Satellite Symposium of the 9th FENS Forum of Neuroscience

Multiple mechanisms of neurodegeneration and progression

MENEDPRO (July 10-11 th, 2014)

VENUE:
Hotel Splendid – Baveno (Lago Maggiore)
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Isola Bella and Palazzo Borromeo
(Lake Maggiore)

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Introduction

Neurodegenerative disorders are relentless progressive diseases and produce care-dependency as well as important health-related public comsumption. So far, we don't know the cause of these disorders and then there are not successful treatments available and some of the drugs use don symptomatic treatments, over time universally become ineffective. Thus, the need to develop more effective treatments for the neurodegenerative disorders is widely recognized, however, as we don't still know the ultimate cause it is difficult to. Moroever, as the degeneration progresses, patients become more and more dependent of the family as well as of the social security, and the truth is that for these diseases there is currently no cure.

The pathogenesis of different neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's (PD) shares several common features. One of these is the abnormal accumulation and aggregation of disease-specific proteins, which is suggested to lead to neurodegeneration. Recent evidence also indicates that the aggregated proteins may spread from one cell or brain area to another and function as seeds to instigate protein misfolding and aggregation in these previously unaffected cells or areas. Genetic mutations or different environmental factors, such as oxidative or metabolic stress, have been suggested to promote protein mis-folding and aggregation in different neurodegenerative diseases. In fact, the combination of genetic mutations and enviromental factors can induce protein misfolding and aggregation; however, the exact underlying mechanisms of protein aggregation in different neurodegenerative disorders are still not completely understood.

The degeneration factors together with physiological aging and other factors involved in the pathogenic mechanisms underlying these neurodegenerative disorders (such as inflammation and oxidative or metabolic stress and pathogenic disease-associated mutations) could play an important role in determining the onset and progression of the disease and finally causing widespread neuro degeneration in specific brain regions.

Then, this sattelite event looks for discuss the multiple mechanisms of neurodegeneration in different diseases as well as to analyze the posible causes of the cell death progression. This may provide novel opportunities to better understand the disease pathogenesis and subsequently to identify new disease biomarkers and therapeutic targets for an earlier diagnosis and treatment of patients suffering from different neurodegenerative disorders. The speakers will be able to discuss offering their expertise in several fields of neurodegeneration and all together can explain to each other some causes of the gradual progression of the disease pathology in the brain over time (at least in the case of the most prevalent neurodegenerative disorders).

SCIENTIFIC PROGRAM

Multiple mechanisms of neurodegeneration and progression

(MENEDPRO)

JULY 10TH

PATHOGENESIS OF DIFFERENT NEURODEGENERATIVE DISEASES

09.00–11.00 h:

TOPIC I - Prenatal Factors.

- **Marta C. Antonelli.**
  Prenatal stress imprinting in the development of dopamine related neurodegenerative diseases.
- **Sandra Ceccatelli.**
  Long-term effects of mild neurodevelopmental insults.
- **Andrew Tasker.**
  Neonatal origins of progressive neurodegeneration and brain dysfunction.
- **Micaela Morelli.**
  Environmental factors influencing MDMA neurotoxicity and neuroinflammation: role of age.
- **Student presentation.**
  Title TBC.

11.00–11.20 h: Coffee-break.

11.20–13.30 h:

TOPIC II - Chemical stress.

- **Alessandro Usiello.**
  Role of D-aspartate in brain aging.
- **Juan Segura Aguilar.**
  Dopamine oxidation and Parkinson´s disease.
- **Luigi Zecca.**
  The role of neuromelanin organelle pathways in Parkinson's disease.
- **Rita Raisman Vozari.**
  Could antibiotics be used to fight neurodegeneration?
- **Student presentation.**
  Title TBC.

13.30–15.30 h: Lunch. Tables will be organised distributing each two speakers with a group of students.

15.30–17.30 h:

TOPIC III - Genetic and epigenetic factors.

- **Stefano Gustincich.**
  Long non-coding RNA in neurodegeneration.
- **Harry Steinbusch.**
  Epigenetic changes in neurodegeneration. Brainstem involvement in neurodegeneration.
- **Rosario Moratalla.**
  Plasticity mechanisms induced in the striatum after dopaminergic degeneration in animal models.
- **Dora Reglodi.**
  Recent advances in the neuroprotective effect of PACAP in models of Parkinson's disease.
- **Student presentation.**
  Title TBC.

17.30–17.50 h: Coffee.

17.50–18.50 h: General open discussion of the causes of degeneration.

20.30 h: Social Dinner.

22.00 h: Young Investigator Award and diploma (presentation).

Isola Bella and Palazzo Borromeo (Lake Maggiore)

JULY 11TH
NEURODEGENERATION'S PROGRESSION

08.30–10.30 h:

TOPIC IV - Inflammation and the immune system.

- **Gilles Guillemin.**
  Involvement of the kynurenine pathway in neurodegenerative disorders.
- **Malu Tansey.**
  The role of the immune system in risk and progression of Parkinson's disease.
- **Anna Carta.**
  Modulating microglia activity through PPAR-: a new target for neuroprotection.
- **María Trinidad Herrero.**
  Matalloproteases, synaptosomes and progression of the neurodegeneration.
- **Anthony Tsarbopoulus.**
  Protein aggregation processes involved in *Alzheimer's Disease* and novel molecules.

10.30–10.50 h: Break.

10.50–12.50 h:

TOPIC V - Traslational and prevention aspects.

- **Benjamin Dehay.**
  Targeting the unfolded protein response in neurodegenerative diseases.
- **Michael Zigmond.**
  Slowing the progression of Parkinson's disease though physical exercise.
- **Silvia Mandel.**
  Strategies for the prevention of neurodegenerative diseases.
- **Peter Jenner.**
  New directions in the research and treatment of neurodegenerative diseases.

12.50–13.00 h: Concluding remarks.

End of the MENEDPRO FENS Satellite Symposium.

