2nd Russian–French Workshop

NEURODEGENERATIVE DISEASES: FROM THE PATHOGENESIS TO THE DIAGNOSIS AND TREATMENT

October 9 – 11, 2017
Moscow
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Organizers:

Russian Federal Agency for Scientific Organizations
Russian Academy of Sciences
Embassy of France in Russia
Russian Society of Neurochemistry
Institute of Developmental Biology RAS, Moscow, Russia
N.N. Burdenko Neurosurgery Institute, Russian Ministry of Healthcare, Moscow, Russia
Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Moscow, Russia
National Research University - Higher School of Economics, Moscow, Russia

Sponsors:

Russian Federal Agency for Scientific Organizations
Russian Foundation for Basic Research
Embassy of France in Russia
National Research University - Higher School of Economics, Russia
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English is official language (Translation is possible)
2nd Russian – French Workshop

October 9 (Monday)

Meeting Venue
Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, 16/10 Miklukho-Maklaya str., Moscow

Registration: 9.30 – 10.00

Welcome words: 10.00 – 10.20

Introduction

10.20 – 10.30 Michael UGRUMOV. Neurosciences in Russia with emphasis on neurodegenerative diseases. Institute of Developmental Biology RAS, Moscow, Russia

10.30 – 10.40 Etienne HIRSCH. Overview of Neurosciences in France and National plan on neurodegenerative diseases. Brain and Spine Institute, Pitié-Salpêtrière Hospital, Paris, France

Parkinson’s Disease

Chairs: Etienne HIRSCH (France) and Michael UGRUMOV (Russia)

10.40 – 11.05 Lydia KERKERIAN. Novel insights onto the pathophysiology and pathogenesis of Parkinson’s disease from optogenetics and disease modeling in rodents. Developmental Biology Institute of Marseille, UMR7288 CNRS/Aix-Marseille University, Marseille, France

11.05 – 11.30 Alexander KIM. Modeling of Parkinson's disease at the preclinical and early clinical stages as an instrument of translational medicine. Institute of Developmental Biology RAS, Moscow, Russia
11.30 – 11.55 Peter SLOMINSKY. Whole-transcriptome analysis of an MPTP induced murine model of the earliest stages of Parkinson’s disease. Institute of Molecular Genetics RAS, Moscow, Russia

11.55 – 12.15 COFFEE BREAK

12.15 – 12.40 Alexey USTYUGOV. The use of transgenic methods to study proteinopathies. Institute of Physiologically Active Compounds RAS. Chernogolovka, Russia

12.40 – 13.05 Sergei ILLARIOSHKIN. Induced pluripotent stem cells-based models of neurodegenerative disorders. Research Center of Neurology, Moscow, Russia

13.05 – 13.30 Stéphane HUNOT. Neuro-immune cell interactions in Parkinson's disease: new insights from analysis of perivascular macrophage function. Brain and Spine Institute, Pitié-Salpêtrière Hospital, Paris, France


13.55 – 14.20 Sofya PCHELINA. Genetic and biochemical markers of Parkinson’s disease. First Pavlov State Medical University of St. Petersburg, Nuclear Physics Institute, St. Petersburg, Russia

14.20 – 16.00 LUNCH AND VISIT TO LABORATORIES

Chairs: Sergei ILLARIOSHKIN (Russia) and Lydia KERKERIAN (France)

16.00 – 16.25 Svyatoslav MEDVEDEV. Complementarity of PET, MRS, and ERP data for studying the mechanisms of development of cognitive impairment in patients with Parkinson's disease. Institute of the Human Brain RAS, St. Petersburg, Russia

16.25 – 16.45 Pierre-Olivier FERNAGUT. Unravelling mechanisms of motor and non-motor side effects of dopamine replacement
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therapy in Parkinson’s disease. Institut des Maladies Neurodégénératives. CNRS UMR5293 & Université de Bordeaux, France.

16.45 – 17.10 Anna GAMALEYA. Cribrous subthalamic nucleus as a target for neurostimulation in Parkinson's disease. N.N. Burdenko Neurosurgery Institute, Russian Ministry of Healthcare, Moscow, Russia

17.10 – 17.35 Philippe HANTRAYE. Dopamine replenishment therapy for Parkinson’s disease: from bench to patients. Molecular Imaging Research Center, UMR9199 CEA CNRS, Institute Francois Jacob, Fontenay-aux-Roses, France

17.35 – 18.00 Michael UGRUMOV. Novel paradigm of the development of preclinical diagnosis of Parkinson’s disease. Institute of Developmental Biology RAS, Moscow, Russia
October 10 (Tuesday)

Meeting Venue:
Central office of the Russian Federal Agency for Scientific Organizations & Russian Academy of Sciences; 
32A Leninsky Prospect, President Hall

Alzheimer Disease

Chairs: Alexander MAKAROV (Russia) and Stéphane HUNOT (France)

10.00 – 10.25  Sergey KOZIN. Modulation of cerebral amyloidogenesis in Alzheimer’s disease. Engelhardt Institute of Molecular Biology RAS, Moscow, Russia

10.25 – 10.50 Natalia BOBKOVA. The YB-1 as a potential drug for treatment of Alzheimer’s disease. Institute of Cell Biophysics RAS, Pushchino, Russia

10.50 – 11.15 Nicolas SERGEANT. In vivo therapeutic strategies against tau pathology: gene therapy and small molecules. INSERM UMRS1172, Alzheimer & Tauopathies, University Nord de France, Lille, France

11.15 – 11.40 Guillaume DOROTHEE. Interplay of innate and cellular adaptive immunity in Alzheimer’s disease: therapeutic potential of T-cell-targeting immunomodulatory approaches. INSERM UMRS 938 – Centre de Recherche Saint-Antoine Hôpital Saint-Antoine, Paris, France

11.40 – 12.00 COFFEE BREAK

12.00 – 12.25 Natalia GULAYEVA. Molecular mechanisms of neuroplasticity / neurodegeneration underlying comorbidity of dementia
2nd Russian – French Workshop

and depression: "my mirror twin, my next of keen". Institute of Higher Nervous Activity and Neurophysiology RAS, Moscow, Russia

12.25 – 12.50 Nikolai YAKHNO. Heterogeneity of pre-mild cognitive decline. I.M. Sechenov 1st Moscow State Medical University, Moscow, Russia

12.50 – 13.15 Igor ZHURAVIN. Brain development, cognitive functions and neurodegeneration after prenatal hypoxia. I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry RAS, St. Petersburg, Russia

Huntington’s Disease

13.15 – 13.40 Sandrine HUMBERT. The developing Huntington’s disease brain. Grenoble Institute of Neurosciences, GIN - INSERM U1216 - Grenoble Alpes University, Grenoble, France

13.40 – 14.05 Vladimir VIGONT. Huntington’s disease: deregulation of calcium homeostasis in patient-specific neuronal model. Institute of Cytology RAS, St. Petersburg, Russia

14.05 – 15.00 LUNCH

Amyotrophic Lateral Sclerosis

Chairs: Nicolas SERGENT (France) and Natalia GULYAEVA (Russia)

15.00 – 15.25 Séverine BOILLEE. Contribution of microglial cells/macrophages to motor neuron degeneration in ALS. INSERM U1127, CNRS U7225, UPMC ICM, Pitié-Salpêtrière Hospital, Paris, France

15.25 – 15.50 Marat MUKHAMEDYAROV. Dysfunction of neuromuscular synaptic transmission in transgenic model of amyotrophic lateral sclerosis. Kazan State Medical University, Kazan, Russia
15.50 – 16.15  Luc DUPUIS. Mechanisms of FUS mediated amyotrophic lateral sclerosis: insights from mouse genetics. INSERM UMR-S1118, Faculté de Médecine, Strasbourg, France.

