to clinical stage;
- Search for ***peripheral biomarkers*** for the development of preclinical diagnostics of Parkinson's disease;
- Development of the ***provocation test*** for the diagnostics of Parkinson's disease at the preclinical stage;
- Development of ***preventive therapy*** in Parkinson's disease:
  - ✓ to slow down neurodegeneration;
  - ✓ to control and improve neuroplasticity;
  - ✓ to deactivate triggers of motor disorders.

Ugrumov (2009) *J Chem Neuroanat. 38, 241–256*; Ugrumov.(2011) *Neuroscience 181, 175-188*; Kozina et al. (2014) J Neurol Sci. *340,198-207;* Ugrumov (2013) *Adv Pharmacol. 68:37-91*.

MPTP models in mice of PD at the preclinical and early clinical stages & untreated PD patients at the early clinical stage:

Biomarkers in blood

Substance	Preclinical stage *(mice)*	Clinical stage *(mice)*	Clinical stage, tremor *(patients)*	Clinical stage, rigidity *(patients)*	Clinical stage *(patients, literature)*
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L-DOPA	↓	↓	↓	↓	No data
DOPAC	→	↓	→	↓	No data
Prolactin	→	↓	↓	↓	→ Murri et al. 1980; ↓ Laihinen et al. 1986
Cortisol			↑	↑	→ ↓ ↑ Laihinen et al. 1986; Swerdlof, 2012
Gene expression of D3 receptor			↓	↓	↓ Nagai et all., 1996

Nigmatullina et al. in preparation

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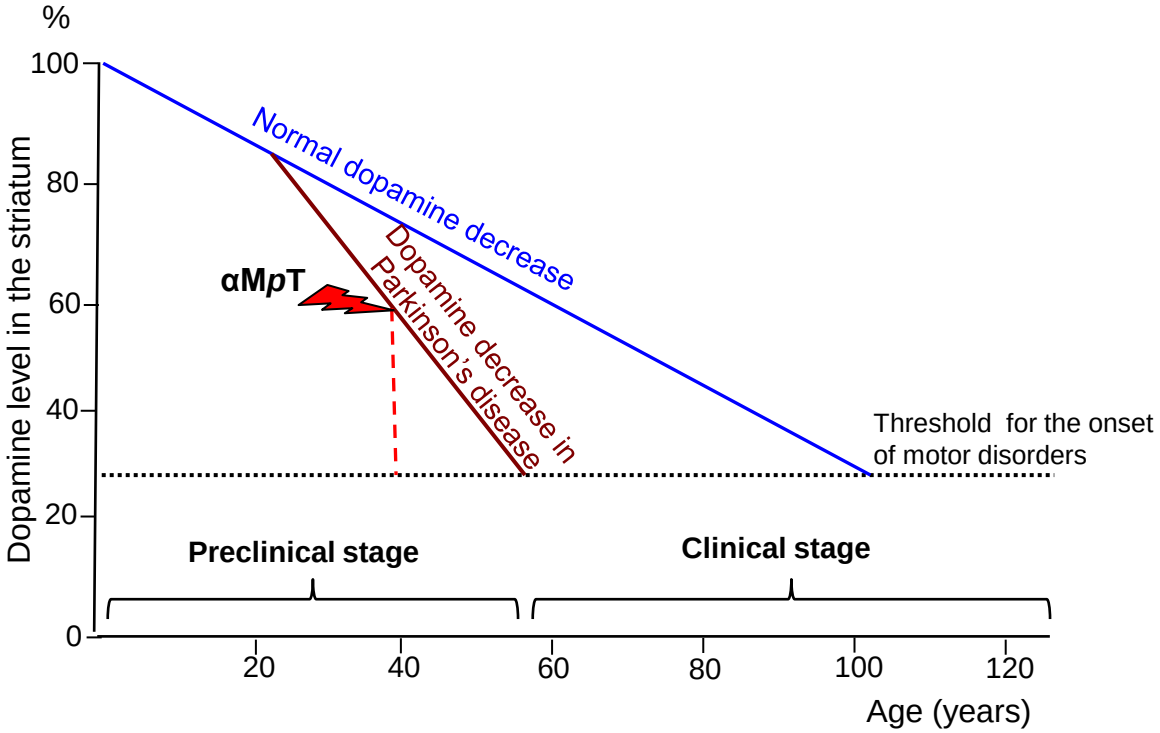
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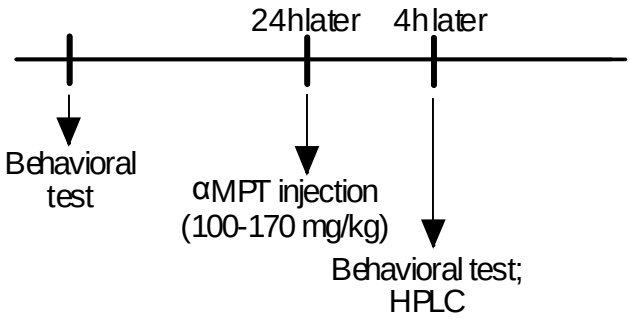
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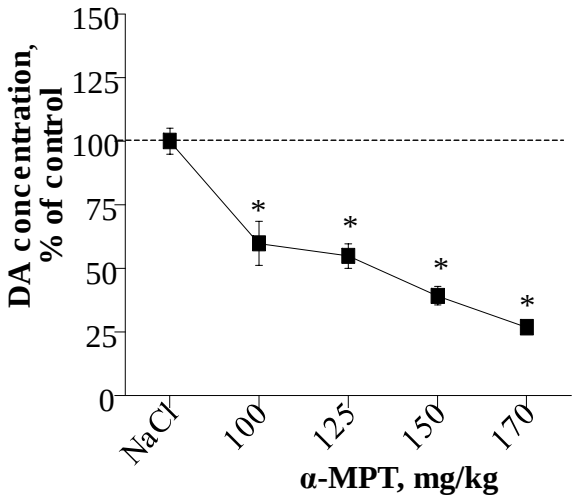
Conclusions and prospect.