ABSTRACTS
Oral presentations

Prenatal stress imprinting in the development of dopamine related neurodegenerative diseases

Marta C. Antonelli

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Facultad de Medicina. Universidad de Buenos Aires. Buenos Aires. ARGENTINA

Prenatal stress exerts a strong impact on fetal brain development in rats impairing adaptation to stressful conditions, subsequent vulnerability to anxiety, altered sexual function and enhanced propensity to self-administer drugs. Most of these alterations have been attributed to changes in the neurotransmitter dopamine (DA). In humans, dysfunction of the dopaminergic system is associated with development of several neurological disorders such as Parkinson disease, schizophrenia, attention-deficit hyperactivity disorder and depression. Evidences provided by animal research, as well as retrospective studies in humans, pointed out that exposure to adverse events in early life can alter adult behaviors and neurochemical indicators of midbrain DA activity, suggesting that the development of the DA system is sensitive to disruption by exposure to early stressors. In our laboratory, repeated restraint during the last week of pregnancy was used as a model of prenatal stress in rats. The offspring were tested at different postnatal ages to evaluate several aspects of the dopaminergic metabolism. Our results show that prenatal stress exerts impairments on the D2 dopamine receptors, dopamine release, specific transcription factors and tyrosine hydroxylase (TH) as well as morphological disruptions in dopamine related areas. These changes were mostly observed after puberty suggesting that perinatal events might render the DA circuitry more vulnerable to puberty variation of the hormonal circulating levels. Moreover, most effects were found in limbic areas, an observation that we confirm when we observed that cells expressing TH in the ventral tegmental area were more susceptible than motor areas to a neurochemical insult with 6-hydroxydopamine (6-OHDA). Taking into consideration our results and those of the literature, we speculate that changes exerted on the dopaminergic limbic system by prenatal insults might mainly be associate with behavioural disorders or neuropsychiatric pathologies with adolescent or young adult onset.

Long-term effects of mild neurodevelopmental insults

Sandra Ceccatelli

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Exposure to adverse environmental cues in early life can influence the risk of later disease, a concept defined as ''early life programming''. Prenatal exposures to drugs and chemicals are among the insults that can alter fetal programming. Using a combined strategy ranging from cellular to behavioural analyses, we have investigated the long-lasting effects of prenatal exposure to adverse developmental stimuli. My presentation will give an overview of our major *in vitro* and *in vivo* studies on the effects of exposure to glucocorticoids or chemical contaminants (i.e. methylmercury). By using neural stem cells, we have been able to identify relevant mechanisms underlying developmental neurotoxicity, including altered expression of cell cycle regulators, senescence-associated markers and genes encoding mitochondrial respiratory chain proteins. These alterations are associated to increased susceptibility to oxidative stress and higher levels of intracellular reactive oxygen species. We performed genome-wide analysis of differentially methylated DNA regions (DMRs) in neural stem cells. We selected a number of genes identified as DMR-enriched for validation of the changes in methylation pattern, and found that the changes are long-lasting and persist in ''daughter'' cells never directly exposed to the insults. The results point to epigenetic modifications playing a critical role in the programming effects leading to long-term consequences, which can predispose to neurodevelopmental disorders and/or neurodegeneration.

Neonatal origins of progressive neurodegeneration and brain dysfunction

R. Andrew Tasker[1], Amber L. Marriott[1], Daphne A. Gill[1], Anabel Perez-Gomez[1] and Tracy A. Doucette[2]

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Many neurological diseases and disorders, including epilepsy and schizophrenia, are known to often originate during perinatal life. Studies of populations with greater than normal prevalence have identified genetic abnormalities associated with increased risk, but chemical and/or environmental insults at the time of birth or during infancy have been shown to initiate a currently ill-defined neurodegenerative process that culminates in a clinical phenotype. For the past 10 years we have been investigating a rat model in which we use low doses of the AMPA/kainate agonist domoic acid (DOM) as a chemical manipulation of brain development during the second week of postnatal life. Longitudinal studies from 1-18 months of age in these rats have revealed a reproducible and time-dependent sequence of neuropathological, neurochemical and neurobehavioural abnormalities consistent with a slowly progressing model of epilepsy and schizophrenia co-morbidity. More recently we have combined DOM treatment with post-weaning social isolation to investigate the ''two hit'' hypothesis of neuropsychiatric disease, and have conducted parallel studies of hippocampal development in the presence of low concentrations of DOM using organotypic hippocampal slice cultures to reveal molecular mechanisms of progressive neurodegeneration and neuroplasticity. This presentation will summarize our published work to date on these models and present our most recent and currently unpublished findings on the long-term consequences of altered brain development in rats with relevance to progressive neuropsychiatric disease.

Research supported by NSERC, Innovation PEI and the Atlantic Innovation Fund. ALM is the recipient of an NSERC Canada Graduate Scholarship and the Regis Duffy Graduate Scholarship. TAD is supported by the Jeanne and J Louis Levesque Research Professorship.

Environmental factors influencing MDMA neurotoxicity and neuroinflammation: role of age

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Clinical observations report a greater propensity to develop Parkinson's disease (PD) in amphetamine users. 3,4-Methylenedioxymethamphetamine (MDMA; "ecstasy") is an amphetamine-related drug that is largely consumed by adolescents which may have neuroinflammatory and neurotoxic effects.

In order to evaluated in mice whether consumption of MDMA during adolescence induces neuroinflammatory and neurotoxic effects later in life and if these effects are influenced by the combined administration of caffeine, a substance often associated with MDMA consumption, we studied in young and adult mice the effects of a treatment with MDMA either acutely or chronically, alone or in combination with caffeine. MPTP was administered in adult mice treated during adolescence with MDMA. Glial activation was studied through GFAP (astroglia) or CD11b (microglia), while dopamine neuron degeneration was evaluated through tyrosine hydroxylase (TH), in striatum and substantia nigra (SN).

Caffeine in association with MDMA worsened dopamine neuron degeneration elicited by MDMA in adolescent but not in adult mice in striatum and SN and potentiated MDMA-induced astroglia without altering microglia in adolescent mice. Moreover, chronic administration of MDMA during late adolescence exacerbated the neurodegeneration and neuroinflammation caused by MPTP.