16.15 – 16.40  Maria ZAKHAROVA. Amyotrophic lateral sclerosis: new insights in pathogenesis. Research Centre of Neurology, Moscow, Russia

16.40–18.15  Visit to the Institute of Molecular Biology RAS and Institute of Developmental Biology RAS
October 11 (Wednesday)

Meeting Venue:
Presidium of the Russian Academy of Sciences
14 Leninsky Prospect

Round Table Discussion
What is the prospect for Russian-French scientific cooperation in the study of Neurodegenerative diseases and other global challenges for society

Chairs: Etienne HIRSCH (France) and Michael UGRUMOV (Russia)

Participants of the round table – representatives of:

- Russian Federal Agency for Scientific Organizations
- Ministry of Education and Science of RF
- Russian Academy of Sciences
- Embassy of France in Russia
- Russian Scientific Foundations
- Speakers at the Russian-French workshop
ABSTRACTS
Parkinson’s Disease
Unravelling mechanisms of motor and non-motor side effects of dopamine replacement therapy in Parkinson’s disease

P.-O. Fernagut

Institut des Maladies Neurodégénératives. CNRS UMR5293 & Université de Bordeaux, Bordeaux, France; pierre-olivier.fernagut@u-bordeaux.fr

Chronic dopamine replacement therapy (DRT) in Parkinson’s disease (PD) leads to a number of disabling side effects. L-Dopa induced dyskinesia (LID) are the main motor side effect of L-Dopa therapy, while impulse control disorders (ICD, such as pathological gambling, binge eating, hypersexuality) are the main non-motor side effects occurring in 20% of patients treated with D2/D3 agonists. Some patients may also develop treatment addiction known as dopamine dysregulation syndrome. The pathophysiological basis of these non-motor side effects is poorly known but likely involve interactions between DRT, the disease process and individual vulnerability traits. We have demonstrated that LID are not related to increased striatal dopamine levels and likewise involve non-motor domains. Using selective neuronal inhibition, we further demonstrated that neurons from the lateral habenula and bed nucleus of the stria terminalis contribute to the expression of LID. Regarding non-motor symptoms, we showed that nigrostriatal degeneration is mandatory for the development of psychostimulant-like properties of L-Dopa that may promote the subsequent development of dopamine dysregulation syndrome in vulnerable individuals. With regards to ICD, we have shown that pre-morbid impulsivity is associated with a reduced vulnerability to neurodegeneration. Despite such reduced vulnerability, high impulsive rats display and enhanced sensitivity to the deleterious effects of D2/D3 agonists on inhibitory control, thereby highlighting critical interactions between vulnerability endophenotypes, neurodegeneration and DRT. Altogether, the identification of mechanisms underlying motor and non-motor sides effects of DRT will pave the way toward the refinement of symptomatic therapeutic options in PD.
Cribroussubthalamic nucleus as a target for neurostimulation in Parkinson's disease

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Introduction: Perivascular spaces (Virchow-Robin, PVS) are pial-lined slit-like spaces that surround small perforating cerebral arteries and arterioles. Small PVS (<2mm) can be detected in MRI in all ages. Enlarged PVS are considered a sign of brain aging and are commonly asymptomatic. Presence of numerous or strikingly dilated PVS has received a name “etatcrible” (cribriform state) and is associated with neurological symptoms. In patients with Parkinson's disease (PD), enlarged PVS are often found. Relationship between overextended PVS and PD is still unclear. In literature, both amelioration and worsening of parkinsonian symptoms in contralateral extremities are described.

Objective: We report a case of successful deep brain stimulation (DBS) in a parkinsonian patient with cribroussubthalamic nucleus (STN).

Methods: A 57-year-old woman had an 11-year history of PD. Motor symptoms manifested on the right side. After 9 years, disabling pharmacologically resistant motor fluctuations and dyskinesia appeared, and she was assigned for evaluation for DBS-surgery. On admission, patient had bilateral akinetic-rigid syndrome with marked predominance in the right extremities, moderate postural instability, and slight cranial and cervical dystonia. Hypokinesia and rigidity could be significantly ameliorated by intake of levodopa. Levodopa also improved gait and balance. Moderate dyskinesia was observed mainly in the right arm and leg. Clinical evaluation was performed according the standard scales (UPDRS, PDQ-39, Schwab&England). Standard MRI revealed striking lacunar cysts in the left brain peduncle and left striatum, which could limit stereotactic placement of DBS-electrode.
Results: We performed comprehensive neuroimaging evaluation in order to assess patient’s suitability for DBS-surgery. Direct visualization of basal ganglia was performed using 3T MRI and MR-tractography (DTI). Overextended PVS were detected in the left subthalamic nucleus and posterior subthalamic area. However, shape and size of cribrous STN were within normal range and internal capsule was not dislocated. Subsequently, DBS-electrodes were implanted in STN bilaterally using standard procedure. Position of the left STN-electrode was calculated by the anterolateral margin of PVS. Microelectrode recording performed during the surgery showed no significant difference in neuronal electrical activity of the cribrous and normal STN. Moreover, high presence of typically firing STN-neurons was noticed at the border of PVS. Following DBS STN, we observed pronounced improvement in clinical state. At six-month follow-up, UPDRS-III score in off-medication state reduced by 79%, UPDRS-II score – by 85%. Daily activity in off-state increased by 40%, PDQ-39 improved by 20%. Levodopa equivalent daily dose decreased by 61%. Motor fluctuations and dyskinesia significantly improved.

Conclusion: Expanded perivascular spaces in midbrain and basal ganglia region may accompany clinical manifestation of PD. Nevertheless, particular interconnection or interdependence of these events needs to be verified in the larger observational studies. Most likely, presence of moderate cribrous changes does not hinder stereotactic implantation of electrodes and does not predict an unfavorable outcome of DBS STN.
Gene therapy approaches for Parkinson’s disease have provided encouraging results in early-phase clinical trials. In a preclinical study (Jarraya et al., 2009), we evaluated the potential therapeutic efficacy of a dopamine replenishment strategy using ProSavin®, a lentiviral vector developed and manufactured by the company OxfordBiomedica Ltd and delivering three key enzymes in the dopamine biosynthetic pathway to non-dopaminergic striatal neurons of the sensorimotor putamen. We assessed the potential therapeutic efficacy of this approach in a semi-chronic MPTP macaque model of PD and showed significant improvements in clinical scores as well as in video-assessed spontaneous activity for animals receiving ProSavin, compared to those receiving either a control vector (LacZ) or no treatment (MPTP only). We also assessed whether the same gene-therapy approach could generate the same motor recovery in MPTP-monkeys with levodopa-induced dyskinesias (LID-MPTP model). Interestingly, not only ProSavin was able to restore motor activity in LID-MPTP monkeys but the treatment was able to reduce by 80% the dyskinetic movement induced by a 40 mg/kg L-Dopa which was not the case in LID-MPTP monkeys receiving the LacZ control vector. Together, these first results suggested that local and continuous production of dopamine in the sensorimotor putamen could reduce in the long term motor deficits, in parkinsonian monkeys without inducing dyskinesia. These data permitted the launch of a first phase I/II clinical trial (Palfi et al., 2013). Again, ProSavin injections in the sensorimotor putamen led to an improved motor function in patients with the highest dose of ProSavin vector injected. $^{11}$C-raclopride PET imaging unambiguously provided evidence of increased dopamine production in the sensorimotor putamen of patients receiving the highest dose of ProSavin. However, the magnitude of the effect suggested that optimal levels of dopamine replacement may have not been achieved. In a translational bed to bench approach, a new lentiviral vector (OXB-102®) with an optimized expression cassette for the three dopamine biosynthesis enzymes...
was developed and tested in vitro, providing significantly higher dopamine-producing capacity than ProSavin. As a last step before clinical evaluation, the therapeutic efficacy of OXB-102 was therefore assessed in vivo in the same MPTP macaque model of PD. Analysis of behavioural recovery showed similar significant improvements in clinical ratings scores (CRS) and video-assessed spontaneous locomotor activity for animals receiving either ProSavin or a high dose (HD) or low dose (LD) of OXB-102, compared to those receiving a vector control (EIAV-Null) at 6 months post vector administration. Positron emission tomography (PET) analysis of $^{18}$F-FMT binding showed a significant increase in binding potential (Ki) for OXB-102 and ProSavin treated animals compared with controls. The greatest increase was observed in OXB-102 HD group, indicating the highest levels of dopaminergic activity. The safety and immunogenicity of OXB-102 was also investigated in a preclinical toxicity study in macaques where the vector was demonstrated to be well-tolerated, with no associated clinical or behavioural abnormalities, and no immune response mounted against the three transgene products. Overall, these data support the further clinical development of OXB-102 for the treatment of Parkinson’s disease patients.