A challenge test for preclinical diagnostics of chronic brain diseases: first trial at the model of Parkinson's disease

Search for a minimum dose of α-methyl-p-tyrosine (αMPT) that provokes motor disorders in intact mice

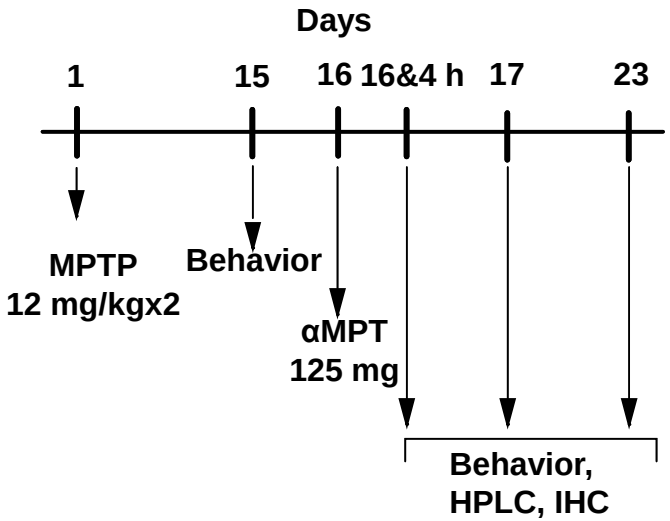


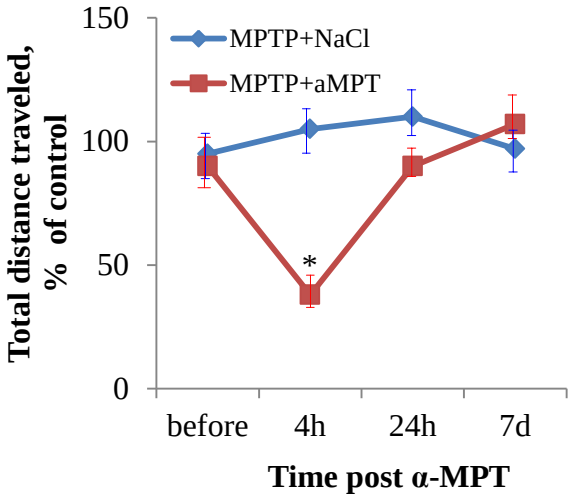


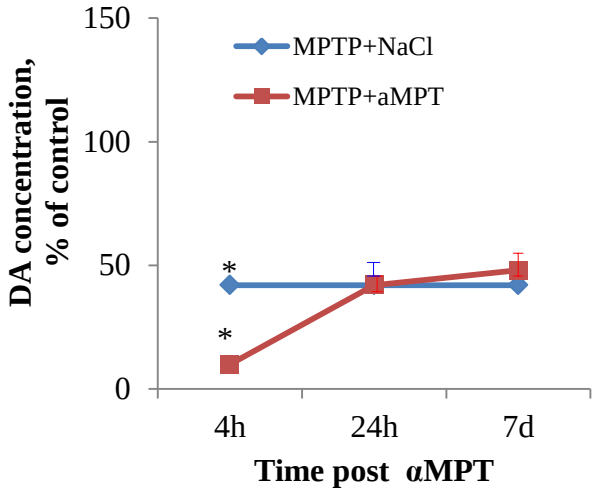


Khakimova et al., submitted

Trial of the provocation test using the model of preclinical stage of PD

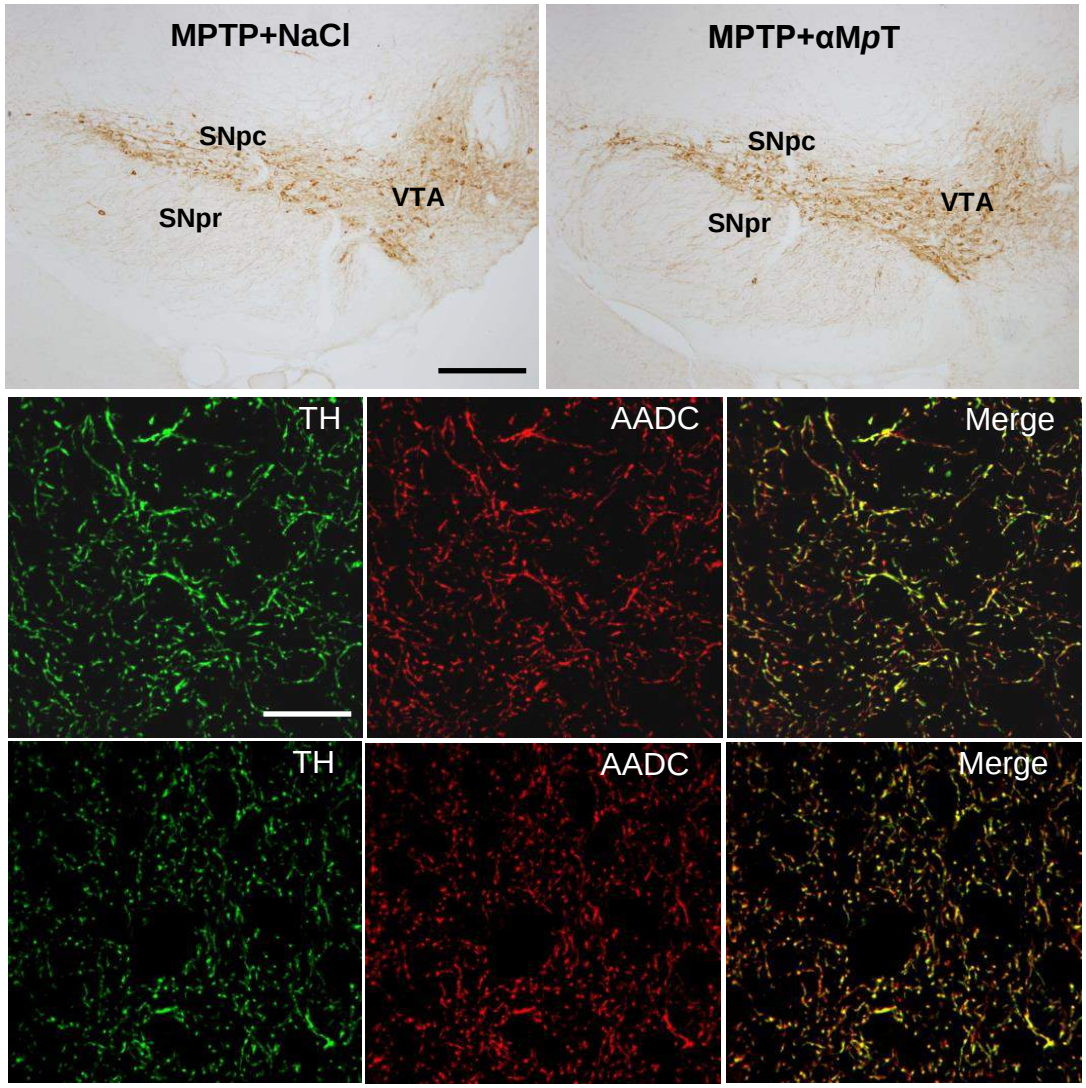


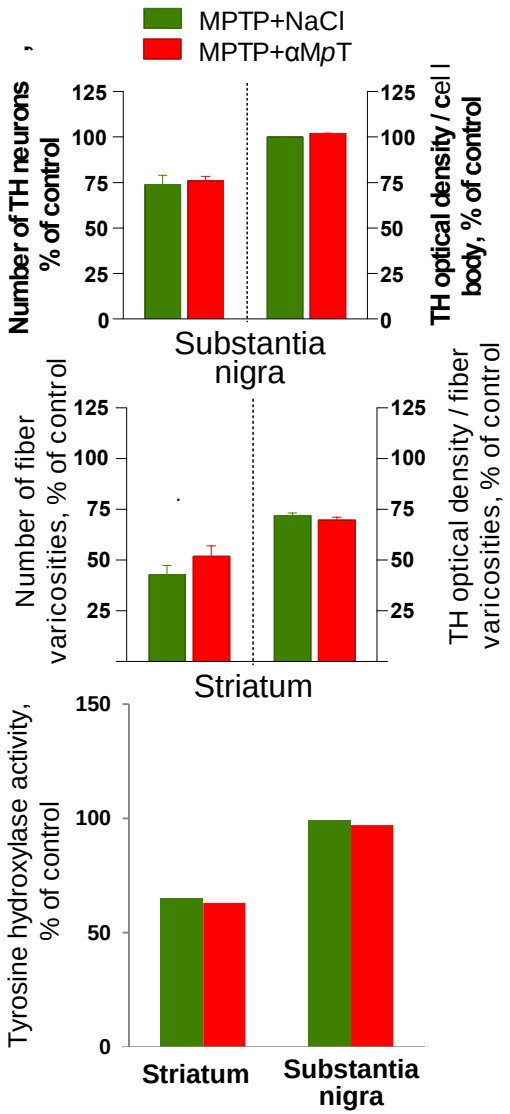




Khakimova et al., submitted

Consequences of the αM*p*T action on the nigrostriatal dopaminergic system





AADC, aromatic L-amino acid decarboxylase; αM*p*T, α-methyl-p-tyrosine; TH, tyrosine hydroxylase; SN, substantia nigra; VTA, ventral tegmental area.