The results demonstrate that the utilization of caffeine in association with MDMA during adolescence may worsen the toxicity elicited by MDMA and that chronic administration of MDMA during adolescence exacerbates the neurodegeneration and neuroinflammation caused by MPTP.

Role of d-aspartate in brain aging

Alessandro Usiello

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D-aspartate (D-Asp) is highly expressed in the whole brain during embryonic period, to strongly decrease at adulthood. The drastic postnatal decline of D-Asp levels in the mammalian brain has been correlated with the onset of D-Aspartate Oxidase (DDO) activity, the only enzyme that selectively metabolizes this D-amino acid and directly regulates its endogenous levels. To understand the biological significance of this D-amino acid in mammalian brain a mouse model with deregulated high levels of D-Asp have been generated by *Ddo* gene-targeting. Such genetic manipulations produced a significant increase of D-Asp in adult brain regions, like hippocampus, cortex and striatum, which normally display very low levels of this molecule. In line with early *in vitro* binding studies, we show that higher brain levels of D-Asp in these mice have unmasked a robust modulatory role for this atypical amino acid at N-Methyl D-Aspartate receptors (NMDARs). Accordingly, augmented D-Asp content through stimulation of NMDARs is able to greatly increase NMDAR-dependent Long-Term Potentiation (LTP) and to facilitate spatial memory in young DDO mutant mice.

On the other hand, prolonged deregulation of D-asp content in adult animals induced a dramatic acceleration in the age-dependent decay of hippocampal functions and morpholgy.

In conclusion, our findings indicate that D-Asp in the mammalian brain acts as an endogenous NMDAR agonist and, in turn, highlight a strong implication for the "ancient" enzyme DDO in preventing an aberrant over-usage of NMDARs associated to a precocious deterioration of hippocampus-dependent functions.

Role of dopamine oxidation in Parkinson´s disease

Juan Segura-Aguilar, Patricia Muñoz, Irmgard Paris, Monica Villa, Sandro Huenchuguala, Carlos Cuevas and Andrea Herrera

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The molecular mechanism involved in neurodegeneration of dopaminergic neurons containing neuromelanin lost in Parkinson's disease that induce motor symptoms still remain unclear. However, the discovery of certain proteins associated with familiar Parkinson´s disease such as alpha synuclein, parkin, DJ-1, LRRK2, etc. have been an enormous advance in this field and there is a general agreement that mitochondrial dysfunction, alpha-synuclein aggregation to neurotoxic protofibrils, protein degradation dysfunction, oxidative stress and neuroinflammation are involved in the molecular mechanism for degeneration of dopaminergic neurons containing neuromelanin in Parkinson´s disease. The slow process of degeneration and disease progression suggest that an endogenous neurotoxin must be responsible for the loss of dopaminergic neurons containing neuromelanin. Dopamine oxidation to aminochrome and its polymerization to neuromelanin involve the formation of three *o*-quinones: dopamine *o*-quinone, aminochrome and 5,6-indolequinone that are formed in a sequential manner. These *o*-quinones have been proposed to be direct involved in the degenerative process of dopaminergic neurons in Parkinson´s disease. Aminochrome the most stable of these *o*-quinones have been found to induce (i) mitochondria dysfunction by inactivation complex I; (ii) dysfunction of autophagy by inhibiting the formation of microtubules required for the fusion of autophagosomes and lysosomes; (iii) dysfunction of lysosomes by increasing its pH; (iv) oxidative stress by forming hydroxyl radicals; (iv) the formation and stabilization of neurotoxic alpha synuclein protofibrils. Evidences obtained from cell cultures and *in vivo* experiments are presented. Our result support the idea that dopamine oxidation play a essential role in the lost of dopaminergic neurons containing neuromelanin. Supported by FONDECYT 1100165.

The role of neuromelanin organelle pathways in parkinson's disease

Luigi Zecca[1], Carolina Cebrián[2], Emanuele Ferrari[1], Chiara Bellei[1], Francesca A. Cupaioli[1], Antonella De Palma[1], Pierluigi Mauri[1], Luigi Casella[1], David Sulzer[4] and Fabio A. Zucca[1]

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The accumulation of organelles containing neuromelanin occurs during aging in different brain regions, particularly in substantia nigra (SN) and locus coeruleus (LC), which are mainly targeted in Parkinson's disease (PD). Investigating aging mechanism is pivotal for understanding neurodegenerative processes of PD. To this end, we have studied protein and lipid pathways of neuromelanin organelles of human SN by LC-MS, western blot and immunoelectron microscopy to clarify their biosynthetic pathways and role in neurodegeneration. Neuromelanin organelle appears as abnormal lysosome with reduced enzymatic activity and capability to fuse with lysosomes or autphagosomes. Typical proteins and double membrane show that this organelle has autophagic nature and engulfs neuromelanin precursors, proteins and lipids from cytosol. Neuromelanin synthesis starts in cytosol from dopamine-protein adducts oxidized to form protein-melanin compounds which bind metals, then uptaken into organelle. Here this complex is cleaved by proteases and reacts with dolichols to form neuromelanin.

In SN and LC catecholamine neurons, as well as in their neuromelanin organelles, we found high levels of major histocompatibility class I complex (MHC-I), which can bind antigens derived from foreign proteins, presenting them on neuronal membrane. Then $CD8^+$ cytotoxic T-cells, which were observed in proximity of MHC-I presenting neurons of SN and LC in both control and PD subjects, can target these neurons inducing neuronal death. Infiltration of T-cells occurs in SN and LC of PD subjects. The presence of MHC-I in catecholamine neurons containing neuromelanin could explain their selective vulnerability in PD, revealing a novel inflammatory T-cell mediated neurodegenerative process of PD.

Could antibiotics be used to fight neurodegeneration?

Rita Raisman Vozari[1] and Elaine Delbel[2]

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Parkinson's disease (PD) is one of the most common progressively disabling neurodegenerative disorders characterized by progressive motor decline accompanied by other déficits. Research aims to find substances that exert protective effects on dopaminergic neurons with the goal of slowing down their degeneration. Microglial activation is a prominent feature of PD and also induced by lesioning dopaminergic neurons in experimental models.