Acknowledgements. Dr Romina ARON BADIN, Nadja VAN CAMP, the staff of MIRCen for their scientific and technical competences to the project. Prof. Stephane PALFI, Neurosurgery department, Henri Mondor Hôpital Créteil France for fruitful collaboration. This work was done in collaboration with Oxford Biomedica Ltd who owns the gene therapy products ProSavin and OXB102.
In the field of neurodegenerative diseases, the concept of non-cell autonomous disease mechanisms suggests that neurodegeneration is not just mediated by damages within the affected neurons but is also influenced by interactions with neighboring glia and immune cells. This so-called neuroinflammatory response produces both neurotoxic and neurotrophic effects and it has been suggested that its imbalance could be at the core of the detrimental progressive nature of many neurodegenerative diseases including Parkinson’s disease (PD). Evidence from epidemiological, genetic as well as postmortem and animal studies suggest that neuroinflammatory processes primarily associated with microglial cell activation could be intricately linked not just to disease progression but also to selectivity of neurodegeneration. In addition to these now well-recognized culprits, latest developments in the field have revealed that adaptive immune response to neurodegeneration may play an equally important role in PD pathogenesis. This later observation highlights the importance of brain extravasation of peripheral immune cells (both myeloid and T cells), a process largely dependent on the chemokine axis. Yet, all immune cell responses to neurodegeneration may not be detrimental during PD. For instance, brain perivascular macrophages, a specialized and highly phagocytic macrophage population strategically positioned along the “glymphatic system”, are likely to play a beneficial role. Therefore, future therapeutic approaches aimed at curtailing deleterious neuroinflammation in PD will need to selectively target the cytotoxic arm without altering the beneficial function of immune cells.
Induced pluripotent stem cells-based models of neurodegenerative disorders

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Major clinical symptoms of Parkinson’s disease (PD), Huntingtons’ disease (HD) and other neurodegenerative disorders are caused by degeneration of relatively restricted areas in the brain, such as brainstem dopaminergic area, substantia nigra, in PD. Creation of reliable cell models for these conditions is of crucial importance for studying their molecular mechanisms and for the development of new therapeutic strategies, including restoration of viable cell population via neurotransplantation. At present, one of the most attractive cell types for modeling PD and other neurodegenerative disorders are induced pluripotent stem cells (iPSCs) that can be obtained from mature somatic cells with the use of a mix of reprogramming peptide factors. Important advantages of iPSCs over other cells sources are immune identity of the transplant and the host, as well as the lack of serious ethic problems and possibility to generate “personalized” cell models for patients carrying mutations in different causative genes. We created iPSCs from patients with PD and HD carrying mutations in the PARK2, LRRK2, GBA and HTT genes, as well as from patients with sporadic PD. On the next stage, neuronal precursors and differentiated dopaminergic neurons or GABAergic striatal neurons were obtained from iPSCs. We showed the disbalance of apoptotic and antiapoptotic factors in iPSCs-derived dopaminergic cells from a patient with PARK2 mutations. Neurons carrying mutations in the “parkinsonian” genes were characterized by functional failure of dopamine transport systems, while HTT-expressing cells had mutant huntingtin aggregates, increased number of autophagosomes, nuclear indentations, abnormal calcium entry and enhanced neuronal death during cell aging, thus recapitulating the disease pathology. Genome editing using CRISP/Cas9 system restored many cellular functions under study. We obtained first experience of neurotransplantation of iPSCs-derived specialized neurons intro the bran of rats with toxic models of PD and HD. Physiological and immunocytochemical studies showed that such neurotransplantation led to improvement of motor functions and partial restoration of target cells in the striatum and other brain regions.
Acknowledgements. The author wants to thank his colleagues from Research Center of Neurology, Institute of Molecular Genetics, Institute of Physical-Chemical Medicine and Institute of General Genetics for their contribution to this work.
Novel insights onto the pathophysiology and pathogenesis of Parkinson’s disease from optogenetics and disease modeling in rodents

Lydia Kerkerian
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The massive and progressive degeneration of the nigral dopamine neurons innervating the striatum, the main basal ganglia input station, is a main neuropathological feature of Parkinson’s disease (PD). The hypokinetic parkinsonian syndrome is viewed as the consequence of imbalanced activity of the direct and the indirect pathways by which the striatum controls the basal ganglia output structures, reinforcing the inhibitory tone exerted by these structures via the thalamus onto the motor cortical outflow. I will illustrate two aspects of our work in rodent models of PD. First, I will present our recent data, combining optogenetics with pharmacological, behavioral and electrophysiological approaches, that provided direct evidence for a causal role of striatal cholinergic interneurons in PD motor symptomatology and identified the direct pathway as a main neural substrate [Collaboration M Amalric, Laboratory of Cognitive Neurosciences (LNC), Marseille; K Deisseroth, Stanford University; Maurice et al., Cell Rep 2015; Ztaou et al., J Neurosci 2016]. Second, I will share preliminary results supporting a role of the stress-induced protein TP53INP1, a multifaceted protein endowed with a dual role of tumor suppressor and regulator of autophagy whose deficiency has been implicated in cancer and metabolic syndrome, in limiting nigral dopamine neuron loss during normal ageing and in a model of progressive parkinsonism [Collaboration A Carrier, Cancer Research Center of Marseille (CRCM); O Corti, Brain and Spine Institute (ICM), Paris].

Support. CNRS, Aix-Marseille University, ANR, Fédération pour la Recherche sur le Cerveau (FRC), Fondation de France, France Parkinson, University Hospital Federation DHUNE
Modeling of Parkinson's disease at the preclinical and early clinical stages as an instrument of translational medicine

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Parkinson's disease (PD) is characterized by the appearance of motor symptoms many years after the onset of neurodegeneration, which explains low efficiency of current therapy. Therefore, the development of the early (preclinical) diagnosis and preventive treatment of PD, based on the knowledge of molecular mechanisms of neurodegeneration and neuroplasticity in the nigrostriatal system, is a high priority. However, because of the inability to diagnose PD at the preclinical stage, such studies can be performed only in experimental models. The goal of this study was to evaluate molecular mechanisms of neurodegeneration and neuroplasticity in the nigrostriatal system of MPTP-treated mice at original models of presymptomatic and symptomatic parkinsonism. Despite the partial loss of dopaminergic (DAergic) neurons in mice at both stages of parkinsonism, there was no change in major functional indicators, such as tyrosine hydroxylase activity, dopamine (DA) content, spontaneous and stimulated release of DA, DA uptake, MAO A and B activity, vesicular monoamine transporter 2 (VMAT2) expression in the substantia nigra. This is apparently due to compensatory processes in the survived individual neurons. In fact, the content, synthesis, uptake, spontaneous and stimulated release of DA, as well as the VMAT2 expression were increased in individual cell bodies. In contrast to the substantia nigra, the striatum of mice showed a functional failure already at the presymptomatic stage that was manifested by a subthreshold (61%) decrease in the intracellular concentration of DA at the loss of 59% of DAergic axons and DA content, as well as in decreased expression of DA transporter and VMAT2. However, the decrease in the intracellular concentration of DA to the threshold level and the manifestation of motor disorders are prevented in the entire striatum at the presymptomatic stage by compensatory processes, such as the maintenance at the control level of spontaneous and stimulated release of DA and DA uptake, as well as a decrease in MAO B activity. This is possible due to a compensatory increase in DA synthesis, release and uptake in survived individual axons. The threshold loss of DA in tissue and intracellular
space at the symptomatic stage is explained by a decrease in functional activity of individual DAergic axons. Indeed, the content and stimulated release of DA decreased by two-fold in individual axons, while the activity of MAO A increased. Moreover, the DA uptake was increased, which also lead to a threshold drop in the level of intracellular DA. In addition to the above compensatory processes, we first proved that DA is synthesized in the striatum inpresymptomatic and symptomatic mice not only in DAergic axons, but also in non-dopaminergic so-called monoenzymatic neurons, expressing only tyrosine hydroxylase, or only aromatic L-amino acid decarboxylase, in cooperation. Thus, the failure of the nigrostriatal system at the progressive degeneration of dopaminergic neurons is compensated at the presymptomatic stage by an increase of tyrosine hydroxylase activity, spontaneous and stimulated DA release and uptake, and cooperative synthesis of DA by monoenzymatic neurons, whereas the symptomatic stage is characterized by a decompensation due to increased MAO-A activity, as well as a decrease of DA release and the expression of DA transporter and VMAT2.