Advanced methodology for the development of preclinical diagnostics of Parkinson's disease

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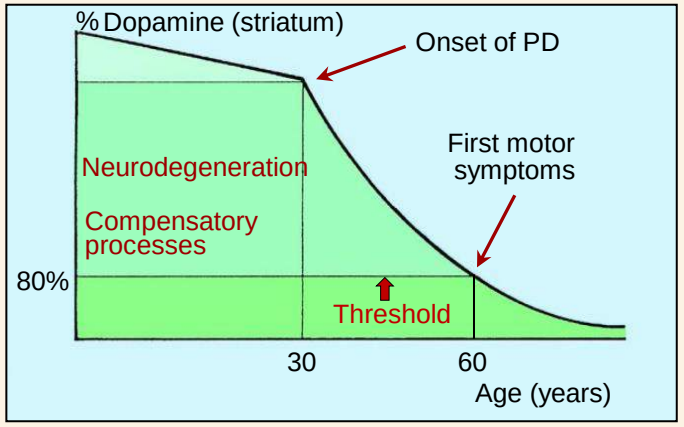
Provocation test as a novel approach to the diagnostic of Parkinson's disease;

➡ **Conclusions and prospect.**

Parkinson's disease: New insight into pathogenesis, diagnostics and treatment

What is known ?

- Parkinson's disease (PD) is developed for a long time without motor symptoms due to compensatory processes;
- Motor disorders first appear after almost complete degradation of dopaminergic input to the striatum that explains low efficacy of current treatment;
- PD is a polygenic and systemic disease; some pathologic processes are developed in the periphery earlier than in the brain.



What should be done ?

Development of preclinical diagnostics and preventive treatment

- A search for peripheral biomarkers in untreated PD patients at the early clinical stage and in experimental models of PD at preclinical stage;
- Clinical trials of the provocation test, the most specific and powerful approach to preclinical diagnostics of PD;
- Development of a two-step preclinical diagnostic of PD - selection of a risk group at examination of healthy population and the final diagnostic with positron emission tomography;
- Development and clinical trials of preventive neuroprotective therapy in PD patients at preclinical the stage aiming to slow down the neurodegeneration.