Doxycycline, a tetracycline-derivative, has been shown to be neuroprotective in *in vitro* and *in vivo* models of neurodegenerative diseases however the mechanism of protective effect of doxycycline seems be distinct from its antimicrobial action. It has been associated with inhibition of matrix metalloproteases, nitric oxide synthase and interleukin-1b-converting enzyme and cleaved caspase-3 protein expression. Doxycycline is one of the tetracyclines most commonly used in conditional transgene expression systems in lab animals and is also used for the treatment of central nervous system infections as its high lipid solubility results in good brain penetration.

We first demonstrated that doxycycline confers neuroprotection in a PD mouse model by restraining glial cell activation and secondly we explored the potential of doxycycline against neuroinflammation, using microglial cell-enriched cultures prepared from post-natal mouse brain. More specifically, we evaluated the impact that a 4 hour pretreatment with doxycycline exerts on microglial cells exposed for the next 24 hours to the bacterial inflammogen LPS (1 ng/ml). Our results show that the calcium-binding protein Iba-1 used as a marker of microglial cell activation, was detectable in 80% Mac-1+ cells after LPS treatment and that this number dropped to 20% with an optimal concentration of doxycycline, i.e., 200µM. Coherent with this result, doxycycline decreased the release of two pro-inflammatory cytokines, TNF-α and IL-1β and that of nitric oxide, a gaseous mediator of neuroinflammatory responses. Finally, doxycycline was also found highly effective in a situation where microglial cells were exposed to 10 ng/ml LPS, i.e., a concentration of the inflammogen required to adequately stimulate the production reactive oxygen species in microglial cells. Taken together our results suggest that doxycycline could operate as an efficient neuroprotective agent in neurodegenerative processes through a repressive effect on microglial cells.

Antisense transcription in neurodegenerative diseases

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ENCODE and FANTOM projects have been proving that the majority of the mammalian genome is transcribed generating a vast repertoire of transcripts that includes mRNAs, long non-coding RNA (lncRNA) and repetitive sequences, such as SINEs (short interspersed nuclear element) and LINE (long interspersed nuclear element).

We have combined transgenic labeling, Laser Capture Microdissection and nanoCAGE (Cap Analysis Gene Expression) to describe the transcriptional landscape of dopaminergic A9 neurons of the Substantia Nigra, the cells that degenerate in Parkinson's disease.

Together with the identification of the unconventional expression of alpha- and beta- chains of hemoglobin as well as of olfactory receptors, we have devoted special attention to gene networks discovery and to the expression of repetitive elements.

Analyzing the non-coding part of the transcriptome, we have identified a group of natural and synthetic antisense non-coding RNAs that activate translation of their sense protein-encoding genes. These molecules have been named SINEUPs since their function requires the activity of an embedded inverted SINEB2 sequence to UP-regulate translation. SINEUPs are thus the first example of gene-specific inducers adding an unexpected layer to post-transcriptional gene regulation and providing a versatile tool to increase protein synthesis of potentially any gene of interest.

By taking advantage of FANTOM5 database, we have mapped antisense transcription in the large majority of genes involved in hereditary neurodegenerative diseases identifying 37 previously unknown transcripts.

Brainstem Dysfunction in Neurodevelopmental and Neuropsychiatric Disorders - Moving from the Forebrain to the Brainstem

Harry W. M. Steinbusch[1,3], Daniel Van den Hove[1,3], Fred W. Van Leeuwen[1,3] and David A. Hopkins[2]

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Despite the role of the brainstem in regulating vital functions such as arousal, breathing, and autonomic nervous system activity as well as regulating higher cerebral functions via neurotransmitter projections systems originating in the brainstem, this structure has received little attention in most neuropsychiatric disorders. In the brainstem, the raphe nuclei, are comprised mainly of serotonin-producing neurons that project to the forebrain. In addition, the locus coeruleus, the substantia nigra and the tuberomammillary nuclei containing noradrenergic, dopaminergic and histamine-producing neurons, respectively, give rise to ascending projections.

The incidence of neurodegenerative disorders (ND's), i.e. AD, PD and MD are increasing. Exposure to stress is related to MD's and also with ND's. Stress, probably through toxic effects of corticosterone, decreases neurogenesis and cell survival while antidepressants enhance these processes. Most theories on developmental and degenerative neuropsychiatric illnesses pose that the central pathologies occur in forebrain regions and the limbic system. However, neurons projecting from brainstem nuclei to forebrain regions are among the first showing signs of neurodegeneration. In the area of depression, several observations have been made in relation to changes in one particular brain structure: the dorsal raphe nucleus (DRN). The DRN is also related in the circuit of stress regulated processes and cognitive events. We will demonstrated the neuroanatomical basis of the brainstem dysfunctioning in connection with forebrain regions using molecular, cellular and imaging technologies, in neurodevelopmental (i.e. autism, schizophrenia) and ND's, consequences related to vegetative aberrations such as respiratory dysfunction of AD and PD patients.

Methamphetamine causes dopamine cell loss in the mouse evidenced by silver staining