Acknowledgments. This work was supported by the Russian Foundation for Basic Research ("Molecular mechanisms of brain neuroplasticity under functional insufficiency (degeneration) of dopaminergic neurons" № 17-04-00479)
Complementarity of PET, MRS, and ERP data for studying the mechanisms of development of cognitive impairment in patients with Parkinson's Disease

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Different neuroimaging techniques allow researcher to study the pathogenesis of neurodegeneration from different point of view that is of great importance for its understanding.

Objective. The study was aimed to matching the different neuroimaging technics data in patients with Parkinson's Disease (PD) with and without cognitive impairment.

Methods. We implemented positron emission tomography with 18F-fluorodeoxyglucose (FDG PET), protone magnetic resonance spectroscopy (H-MRS) and event-related potentials (ERPs) during the execution of the two-stimulus task in the Go/NoGo paradigm in 40 patients with PD and age-matched healthy control group. Application of the novel method of blind source separation to the ERPs allowed to isolate latent components of the ERPs which are generated in certain regions and assumed to reflect various processes. To evaluate cognitive state, the Mini mental state examination (MMSE), the Frontal assessment battery (FAB) and the clock drawing test were used.

Results. In PD patients, cerebral glucose metabolism was decreased in frontal (Brodmann areas (BA) 9, 10, 11, 46, 47), parietal (BA 39), temporal (BA 20, 37), and cingulate cortex (BA 32) compared to the control group. In the group of cognitively impaired PD patients glucose metabolism was decreased in the frontal (BA 8, 9, 10, 45, 46, 47), parietal (BA 7, 39, 40) and cingulate cortex (BA 23, 24, 31, 32).

H-MRS revealed decrease of NAA/Cr ratio (neuronal integrity marker) in supraventricular white matter of left hemisphere in PD patients compared to control. NAA/Cr ratio correlated with cerebral glucose metabolism in frontal (BA 46) and cingulate cortex (BA 23,24,32).

The analysis of ERPs in PD patients revealed decrease of P300 amplitude in Go and NoGo conditions compared to controls. Also changes in the amplitude of latent components generated in the occipital, inferior temporal, parietal and
frontal cortex were revealed. These components are supposed to reflect the stages of visual stimuli processing and operations of the executive system.  

**Conclusion.** Thus, implemented neuroimaging methods allowed revealing different aspects of cognitive impairment pathogenesis in PD. Follow-up studies may possibly help to develop criteria for early diagnostics of cognitive impairment and treatment efficiency evaluation.
Prion-like propagation of pathogenic protein assemblies in neurodegenerative diseases

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Protein intracellular inclusions within the central nervous system are hallmarks of several progressive neurodegenerative disorders in man. The protein constituents of these deposits and the affected regions within the brain differ from one neurodegenerative disorder to another. Until recently, the vicious circle consisting of spread, seeded assembly and accumulation over time within the central nervous system of misfolded proteins aggregates was thought to be restricted to the prion protein PrP. Recent reports suggest that other protein aggregates spread and amplify within the central nervous system leading to distinct diseases. I will present data illustrating the propagation propensities of alpha-synuclein, Huntingtin Exon 1 and tau protein aggregates. I will discuss the nature of protein assemblies that are “Infectious”, how they bind to the cell membranes, what they bind to and the cellular consequences of binding. I will present a quantitative assessment of their uptake, transport and export. I will show data demonstrating that pathogenic protein assemblies disrupt the endo-lysosomal membranes to reach the cystosol where they amplify. Finally, I will describe how and why different alpha-synuclein, Huntingtin Exon 1 and tau polymorphs cause distinct diseases. Strategies targeting the propagation of protein assemblies involved in age-related dementias will be presented and discussed.

References:
The intranasal administration of lactacystin: a new rodent model for a search of non-motor signs of the early stage of Parkinson’s disease

I.V. Ekimova¹, D.V. Plaksina¹, V.V. Simonova¹, M.N. Karenko¹², A.R. Gazizova¹, M.B. Pazi¹, Y.F. Pastukhov¹
¹ I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry RAS; irina-ekimova@mail.ru; ² Institute of Experimental Medicine, St. Petersburg, Russia

Objectives: Parkinson’s disease (PD) is the second most common neurodegenerative disorder worldwide. The main clinical manifestations of PD are motor disorders, hypokinesia and tremor. The pathomorphologic PD features include nigrostriatal and extranigral neurodegeneration, the presence of α-synuclein positive neuronal inclusions and increased amount of activated microglia. Late diagnosis and symptomatic treatment are the main reasons for incurability of PD. A number of data available in the literature indicate that the formation of mitochondrial dysfunction, oxidative stress, and disturbances in the ubiquitin-proteasome system (UPS) play the key role in the development of PD. There is a common view that the creation of animal models of the preclinical PD stage can accelerate the search for non-motor markers specific for the early PD stages and new molecular targets for the PD therapy. This work was designed to create a new experimental model of the preclinical PD stage in rats based on the weakening of brain UPS activity.

Methods: The specific UPS inhibitor lactacystin (LC) was intranasally injected to the adult (7-8 months) and old (19-20 months) male Wistar rats with 7-days interval. Beam-walking, rotarod and open-field tests were performed to reveal motor disturbances. Sucrose preference test was used to reveal the features of depressive-like behavior. Continuous EEG recordings were performed using DSI device with 4ET telemetric module to access sleep changes. Immunohistochemical assay was applied to analyze the pathomorphological signs of created model; high performance liquid chromatography with electrochemical detection was used to assess the level of dopamine in dorsal striatum. Intraperitoneal L-DOPA administration was used in the model of clinical (motor) PD stage to establish whether the observed motor disturbances disappear with the use of the clinically effective antiparkinsonian drug.
Results: Our study showed that two LC injections with 7-days interval followed by additional injection caused the appearance of motor deficits, which were counteracted by L-DOPA treatment. The period before the third LC administration (till the day 21) was attributed to the preclinical (premotor) PD stage and chosen for further research. We showed that preclinical PD stage is characterized by: 1) the development of α-synuclein positive neuronal inclusions within olfactory bulbs, locus coeruleus, substantia nigra, and ventral tegmental area accompanied by the neuroinflammation; 2) subthreshold level of nigrostriatal and extranigral neurodegeneration; 3) a subthreshold striatal decrease of dopamine level in dorsal striatum; 4) activation of the compensatory processes in dopaminergic nigrostriatal system; 5) appearance of the signs of anhedonia; 6) sleep disturbances (hypersomnia, sleep fragmentation, decrease in the total time of deep slow-wave sleep).

Conclusions: The model conforms to the key pathogenetic features of the early PD stage and can be applied to investigations of the brain performance at the preclinical stage of PD pathogenesis and to the search for non-motor signs for preventive PD treatment.