Neuroprotective therapy at the preclinical stage of neurodegenerative diseases

Pharmacotherapy

Pathogenesis	Neuroprotector
Oxidative stress	Antioxidants (tocopherol vitamin E, etc.)
Protein aggregation	✓ Inhibitors of the aggregation of α-synuclein, tau, etc.; ✓ Drugs serving to diminish the level of the aggregated proteins; ✓ Stimulator of proteasome and lysosome activity.
Inflammation	Antinflammatory drugs
Apoptosis	Antiapoptotic drugs
Prion-like propagation of pathological proteins	Immunotherapy
Decrease of the level of growth factors	Exogenous growth factors

Yacoubin , Standaert, 2009. *Biochem. Biophys acta 1792, 676-687*.

PROGRAM OF THE RUSSIAN ACADEMY OF SCIENCES

"Basic Research for Medicine"

PROJECT

"Neurodegenerative Diseases : Fundamental and Applied Issues"

Russian participants

1. Inst. of Developmental Biology RAS, Moscow
2. Inst. of the Highest Nervous Activity RAS, Moscow
3. Inst. of Gene Biology RAS, Moscow
4. Inst. of Radio Engineering and Radio Electronics RAS, Moscow
5. Computer Centre RAS, Moscow
6. Inst. of Molecular Genetics RAS, Moscow
7. Inst. of Physiologically Active Substances RAS, Moscow (Chernogolovka)
8. Inst. of Biochemical Physics RAS, Moscow
9. Inst. of Bioorganic Chemistry RAS, Moscow
10. Inst. of the Issues of Transferring Information RAS, Moscow
11. Inst. of Evolutionary Physiology and Biochemistry RAS, St. Petersburg
12. Inst. of Nuclear Physics RAS, St. Petersburg (Gatchino)
13. Inst. of Theoretical and Experimental Biophysics RAS, Moscow (Pushchino)
14. Inst. of Cell Biophysics RAS, Moscow (Pushchino)
15. Central Clinical Hospital RAS, Moscow
16. Inst. of Pharmacology RAMS, Moscow
17. Inst. of Normal Physiology RAMS, Moscow
18. Inst. of General Pathology, RAMS, Moscow
19. Centre for Mental Health, RAMS, Moscow
20. State University Medical School, Moscow

"…If the brain was simple enough to be intelligible, we would not be intelligent enough to understand it…"

J. Neirynck (2009)



Thanks for your attention

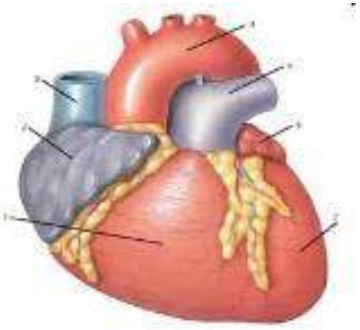
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MPTP models of preclinical and early clinical stages of PD & untreated PD patients at the clinical stage:

Peripheral manifestations



Peripheral markers of Parkinson's disease:

- ✓ Syndrome of the heart sympathectomy;
- ✓ Decrease of the noradrenaline concentration in the heart and plasma (Fornai et al., 2007);
- ✓ Appearance of Lewy bodies and α-synuclein-immunoreactive neurons in the heart (Goldstein, 2003; Micieli et al., 2003);

Nigmatullina et al., in preparation

Sample / Parameter	Preclinical stage *(mice)*	Clinical stage *(mice)*	Clinical stage *(patients, literature)*
Left ventricle of the heart			
Weight	→	↓	↓ Akbar et al., 2014
Contractility	↓	↓	↓ Nigmatullina et al., 2014
Systole duration	↓	↓	→ Imrich et al., 2009
Noradrenaline concentration	↓	↕	↓ Goldstein et al., 2007 Li et al., 2002 → Goldstein et al., 2011
Adrenaline concentration	→	→	No data
Dopamine concentration	↓	→	↓ Fornai et al., 2007
Serotonin concentration	↓	↑	No data
Contractility response to noradrenaline	↑	↓	↕ Imrich et al., 2009
Contractility response to serotonin	↕	↓	No data