Sara Ares-Santos, Noelia Granado and Rosario Moratalla

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Our research shows that methamphetamine, an illicit drug abused by 14-50 million people worldwide, can cause irreversible loss of dopaminergic neurons in the substantia nigra. Since loss of this specific population of nerve cells is the main cause of Parkinson's Disease, our results offer a plausible explanation for the finding that methamphetamine abusers have a significantly higher risk of developing Parkinson's Disease. It has been clear for some time that methamphetamine causes persistent loss of dopamine fibers. Our study addresses a long-standing question in the field: whether methamphetamine destroys not just the fibers, but also the cell bodies in the substantia nigra, causing permanent damage. Our study is the first to provide conclusive evidence that methamphetamine kills dopamine neurons in the substantia nigra. We studied the effect of a single high dose or multiple low doses of methamphetamine on dopaminergic neurotoxicity. The integrity of dopaminergic fibers and cell bodies was evaluated 3 and 12 hours and 1, 3, 7 and 30 days after methamphetamine administration, using a marker to identify dopaminergic neurons and amino-cupric-silver staining to identify degenerating cell bodies and fibers. Strikingly, both treatment protocols resulted in a progressive death of the dopaminergic neurons in the substantia nigra, with 7-15% of these neurons degenerating 1 and 3 days post-methamphetamine, with further small loss of neurons at 7 and 30 days. The total loss of dopamine neurons was 20-25% at 7 or 30 days. Thus, this is the first demonstration of irreversible methamphetamine-induced neuronal loss. As shown previously, both administration protocols also caused significant loss of striatal dopaminergic fibers. Multiple low doses of methamphetamine induced more fiber loss than a single high dose, with the greatest loss occurring 1 day postreatment, followed by a progressive recovery. Despite partial recovery, some deficits in dopamine fibers persisted 30 days after treatment. This neuronal damage had functional consequences: mice exhibited a drastic decrease in movement and motor coordination 1 to 3 days after drug delivery, coincident with the peak nerve fiber loss. Motor activity and motor coordination recovered 7 days after methamphetamine, in parallel with the partial recovery of dopamine nerve fibers. The extent of neuron loss we observed is not sufficient to induce parkinsonism on its own, since the clinical symptoms of Parkinson's Disease appear when there is greater than 60% loss of dopamine. However, the neuronal loss and fiber damage caused by methamphetamine use likely confers a persistent vulnerability to subsequent insults, increasing the risk of developing Parkinson's Disease. Funded by: The Spanish Ministries de Economia y Competitividad, grant BFU 2010-20664 and ISCIII, CIBERNED, grant CB06/05/0055, PNSD and Comunidad de Madrid ref # S2011/BMD-2336.

Recent advances in the neuroprotective effect of PACAP in models of Parkinson's disease

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a neuropeptide originally isolated from the hypothalamus based on its cAMP-elevating effect in the pituitary. During the last 20 years a vast amount of evidence has emerged showing that PACAP acts on different receptors and has diverse biological effects. One of the well-studied effects of this neuropeptide is the strong neuroprotective effect. Its neuroprotective action was first shown in global and focal cerebral ischemia, and subsequently in numerous different in vitro and in vivo models of nervous system pathologies. In vitro, the peptide has been shown to protect neurons and glial cells against oxidative stress, hypoxia, different toxic agents, ceramide and ethanol. In vivo, PACAP has protective effects in animal models of Parkinson's disease, Huntington chorea, retinal degeneration, multiple sclerosis, traumatic brain injury and spinal cord injury. The aim of the present study is to show recent progress in the neuroprotective effects of PACAP. In Parkinson`s disease, our laboratory and others have demonstrated that PACAP-deficient mice show higher susceptibility to toxic agents causing degeneration of the substantia nigra dopaminergic neurons. Using mass spectrometry we have revealed that the expression of numerous proteins is altered in the mesencephalon and striatum of knockout mice. These proteins include ones playing a role in homeostatis/energy balance of the brain and others important in neuronal degeneration. In vitro, we have shown that PACAP is protective against salsolinol-induced neuronal death (an in vitro model of Parkinson`s disease), involving decreases in caspase-3 levels and increases in brain-derived neurotrophic factor and CREB phosphorylation. In summary, recent results support the neuroprotective effects of both endogenous and exogenous PACAP in Parkinson's disease and provide further insight into its antiapoptotic effects in neuronal cells.

This study was supported by OTKA K104984, and TAMOP (4.2.2.A- 11/1/KONV-2012-0024), the European Union and the State of Hungary, and cofinanced by the European Social Fund in the framework of TAMOP 4.2.4.A/2-11-1-2012-0001 "National Excellence Program," Magyary Zoltan Scholarship, Arimura Foundation, PTE-MTA "Lendulet" Program and Bolyai Scholarship, NIH/NIGMS 2 S06 GM08016-39. KTIA-NAP-13-1-2013-0001.

Kynurenine pathway in brain immune cells and its involvement in neuroinflammatory diseases

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The kynurenine pathway (KP) of tryptophan metabolism is one of the major regulatory mechanisms of the immune response. Activation of the KP is implicated in the pathogenesis of a wide range of neuroinflammatory diseases. Several pro-inflammatory mediators can activate indoleamine 2,3 dioxygenase (IDO-1) one of the first and regulatory enzymes of the KP. A prolonged activation of the KP leads to production and accumulation of several neuroactive metabolites including the potent excitotoxin quinolinic acid (QUIN). Every brain cell types appear to express differently the KP enzymes and producing different KP metabolites. Neurons, astrocytes, oligodendrocytes and brain microvascular endothelial cells produce neuroprotective compounds whereas activated microglia, pericytes, infiltrating macrophages synthesize and release neurotoxic KP metabolites. We have shown that the KP is activated and QUIN level in most of the major neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis…) and neuropsychiatric disorders (depression, suicidality, schizophrenia, autism…). This dynamic and complex interplay between KP metabolites from different brain and immune cells is directly involved in the global and progressive inflammatory response involved in the neuropathogenesis of major neurodegenerative diseases and psychiatric disorders.

The role of the immune system in risk and progression of parkinson's disease

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Interactions between environmental triggers and genetic susceptibility genes determine relative risk for PD. Multiple genome-wide association studies report an association between risk for late-onset PD and a common genetic variant in the human leukocyte antigen *(HLA) DRA* gene that is part of the major histocompatibility complex class II (MHC-II) locus in humans involved in antigen presentation for immune defense. Homozygosity for G at the non-coding single nucleotide polymorphism (SNP) rs3129882 in *HLA-DRA* increases one's risk for PD by 1.7-fold. We hypothesized that the GG SNP, present in 40% of the population, would be associated with altered expression of MHC-II in B cells and monocytes, the major types of antigen presenting cells in blood. We isolated B cells and monocytes from healthy controls (HC) and PD patients who were either GG (high-risk) or AA (low-risk) genotype at this SNP. We found that the high-risk allele was associated with increased cell surface expression of HLA-DR and greater inducibility of HLA-DQ expression in HCs, suggesting that the GG SNP may be a marker of immune hyper-responsiveness. Moreover, mRNA expression of MHC-II β-subunit genes was increased 20-30 fold in GG HCs relative to AA HCs; while the inducibility of all measured MHC class II mRNAs was increased 200-300 fold in monocytes from GG PD compared to AA PD patients. Use of this SNP together with peripheral blood immunophenotyping may help identify individuals at higher risk for PD whose differential presentation of pathogenic, immunodominant antigens may result in increased tendency towards generation of pro-inflammatory responses.