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Genetic and biochemical markers of Parkinson’s disease

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Parkinson’s disease (PD) is the second most frequent neurodegenerative disorder after Alzheimer’s disease. About 15% of PD patients suffer from a monogenic form of PD. An impaired metabolism of alpha-synuclein and its abnormal aggregation in neuronal cells is believed to be a critical step in the molecular pathogenesis of the disease. The levels of alpha-synuclein species (total, oligomeric) in CSF and plasma have been proposed as a possible biomarker for PD with inconsistent results, which may be due to disparities in methods, antibodies used, red blood cells (RBCs) and platelets contamination. The aim of our study is to find new genetic markers for PD in North-Western region of Russia and to estimate the possibility to use peripheral alpha-synuclein as suitable PD biomarker. We conducted the screening of LRRK2 G2019S, GBA mutations (L444P, N370S) and non-pathological variants (E326K, T369M) in 762 PD patients and in 400 controls from North-Western region of Russia. In 28 familial cases the sequence of coding region of the SNCA gene was conducted. 25 early-onset PD cases were screened using a 17 akinetic rigid autosomal-recessive PD genes targeted nest-generation sequencing. We identified 12 PD patients with LRRK2 G2019S mutation with the frequencies as 5.8% - in familial PD and - 0.5% in sporadic. The frequency of GBA mutations (L444P, N370S) within PD patients (2.23%) was 6.9 times higher than the 0.3% observed in controls (OR (L444P+ N370S) = 6.9 (95%CI:0.90-53.2) p=0.03). The frequency (4.9%) of E326K and T369M in PD patients was also higher than 2.5% in controls (OR (E326K and T369M) = 2.06(95%CI: 0.97-4.41), p=0.05), supporting that these two variants could modify risk of PD. No mutations were found in the coding region of the SNCA gene as well as in a series of akinetic rigid autosomal-recessive PD genes. Phenotypic analysis showed that GBA mutation carriers had earlier age at onset and cognitive impairment. Moreover, GBA-PD group was characterized with decrease in GBA enzymatic activity and increased lysosphingolipids level measured in dried blood spots (DBS) by LC-MS/MS as well as an increased level of plasma alpha-synuclein estimated be means of ELISA method. We also demonstrated the increased
level of alpha-synuclein (total, oligomeric) in CD45+ blood cells in patients with sporadic PD. LRRK2 and GBA mutations were found to be common risk factors for PD in North-Western region of Russia. Our results suggest that the decrease in enzymatic activity of lysosomal hydrolases in GBA mutation carriers may contribute to PD pathogenesis by increasing the level of lysosphingolipids as well as neurotoxic oligomeric alpha-synuclein species. CD45+ oligomeric alpha-synuclein level may be suggested as PD biomarker.
Whole-transcriptome analysis of an MPTP induced murine model of the earliest stages of Parkinson’s disease

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Because of the impossibility of studying the endogenous processes occurring in the brain of patients with Parkinson’s disease (PD) at early stages, one of the main approaches is to study the changes in the transcriptome in models that reproduce the earliest stages of PD. Models based on the administration of MPTP are the most frequently used. Administration of the MPTP reproduces the death of DAergic neurons in substantia nigra and the subsequent deficit of DA in striatum, as well as the manifestation of symptoms of PD. In our study we utilize a model with two- and four-fold administration of 12 mg/kg MPTP at 2-h intervals allows the modeling of presymptomatic and early symptomatic stages, leading to the loss of 35% and 43% of DA neurons, respectively, in the substantia nigra in 2 weeks after administration of the toxin. A complex analysis of the wholetranscriptome data of MPTP-induced models of the earliest stages of PD pathogenesis was conducted. We were compared the whole transcriptome pattern in four groups of animals after the administration of MPTP: 6, 24 hours and 2 weeks after four-fold administration of 12 mg/kg MPTP and 2 weeks after two-fold administration of 12 mg/kg MPTP The analysis revealed a minimal response of the transcriptome at 6 and 24 hours after four-fold administration of MPTP. At this time, the main changes in the development of pathological processes begin in the striatum and affect this structure to a greater extent than the substantia nigra in all stages modeled. More profound changes in gene expression pattern was wound in two weeks after MPTP administration. The cluster analyses indicate a complex picture of the response to developing neurodegeneration. The substantia nigra and striatum have unique patterns of changes in the representation of differentially represented transcripts (DRTs) at each stage. The data we obtained affirms the independent role of various brain structures, as well as individual parts of nerve cells in the formation of a response to the development of neurodegeneration during PD in the investigated models. The analysis of functional clustering of DRTs with both increased and decreased representation showed the presence of the
processes related to the functioning of mitochondria, transport, and apoptosis in groups of DRTs with either representation. This may indicate that these processes are involved in the development of compensatory effects in response to the administration of MPTP (upregulated expression) and neurodegenerative effects (downregulated expression). Meanwhile, only a fall in the levels of DRTs for the genes within the ubiquitin-binding cluster is observed. The fall in the expression of these genes may serve as a reason for the development of neurodegenerative processes, the accumulation of proteins, and the appearance of inclusions in the late stages of PD. Identifying the myelin sheath cluster during the functional clustering is extremely interesting. The reduction in the myelination of neuron projections can be significant in the earliest stages of the disease and may also be associated with initiating neurodegeneration in PD. The genes included in this cluster are candidates for PD pathogenesis.
Novel paradigm of the development of preclinical diagnosis of Parkinson's disease

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Motor symptoms first appear in Parkinson's disease (PD) many years after beginning of degradation of the nigrostriatal dopaminergic system at loss of threshold amount of dopamine (DA) in the striatum (70%) and depletion of neuroplasticity, which explains low efficiency of current treatment. Therefore, the development of the diagnostics of PD at the preclinical stage is a high priority. Considering the systemic pathogenesis of PD, current methodology is based mainly on finding biomarkers, such as non-motor clinical symptoms and changes in body fluids (blood, CSF) and blood cells in untreated PD patients at the early clinical stage, i.e. after the appearance of motor symptoms. A number of weak points makes this methodology doubtful: (i) there is no guarantee that biomarkers found in body fluids of patients at clinical stage are also characteristic of patients at preclinical stage; (ii) considering that individual biomarkers (non-motor symptoms, changes in body fluids) are non-specific or semi-specific, it is necessary to use a huge battery of biomarkers; (iii) the diagnostic procedure should be too expensive for preventive examination of healthy population. That is why, in addition to patients, we searched for biomarkers in the blood in MPTP-treated mice at the early symptomatic stage and presymptomatic stage of parkinsonism. According to our data, the concentration of some markers in plasma, e.g., L-DOPA, were modified in the same way in PD patients and mice at both stages of parkinsonism. The concentration of others, e.g., taurine differed at the presymptomatic stage in mice from those in mice at the symptomatic stage and patients. Apparently the former markers are much more specific than the latter. Moreover, in experimental models, we developed a new approach to preclinical diagnosis of PD by using a pharmacological provocation test (reversible inhibitor of dopamine synthesis), which induces a reversible increase in failure of the nigrostriatal system and a short-term appearance of motor disorders. The validity of this methodology for the development of preclinical diagnosis of PD was proven by systemic administration of a reversible inhibitor of dopamine synthesis to 1-methyl-4-phenyl-1,2,3,6-
tetrahydropyridine-treated mice at the presymptomatic stage of parkinsonism. Development of preclinical diagnostics of PD basing on the search for biomarkers in the blood in untreated PD patients and experimental models and the use of a provocative test would allow us to use neuroprotective pharmacotherapy for slowing down neurodegeneration and thereby prolongation of an asymptomatic period. Thus, we developed a novel methodology for the development of preclinical diagnosis of PD, basing on a search for biomarkers in blood in patients and experimental models, and a use of the provocation test.