Modulating microglia activity through PPAR-γ: a new target for neuroprotection

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Progression of neurodegeneration in Parkinson's disease (PD) is largely attributed to chronic neuroinflammation. Recent findings have suggested that activated microglia may polarize to a pro-inflammatory M1 phenotype or the alternative anti-inflammatory M2 phenotype, via cytokine production. Using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-probenecid (MPTPp) mouse model of progressive PD and fluorescent immunohistochemistry, we evaluate changes in the M1/M2 microglia ratio along with neurodegeneration, by measuring changes in pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, and anti-inflammatory cytokines, such as transforming growth factor (TGF)-β and IL-10, within Iba-1-positive cells in the substantia nigra compacta (SNc). While a prevalence of microglia expressing anti-inflammatory over pro-inflammatory cytokines was detected in control mice, the neurotoxin treatment was associated with a gradual increase of pro-inflammatory over anti-inflammatory microglia, suggesting that a skewed M1/M2 polarization was associated with disease progression. To investigate whether modulation of microglia polarization may be a therapeutic target for neuroprotection, we evaluated the effect of the peroxisome-proliferator-activated receptor (PPAR)-γ agonist rosiglitazone, which is neuroprotective in PD models and acts as a modulator of peripheral macrophages polarization. Coadministration of rosiglitazone during the last 10 days of MPTPp treatment arrested nigral neurodegeneration, reduced pro-inflammatory cytokines while increasing anti-inflammatory cytokines as compared with the MPTPp treatment. Therefore, the neuroprotective treatment with PPAR-γ agonist modulated microglia polarization correcting the imbalance between M1 over M2 associated-cytokines, offering a novel immunomodulatory approach to neuroprotection.

MMP-9, inflammation and Parkinsonism

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Inflammation, manifested by glia activation and expression of pro-inflammatory mediators, is a predominant aspect of neurodegenerative diseases being important in its progression. Matrix metalloproteinases (MMPs) are extracelular proteases that finely modulate several physiological and pathological/inflammatory processes. MMP-9, a major component of the basement membrane, is highly expressed in the brain in physiological conditions, and is significantly up-regulated in several brain pathologies, including Parkinson's disease. In fact, the overexpression of α-synuclein may stimulate MMP-9 activity, and the overexpression of α-synuclein in rat primary astrocytes increased MMP-9 activity by stimulating microglia to activate PAR-1 and amplify microglial inflammatory signals.

Our studies in animals models of Parkinsonism (C57BL/6 mice, MMP-9 KO mice and monkeys) suggest that sustained neuroinflammation exacerbates degeneration of the dopaminergic nigro-striatal pathway, and additionally, both perpetuation of inflammation and high levels of MMP-9 are associated to continuous DA neuron loss. We conclude that: i) MMP-9 is important for inflammatory glia activation, which in turn exacerbates DA neuron loss; ii) at later stages, when neuroinflammation subsides, MMP-9 may contribute to partial recovery of the nigro-striatal pathway; and iii) treatment with MMP-9 inhibitors could attenuated the neuronal cell death induced by the dopaminergic neurotoxins suggesting MMP-9 as a posible therapeutical target in PD.

Amyloid peptide aggregation: is nature a good source for potential inhibitors?

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The origin of many neurodegenerative disorders like Alzheimer's Disease (AD) lies in protein processing failures, which leads to protein aggregation and accumulation as amyloid fibrils. The Aβ peptide is currently believed to play a central role in the pathogenesis of AD and in light of the suggested link between oxidative stress and neurodegeneration, screening of bioactive antioxidants could lead to novel aggregation inhibitors for the prevention or treatment of AD. Screening of endogenous antioxidants, such as melatonin or natural product-derived bioactive compounds for binding to Aβ was performed by nano-electrospray ionization (ESI) mass spectrometry (MS). The binding strength of the aforementioned interactions was assessed by ESI tandem MS approaches. Finally, *in vitro* screening was complemented with cell viability assays using differentiated neuronal SH-SY5Y cells to assess any potential toxic effects of the selected substances.

The formation of 1:1 noncovalent complexes of Aβ with certain antioxidants such as oleuropein (OE), melatonin (M) and the main crocin constituents isolated from *Crocus sativus* L., which are mono-and bis-esters of crocetin, such as TC-2, TC-3 and TC-4, were observed in this study. The specificity of these noncovalent interactions was also evaluated at low concentration levels, i.e., 5-100 μM, where the occurrence of nonspecific aggregation in the gas-phase can be prevented. In another study, conducting ESI collision-induced dissociation studies on the +5 charged ion of the noncovalent complex assessed the binding strength of the aforementioned interactions. It is clearly shown that it takes more energy to dissociate the Aβ signal with TC-2/TC-3/TC-4/OE than that with M and trans-crocetin; thus, demonstrating the higher binding strength of the former noncovalent interactions over the latter. Furthermore, the ESI MS mapping study revealed that the interacting region of Aβ with the antioxidant ligands lies within its hydrophobic region, which is also responsible for its aggregation. At the cellular level, OE, trans-crocetin and TC-4 did not appear to have a toxic effect at concentrations up to 10μM, at both 24 and 72 hours of incubation. It should be noted that trans-crocetin and TC-4 appear to modestly enhance cell proliferation at 24 hours of incubation with concentrations

between 0.1 and 10 μM. This work demonstrates that natural products may be effective in inhibiting Aβ fibrillogenesis without limiting neuronal cell viability at low concentrations. Implementation of this ESI MS methodology could allow real-time monitoring of the aforementioned noncovalent interactions thus shedding some light into the mechanisms of AD pathology and facilitating the design of novel compounds, which could act as protective or even therapeutic agents against AD.

Prion-Like Mechanisms in Neurodegenerative Diseases: focus on Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative disorder characterized mainly by resting tremor, slowness of movement, rigidity and postural instability, all attributed to a dramatic loss of dopamine-containing neurons in the substantia nigra pars compacta (SNpc).

Besides the progressive loss of dopaminergic neurons, the pathological hallmark of PD is the presence of intraneuronal proteinacious cytoplasmic inclusions, named Lewy bodies (LB). Biochemical analyses have shown that α-synuclein, an aggregation-prone protein, is a major protein component of LB and is part of the fibrillar structure of these structures, thereby linking sporadic and idiopathic forms of PD. The mechanism underpinning the formation of LB and their significance i.e.do they reflect a neuroprotective vs neurotoxic process for the neurodegenerative process remains unknown.

To identify the pathological species of α-synuclein involved in the perturbation of cellular function is a hot topic of current PD research. In the past few years, substantial progress has been made in elucidating how α-synuclein undergoes spontaneous self-aggregation, as well as the process of aggregation of α-synuclein from monomers, by oligomers, into fibrils. These studies established that misfolded α-synuclein, especially in its oligomeric forms, is probably the toxic species in *in vitro* and *in vivo* settings.

Of particular relevance is the recent observation in 2008 by several groups that LB form in grafted dopaminergic neurons in the striatum of PD patients (Kordower et al., 2008; Li et al., 2008). These were embryonic cells that remained apparently healthy and were functional after grafting, suggesting that the PD brain predisposes even young cells to form LB. These findings were a conceptual breakthrough, generating the "host to graft transmission" hypothesis, also called the prion-like hypothesis.

Mounting evidence suggest that α-synuclein, a major protein component of LB, may be responsible for initiating and spreading the pathological process in PD. In the past two years, *in vivo* studies have added a further piece to the puzzle by using artificial α-synuclein fibers to mimick LB-like pathology. However, it remains uncertain whether the pathogenic effects of recombinant synthetic α-synuclein may apply to PD-linked pathological α-synuclein and occur in species closer to humans.

To address these questions, we explored the potential pathogenic effects of inoculating α-synuclein-containing nigral LB extracts from PD patients into the brains of wild-type mice and macaque monkeys. Nigral LB-enriched fractions containing pathological α-synuclein were purified from post-mortem PD brains by sucrose gradient fractionation and subsequently inoculated into the substantia nigra or striatum of wild-type mice and macaque monkeys.

In both mice and monkeys, intranigral or intrastriatal inoculations of PD-derived LB extracts resulted in progressive nigrostriatal neurodegeneration starting at striatal dopaminergic terminals. In LB-injected animals, exogenous human α-synuclein was quickly internalized within host neurons and triggered the pathological conversion of endogenous α-synuclein. At the onset of LB-induced degeneration, host pathological α-synuclein diffusely accumulated within nigral neurons and anatomically interconnected regions, both anterogradely and retrogradely. LB-induced pathogenic effects required both human α-synuclein present in LB extracts and host expression of α-synuclein.

This world premiere study shows for the first time that α-Synuclein species contained in PD-derived LB are pathogenic in both rodents and non-human primates and have the capacity to initiate a PD-like pathological process, including intracellular and pre-synaptic accumulations of pathological α-synuclein in different brain areas and slowly progressive axon-initiated dopaminergic nigrostriatal neurodegeneration.

Overall, the results presented here indicate that insoluble α-synuclein forms contained in PD-derived LB are pathogenic. These results may have important implications for the development of disease-modifying therapies for PD aimed at targeting expression levels, pathological conversion and/or cell-to-cell transmission of α- synuclein.

Slowing the progression of Parkinson's disease though physical exercise

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The motor deficits associated with Parkinson's disease (PD) appear due to the loss of dopamine (DA)-containing neurons projecting from substantia nigra (SN) to striatum. Pharmacological treatments exist that reduce the symptoms of PD. However, they have a limited period of efficacy, often produce side effects, and fail to significantly attenuate the neurodegenerative process. Despite the absence of neuroprotective pharmacological interventions, several labs have shown that physical exercise can reduce the vulnerability of DA neurons to neurotoxins in laboratory animals, suggesting that exercise would be useful in slowing the progression of PD. Furthermore, the impact of exercise seems to be associated with an increase in the concentration of neurotrophic factors (NTFs). My colleagues and I have studied these phenomena in rodents, monkeys, and in several in vitro models. Among our conclusions are that several NTFs are likely to work synergistically to produce their neuroprotective actions and that this involves the activation of the phosphokinases, ERK and Akt, among other intracellular events that retard apoptosis.

Disease modification in Alzheimer's disease: how far are we?

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Vast efforts have been put in the past years by the pharmaceutical companies in developing disease-modifying therapeutic approaches for Alzheimer's disease (AD), centering to a large extent on the amyloid cascade hypothesis. Disappointingly, the amyloid peptide immunotherapy directed trials in AD failed to meet clinical endpoints. This may be explained in part by the advanced stage of the disease at time of treatment to potentially reverse or slow symptoms. Also, the poor antibody design and brain penetrance together with the preclinical data may have misled the clinical translation validity of the treatments. There is still the matter of relevance of the amyloid plaques vs soluble Aβ oligomers in AD pathogenesis. Brain amyloidosis and fibrillated Tau are downstream disease markers and not disease initiators.

It is now well established that the pathology of AD is not the gold standard. It does not correlate with the severity of dementia. Plaques and tangles are present also in cognitively healthy individuals. AD is a multi-etiological disease entity, individuals accumulate pathologies throughout life. Initiation of AD pathology is estimated to begin ~10-15 years prior to the onset of clinical symptoms, and thus there is a long phase that precedes the classical symptomatology (prodromal AD). Individuals at early stages of AD are the most likely to benefit from disease-modifying therapies should they become available. The challenge is to find the meaningful biological targets for new diagnostic and drug development. In my talk I will elaborate on emerging targets and strategies in AD and the perspectives for new therapeutics.