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The use of transgenic methods to study proteinopathies

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Advances in new cell technologies, bioinformatics approaches, and methods of targeted manipulations with genomes led to a substantial progress in biomedicine. As a result, a large number of clinically different neurodegenerative disorders (NDD) were grouped under one term – proteinopathy, and proved to have pathological aggregation of proteins, formation of insoluble fibrillar structures, and their deposition in the form of histopathological inclusions in the nervous system. The major challenge in studying molecular aspects of proteinopathies lies in the multifactorial nature of disease states. Thus, in our studies we use genetic tools to induce aberrant protein aggregation at various levels ranging from cells to whole animal. Our unique transgenic models recapitulate essential NDD molecular pathology providing ample methods to study specific molecular targets as well as aiding the design and development of new therapeutic compounds that are aimed at modulating the key pathogenic step – protein aggregation and cell survival. Our cellular models of proteinopathies are based on the use of neuroblastoma cell line that is transfected with various types of expression vectors carrying an array of known disease-causing protein isoforms: Fus, Tdp-43, synucleins, etc. As a result, cellular models adequately reproduce protein aggregation providing a quick response for the optimization of compounds that are specifically suppressing different stages of the proteinopathy. Meanwhile, transgenic animal models with a specific neuronal overexpression of target proteins are used to establish the potential regulatory mechanisms as well as a long term effect of treatment with prospective compounds on cognitive functions and motor deficiency that are the hallmarks of NDD. In conclusion, over the past decades the use of various types of genetic models advanced our understanding of brain disorders in general, yet we are still limited in reproducing the overall pathogenic state of NDD due to its heterogeneity. Thus, the major upcoming challenges in NDD modeling will be focused on regulating of gene expression of pathogenic proteins and using multiple pathogenic factors to establish molecular links to uncover the relationships in disease states.
Alzheimer Disease
The YB-1 as a potential drug for treatment of Alzheimer's disease

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The Y-box binding protein 1 (YB-1) is a multifunctional DNA/RNA-binding protein with an evolutionarily conserved cold shock domain. It participates in the formation of the neural tube in embryogenesis, in the adult brain YB-1 is detected mainly in neurons of the hippocampus and neocortex, takes part in neurogenesis, and in vitro protects Tau-protein from oligomerization. Its level decreased with aging. The brain functions of YB-1 are not clear. We have proposed that the multifunctional protein YB-1 can protect brain from stress and memory pathology, such as Alzheimer's disease (AD). It is known that AD is a neurodegenerative disease of elderly people and characterized by dementia, induced by neuronal death in brain areas connected with learning and memory. Markers of AD are accumulation of the β-amyloid peptide and hyperphosphorylation of the Tau-protein, which form extracellular β-amyloid plaques and intracellular tangles in the brain. Neurotoxicity of β-amyloid oligomers and disruption of the axonal transport of mRNAs due to the loss of affinity of hyperphosphorylated Tau-protein to the microtubules, as well as low level of neurogenesis are probably causes of AD. We investigated YB-1 effects on the progress of Alzheimer's type neurodegeneration in two in vivo models: transgenic 5XFAD mice (a model of family AD) and olfactory bulbectomized (OBX) mice (sporadic AD model). In both models the chronic intranasal administration of YB-1 (2µg/mouse/day) retains learning ability, prevents memory loss, reduces the amount of β-amyloid, slows the formation of β-amyloid plaques in transgenic mice, and protects neurons from the death in OBX animals. In primary hippocampal culture YB-1 protects neurons from the cytotoxic effect of both the hTau-protein, secreted by 3T3-4R fibroblasts, and synthetic hβ-amyloid peptide. Intranasally administered fluorescently labeled YB-1 penetrates into the brain and localizes predominantly in the cytoplasm of neurons. Intranasally injected YB-1 "envelops" β-amyloid plaques and intracellular β-amyloid fibrils in the brain of transgenic 5XFAD mice. Moreover, YB-1 prevents the formation of β-amyloid fibrils in vitro and partially destroys already formed fibrils. It is interesting the chronic intranasal
administration of YB-1 had positive effect on longevity of wild NMRI mice. The obtained results allowed us to think about common mechanisms of ageing and AD and propose the multifunctional protein YB-1 as a potential drug for treatment of Alzheimer’s disease.

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Interplay of innate and cellular adaptive immunity in Alzheimer’s disease: therapeutic potential of T-cell-targeting immunomodulatory approaches

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Chronic neuroinflammation mediated by microglia and astrocytes is a key feature of Alzheimer’s disease (AD). Growing evidence indicate that such innate neuroinflammatory responses play a complex role in the pathophysiology of the disease, with both beneficial and detrimental impacts that may evolve along the course of disease progression. Besides such innate neuroinflammation, accumulating neuropathological and genetic data now also emphasize an instrumental role of cellular adaptive immunity in AD, although the impact and antigen specificity of the involved T cell populations remain poorly defined. Our recent studies in mouse models of AD-like amyloid and Tau pathology suggest that different T cell sub-populations may contribute to modulate disease progression, at least in part through the modulation of innate neuroinflammatory responses. Interestingly, our data highlight the therapeutic potential in AD of innovative immunotherapeutic strategies, based on T-cell-targeting peripheral immunomodulatory approaches for rebalancing detrimental innate neuroinflammation.
Molecular mechanisms of neuroplasticity / neurodegeneration underlying comorbidity of dementia and depression: "my mirror twin, my next of keen"

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Comorbidity of dementia and depression is frequent, specifically in older adults. The relationship between these pathological is widely discussed by clinicians as potential cause, consequence, or coincidence. The hypotheses and concepts seem different unless consequences of stressful life events and brain molecular mechanisms become the focal focus. Indeed, taking into account the array of neurochemical changes induced by stress, it is not surprising that stressful experiences may induce a variety of illnesses, including depression and neurodegeneration-related dementia. Initiated by glucocorticoid disturbances proinflammatory changes, alterations in neurotransmitter and neurotrophic factor systems, changes in neurogenesis underlie vascular pathology and hippocampal atrophy. Neuroplasticity and neurodegeneration are two sides of the same coin being closely related by common molecular mechanisms. From this perspective, studies on the mechanisms of depression and dementia comorbidity represent a very important piece of translational medicine aimed at the elaboration of evidence based therapy.
Alzheimer’s disease (AD) is closely associated with ageing. In view of the amyloid hypothesis, the key molecular event of AD is structural transition of β-amyloid (Aβ) from the physiologically normal monomer state to soluble neurotoxic oligomers accumulating in the form of insoluble extracellular aggregates (amyloid plaques) in brain tissues. Zinc ions as well as ‘aged’ Aβ species present in the plaques are known to play a pivotal role in triggering pathological conversion of endogenous Aβ. Isomerization of Asp7 is the most abundant age-related spontaneous non-enzymatic modification of Aβ. In silico, in vitro and in vivo studies have shown that Aβ species with isomerized Asp7 (isoAsp7-Aβ) significantly differ in their properties from healthy (non-modified) Aβ molecules [1-4]. We have found that isoAsp7-Aβ might constitute a nucleation seed and initiate formation of the neurotoxic zinc-dependent Aβ oligomers, thus inducing development of cerebral amyloidogenesis and other pathological processes characteristic of AD [5-7]. Moreover, the role of the Aβ metal-binding domain (the N-terminal region 1-16) as the minimal necessary and sufficient pathogenic unit of isoAsp7-Aβ has been strongly suggested [8]. Based on the molecular mechanism of zinc-dependent oligomerization of Aβ isoforms [9, 10], a potential site of zinc-mediated interactions of such oligomers with acetylcholine receptors was established. Intravenous injection of a synthetic analogue of this site led to a sharp slowdown in cerebral amyloidogenesis in the animal model of Alzheimer's disease. In combination, our data suggest that the interaction between isoAsp7-Aβ and acetylcholine receptors plays a crucial role in modulating cerebral amyloidogenesis in AD. Accordingly, suppression of such interactions is a promising strategy for the treatment of Alzheimer's disease.

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References:
In vivo therapeutic strategies against tau pathology: gene therapy and small molecules

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The microtubule-associated Tau protein belongs to the family of microtubule-associated proteins. A single gene and alternative splicing generates six human brain tau isoforms principally expressed in neuron. Besides regulating axonal microtubules architecture and dynamic, tau also play roles in other cell compartments, such as the nucleus and synapse. Intracellular accumulation of abnormally and hyperphosphorylated microtubule-associated tau proteins into filaments is leading to the so-called neurofibrillary degeneration (NFD), a neuropathological feature common to Tauopathies, including Alzheimer’s disease. From the gene to the protein many etiological factors, including mutations, splicing, phosphorylation and environmental factors contribute to the dysfunction of tau and abnormal accumulation. However, mechanisms leading to neuronal cell death remain ill-defined and transgenic mouse model enable to address several of these mechanisms. We will review our current tau-based therapeutic approaches to neurodegeneration and factors modulating NFD in our Thy-Tau22 transgenic mouse model. Tau mis-splicing either direct or indirect may contribute to neurodegeneration. Tau splicing can be modulated by splicing factors and could also be way to modulate tau toxic function. We will discuss innovative therapies such as novel family of small molecules. Since neurofibrillary degeneration is a common neuropathological process to more than twenty neurological disorders therapeutic strategies against Tau pathology could have a broad spectrum of applications.
Insufficient efficacy of current dementia treatment increases interest to cognitive impairment not prominent enough to achieve dementia level: mild cognitive impairment and pre-mild cognitive decline. Early diagnosis of cognitive impairment enhances prevention of dementia and could provide opportunity for early start of disease modifying treatment of neurodegenerative and other central nervous system disorders. Nowadays there is strong trend in behavioral neurology to label all cases of pre-mild cognitive decline as “subjective cognitive decline”. It was shown that the presence of this condition increases the risk of Alzheimer’s disease. However, previously, we as well as some other authors had described condition labeled as “subtle cognitive disorder” which differed significantly with normal data on some neuropsychological scores but did not match criteria for mild cognitive impairment. There is data evidenced heterogeneity of subjective cognitive decline also. We analyzed clinical and neuropsychological characteristics of 602 outpatients of our memory clinic, 424 women and 178 men, mean age 63,2±11,2 years, which did not match diagnostic criteria for mild cognitive impairment. Based on neuropsychological data, these patients were divided on two groups. The patients in first group did not differ significantly from age-matched normal control (144 patients). We labeled these patients as patients with “subjective memory decline”. In patients of 2nd group there were mild decrease of neuropsychological scores, which did not exceed 1 standard deviation from mean of normal age-matched control. These patients were labeled as patients with “subtle cognitive decline”. “Subjective cognitive decline” and “subtle cognitive decline” patients did not differ significantly with regard to age, gender, social, somatic and neurological characteristics. Neuropsychological scores of patients with “subtle cognitive decline” differed from normal scores in almost all neuropsychological tests applied. The most prominent differences were observed in MMSE and FAB scores, literal and category fluency. Trail making test, immediate and delayed cued recall scores differed also. Significance of division of patients with pre-mild cognitive impairment on two groups was supported by cluster and discriminative
analysis. At the same time, anxiety scores of groups did not differ significantly. Thus, according our data, pre-mild cognitive impairment is heterogeneous with regard to neuropsychological scores. Further follow-up with repeated neuroimaging and laboratory CSF evaluations is need for assessing of clinical significance of pre-mild cognitive impairment division on different types.
Brain development, cognitive functions and neurodegeneration after prenatal hypoxia

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Our multifunctional studies aim at elucidating the mechanisms involved in impairment of cognitive functions and development of neurodegeneration in individuals affected by prenatal stress, in particular, hypoxia. Using a model of acute prenatal normobaric hypoxia (7% O₂, 3 hours) on various days of gestation of rats we have demonstrated numerous changes in development of the brain, in particular, of the structures involved in learning and memory, which correlated with the deterioration of cognitive functions observed at various stages of postnatal life. The experimental data accumulated to date allow us to link this cognitive impairment caused by prenatal hypoxia with a decrease in the adaptive potential of the brain, changes in excitability and plasticity of the neocortex due to the disturbances in the process of formation of new contacts between neuronal cell populations during brain development and functioning. Together with increased amyloid precursor protein (APP) expression in rat brain caused by prenatal hypoxia and decrease in the activity of soluble AChE we also observed activation of caspase-3 and reduced levels of a major amyloid-degrading enzyme, neprilysin (NEP), and a transport protein transthyretin (TTR) correlating with decreased levels of a transcriptional regulator AICD (C-terminal fragment of APP produced alongside Aβ) which is readily cleaved by caspases. On the other hand prenatal hypoxia had a significant impact on the expression and activity of NEP and TTR involved not only in metabolism of amyloid-β peptide (Aβ) but also of a number of neuropeptides involved in regulation of brain functions. Since both NEP and TTR play an important role in Aβ clearance, alterations of their expression might lead to disruption of Aβ homeostasis leading to impaired brain functions and could result in accumulation of Aβ leading to development of neurodegeneration. Injections to animals subjected to prenatal hypoxia of...
such compounds as a caspase inhibitor Ac-DEVD-CHO, a histone deacetylase inhibitor sodium valproate or an antioxidant epigallocatechin gallate resulted in an increase of NEP expression and activity, the number of labile synaptopodin-positive dendritic spines and restoration of cognitive functions. This opens an avenue for the search for other compounds which can prevent accumulation of Aβ in the brain and improve cognitive functions and the model of rats subjected to prenatal hypoxia can be used as a tool for assessment of their efficacy.

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Huntington’s Disease
The developing Huntington’s disease brain

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The bulk of interest in the huntingtin protein has centered on the fact that, when mutated, huntingtin causes Huntington’s disease (HD), a devastating neurological disorder. The mutation causing HD is an abnormal polyglutamine stretch in huntingtin. Given the adult onset and dysfunction and death of adult neurons characterizing HD, most studies have focused on the toxic effects elicited by mutant huntingtin in post-mitotic neurons. However, the protein is ubiquitous and expressed in the developing embryo where it plays an essential role as revealed by the early embryonic lethality at day 7.5 of the complete knockout of the huntingtin gene in mouse. Anyway, the roles of the wild-type protein during development have been overlooked. I will discuss how huntingtin regulates several steps of mouse embryonic corticogenesis. I will also show the consequences of the presence of an abnormal polyglutamine expansion in huntingtin during cortical neurogenesis and consider the viewing of HD as a developmental disorder.
Huntington’s disease: deregulation of calcium homeostasis in patient-specific neuronal model

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Huntington's disease (HD) is an autosomal dominant hereditary neurodegenerative disorder which manifests in neural loss predominantly of GABA-ergic medium spiny neurons (GABA-MSNs) in striatum. HD is caused by the mutation in gene encoding huntingtin protein that leads to polyglutamine expansion within N-terminus region of this protein. Our studies of deregulation of the calcium homeostasis in the different cellular models of Huntington’s disease (human neuroblastoma SK-N-SH, mouse neuroblastoma Neuro-2a, primary culture of striatal neurons isolated from mice) indicated that neuronal store-operated calcium (SOC) channels play a significant role in HD pathogenesis and could be considered as a potential target for medical treatment. One of the most promising frontiers of the modern science is an investigation of disease models based on patient-specific induced pluripotent stem cells (iPSCs) that can be used in frames of the patient-oriented therapy paradigm.

Therefore, we examined the SOC currents levels in human GABA-MSNs differentiated from iPSCs, obtained by somatic reprogramming of patient-specific fibroblasts. We studied 3 different GABA-MSN lines from patients suffering from HD. As a control, we used 2 GABA-MSN lines from healthy subjects and 1 line of GABA-MSNs differentiated from healthy embryonic stem cells (ESCs). All HD-GABA-MSNs had an endogenous expression of mutant Huntingtin with low-length polyglutamine tract that differs from wild type protein by only a few additional glutamine residues. Nevertheless, electrophysiological recordings indicated the 2-fold increase in SOC current level in each of these HD cell lines compared to control cells. In addition we found no differences in SOC currents in control neurons differentiated from iPSCs or ESCs. Using molecular biological approaches such as allele-specific knockdown and lentiviral infection we established that increased currents mediated by SOC channels are caused by the mutant huntingtin expression. Further we showed that promising neuroprotective drug EVP4593 (quinazoline-derived compound) can decrease abnormal SOC entry both in
HD-GABA-MSNs and control GABA-MSNs. Our data indicate a validity and well-reproducibility of iPSCs-based HD model and strongly support previous findings of calcium deregulation in HD. Therefore, the iPSCs-based model could be considered as one of the most adequate HD models and may provide a useful platform for future fundamental studies of HD and drug development.

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Amyotrophic Lateral Sclerosis
Amyotrophic Lateral Sclerosis (ALS) is the most common adult-onset motor neuron disease. Although mainly sporadic, more than 20 genetic causes have been identified. Our team is interested in new causes and mechanisms implicated in ALS disease progression: We have analyzed the expression and contribution of ALS genes to motor neuron degeneration, have developed new ALS mouse models and have helped define the genetics of the French ALS population. Importantly, we have also shown that ALS is a non-cell autonomous disease with glial/immune cells surrounding motor neurons and especially microglial cells, implicated in disease progression in ALS mice. Therefore, our research focuses on the interactions between motor neurons and microglia/macrophages as contributors to the neurodegenerative process. Specifically, we have found that microglial/macrophage expression of the glutamate transporter system xc-, not expressed by motor neurons, was implicated in disease progression in ALS mice and modified microglial functions. We also showed that complement C1q stabilized synaptic loss in ALS mice. In order to analyze the contribution of ALS causing mutations directly in human motor neurons and the non-cell autonomous contribution of microglia/macrophages to motor neuron degeneration, we have used human motor neuron cultures derived from our French patient’s iPSc to compare, in the same conditions, human motor neurons carrying mutations in different ALS genes and sporadic cases. Complementary, we have also obtained blood-derived human macrophages from familial and sporadic cases to analyze their neurotoxic potential, with our main objective being to find new therapeutic targets to slow down ALS motor neuron degeneration.
Mechanisms of FUS mediated amyotrophic lateral sclerosis: insights from mouse genetics

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FUS mutations cause early, severe amyotrophic lateral sclerosis (ALS). In ALS-FUS patients, cytoplasmic FUS aggregates are often associated with loss of nuclear FUS. Whether loss of nuclear FUS function, gain of a cytoplasmic function, or a combination of both lead to neurodegeneration remains elusive. Furthermore, while motor neuron-extrinsic mechanisms have been shown to participate in the pathogenesis of ALS-SOD1, it remains unclear whether such mechanisms contribute to FUS-associated ALS. We generated knockin mice expressing mislocalized cytoplasmic FUS conditionnally and complete FUS knockout mice. In homozygous state, knock-in and knock-out FUS mice display perinatal lethality with respiratory insufficiency, and largely similar alterations in gene expression and mRNA splicing patterns, indicating that mislocalized FUS results in loss of its normal function. However, FUS knockin mice, but not FUS knockout mice, present reduced numbers of motor neurons at birth, which can be rescued by cell-specific CRE-mediated expression of wild-type FUS within motor neurons. Heterozygous FUS knock-in mice, but not mice heterozygous for a Fus null allele, develop similar pathology as ALS-FUS patients and a mild motor neuron phenotype. Most importantly, CRE-mediated rescue of the Fus mutation within motor neurons prevented degeneration of motor neurons, but only delayed appearance of motor symptoms. Interestingly, heterozygous FUS knock-in mice also develop progressive atrophy of the frontal and temporal lobes associated with social disinhibition. Thus, loss of FUS is not sufficient to drive motor neuron degeneration. Mislocalized FUS triggers toxic events in both motor neurons and neighboring cells to elicit motor neuron disease and fronto-temporal related deficits.
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Dysfunction of neuromuscular synaptic transmission in transgenic model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by degeneration of motoneurons and development of paralysis and atrophy of skeletal muscles. The rapid progression of the disease and the lack of effective treatment make ALS a serious medical and social problem of modern society. Several hypotheses of the ALS pathogenesis considering the interaction of the central and peripheral pathogenic mechanisms in the development of the disease are developed. In this study, we examined the time course of development of dysfunction of neuromuscular synaptic transmission, contractile dysfunction and impairment of electrogenesis of skeletal muscle fibers in the ALS transgenic mouse model of different ages. For ALS modeling transgenic mice expressing mutant human gene of Cu/Zn-superoxide dismutase SOD1 (mSOD1) were used. Experiments were done on diaphragmatic muscles of ALS transgenic mice at presymptomatic (3-4 months), symptomatic (6-7 months) and terminal (8 months) stages of the disease. In electrophysiological and fluorescent experiments, it was found that dysfunction of neuromuscular synaptic transmission characterized by decrease in the quantal content of endplate potentials and slowing of recycling of synaptic vesicles. Impairment of skeletal muscle electrogenesis and contraction was found as well. Thus, features of dysfunction of "nerve-muscle" system of ALS mice were found in all studied ages; the character of the dysfunction of "nerve-muscle" system depends on the stage of disease. The sequence of development of revealed disorders suggests that dysfunction of neuromuscular synaptic transmission in ALS model occurs earlier and is likely to provoke/promotes contractile dysfunction. Obtained results greatly expand our understanding of the mechanisms of ALS pathogenesis.

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Amyotrophic lateral sclerosis: new insights in pathogenesis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurologenerative disease, characterized by the degeneration of both upper and lower motor neurons in the brain and spinal cord. ALS is a complex disease associated with numerous pathologic mechanisms, including oxidative stress, mitochondrial dysfunction, excitotoxicity, axonal damage, protein aggregation, microglial activation and inflammation. Molecular and genetic heterogeneity is the main reason of lack of specific biomarkers and treatment in ALS. The majority of cases are sporadic and only 10% of cases of ALS demonstrate direct in the heritance (familial ALS). The most prominent genes where mutations have been identified are Cu/Zn superoxide dismutase 1 (SOD1) gene, TAR DNA binding protein (TARDBP), Fused in Sarcoma (FUS) and Chromosome 9 open reading frame 72 (C9orf72). 310 Russian patients (Research Center of Neurology, Moscow) with amyotrophic lateral sclerosis (ALS) including 29 familial ALS patients and 281 sporadic ALS patients were screened for mutations in SOD1 and C9orf72. Mutations in SOD1 were found in approximately 20,7% of familial ALS cases and approximately 5% of sporadic ALS cases. The most frequent mutation among familial ALS cases is L84V (10,3%), and the most frequent mutation among sporadic ALS cases is D90A (1,4%). A pathogenic hexanucleotide (G4C2) repeat expansion (>50 repeats) in the first intron of C9orf72 was found in approximately 6,9% of familial ALS and approximately 3,2% sporadic ALS cases. The search for biomarkers related to ALS is the other important field in ALS research, because at present current diagnostic measures rely upon only clinical examination and electrophysiological measurements, which in most cases enable early diagnosis of ALS. In the Centre of Neurology we have confirmed the results of other authors that increase of phosphorylated neurofilament heavy chains (pNFM) in the CSF of patients with ALS is the diagnostic marker for disease progression. The prominent feature of ALS is aggregation of more than 50 proteins and formation of pathological inclusions. 50% of these proteins are RNA – binding proteins with several functions in processing and maturation of RNAs. The mechanisms of aggregation of proteins are heterogenous, including
dysfunction of UPS (ubiquitin proteasome system) and autophagic process. In our Centre we have analysed the markers of autophagy in blood cells of patients with ALS and confirmed the disturbances of autophagic process, especially in rapid progressive forms. The understanding of mechanisms of selective vulnerability of motor neurons in ALS and disease progression are the major interesting aspects of ALS problem.
Meeting values:
address and transport
October 9 (Monday)

1. Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, 16/10 Miklukho-Maklaya str.
2. Bus: 145k, 261, 273, 295, 330, 404, 752, 816, s2; Station - “Vityaz Cinema”
3. Bus: 261, 330, 404; Station - “Belyaevo”
4. Subway: Station - “Belyaevo”
October 10 (Tuesday)

1. Central office of the Russian Federal Agency for Scientific Organizations & Russian Academy of Sciences; 32A Leninsky Prospect
2. Subway: Station - “Leninskiy Ave”
2nd Russian – French Workshop

October 11 (Wednesday)

1. Presidium of the Russian Academy of Sciences
   14 Leninsky Prospect
2. Bus: 4, 7, 111, 196, m1, m4, m4k, n1; Station - “Bol'nitsa Svyatitelya Aleksiya”