New directions in the research and treatment of parkinson's disease

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Attempts to develop a neuroprotective approach to Parkinson's disease (PD) have so far proved unsuccessful. Despite promising findings in preclinical models of PD, translation in to clinical effect has so far failed. The reasons for this are still not fully understood but new concepts of the cause and progression of PD are leading to novel approaches to treatment. The classical view of nigral dopaminergic cell loss and basal ganglia dysfunction as being the underlying cause of PD has given way to the concept that it is a multiple pathology and multiple biochemical defect disorder that progresses through the brain.

No single pathogenic mechanism has so far been identified as underlying cell death in PD although disruptions in protein handling and in mitochondrial function appear most likely. One view is that there is no single cause of PD and consequently, no single drug treatment would prevent disease progression in all affected individuals. In addition, animal models of PD have focussed largely on dopaminergic cell death and in future, need to reproduce the full spectrum of pathology of PD and its progression.

As a consequence, approaches to neuroprotection in PD that focus on mechanisms identified as common to the degenerative process underlying the spread of pathology are under investigation. For example, prevention of the toxicity of α-synuclein and the blockade of calcium channels is under investigation. Early identification of those individuals developing PD is also essential as neuronal damage may be too advanced for effective treatment once motor symptoms have appeared.

ABSTRACTS
Students and attendants

The inhibition of *SIRT2* decetylase improves cognitive performance in transgenic mice models of Alzheimer's disease

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Alzheimer's disease (AD) is a frequent cause of dementia characterized by cognitive decline. Recent studies have shown that the modulation of sirtuin pathway can be relevant for aging processes and neurodegeneration (*Albani D. et al, 2010*). Our attention was focused on SIRT2, as its inhibition or downregulation seems to provide protection in neurodegenerative disease models, including Parkinson's and Huntington's disease (*Donmez et al, 2013*). **Materials and methods.** To examine the effect of SIRT2 inhibition in brain, two different animal models (APP23 transgenic mice harboring the double Swedish mutation in APP gene and the triple-transgenic mice 3XTG-AD harboring a knock-in mutation of presenilin 1, the Swedish mutation and a mutation in tau) were treated daily twice for 15 days with a specific brain-permeable SIRT2 inhibitor (AK7, 20mg/kg) or vehicle. To test the effect of AK7 treatment on memory, the animals underwent Novel Object Recognition Test (NORT). **Results.** In both transgenic models, NORT revealed long-term recognition memory impairment in TG-Veh, mice that were not able to discriminate between the novel and the familiar object, at difference from WT-Veh showing a preference for the novel object. The AK7 treatment counteracted the impairment, as TG-AK7 mice showed a discrimination index (DI) statistically different from TG-Veh mice (TG-AK7 DI: 0.23, TG-Veh DI: 0.02 in APP23 mice and TG-AK7 DI: 0.202, TG-Veh DI: 0.015 in 3xTg mice). **Conclusions**. We were able to demonstrate that cognitive impairment in TG mice can be reversed by SIRT2 inhibition ensuing AK7 treatment. We are now going to investigate the molecular mechanism and the clinical optimization of our treatment.

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Tau-protein potentizes the toxic effect of beta-amyloid

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The idea about the interaction between Aβ and Tau protein toxic effects for a long time was not finding support. Study of the functional role of these proteins separated researchers at two ''camps'' in accordance with the accepted hypothesis of the pathogenesis of Alzheimer's disease (AD) - ''amyloid cascade hypothesis'', insisting on a key role of Aβ [Selkoe, 2002], and "tau-hypothesis'', giving priority to Tau protein [Trojanowski and Lee, 2002]. Here we check the character of this interaction.

Previously we developed *in vitro* cell model of process of formation of the fibrillar Tau-protein forms by line of cell 3T3-4R-Tau, constantly expressing human Tau-protein (4R-form). Test system was primary culture from the hippocampus of newborn rats. Cells in culture were stained with Hoechst 33342 which is cell-permeable and able to bind to DNA (excitation - 350 nm) or with propidium iodide which is permeable for dead cells (excitation - 536 nm).

Co-cultivation 3T3-4R-Tau cells with primary culture of the hippocampus showed an increase number of dead cells. The preincubation of 3T3-4R-Tau cells with Aβ (1-42) and following co-cultivation 3T3-4R-Tau cells with primary culture increased their toxicity and induces massive extinction of the hippocampal cells.

Thus, we have established toxic effect of Aβ is mediated through protein Tau, that allows for new insights into the pathogenesis of AD.

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The conditioned medium of murine adipose mesenchymal stem cells is able to prevent oxidative damage in human neuronal cells SHSY5Y

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Neurodegenerative diseases are characterized by a long-lasting degenerative process in which the nervous system progressively and irreversibly deteriorates. There are several reasons behind neurodegeneration, including oxidative stress that is a main feature in post-mortem autoptic brains from diseased cases. There are currently no therapies other than symptomatic to modify the progression of these diseases, but a very promising field has been explored by developing new therapies that target the immune system, such as the regulation of neuroinflammation by Mesenchymal Stem Cells (MSCs) (Wei et al. 2013). MSCs create a favorable environment for regeneration, and they are able to promote the production of beneficial bioactive factors through paracrine signaling. MSCs - Conditioned Medium (MSC-CM) increases expression of several cytokines, neurotrophins and growth factors (BDNF, GDNF, NGF; Das at al. 2013). Our purpose was to evaluate, in an *in vitro* model (SHSY5Y human neuroblastoma cell line), whether the MSCs – CM gave cytoprotection against agents that cause oxidative stress such as H_2O_2 or 6-OHDA, the latter being a toxin relevant for Parkinson's disease. **Materials and methods.** SHSY5Y cells were treated for 24 hr with conditioned medium of adult rat adipose-derived mesenchymal stem cells (at different dilution rates) and after 24 hr H_2O_2 or 6-OHDA were added to cells at different concentrations. Finally, the cellular viability was evaluated by MTS assay. **Results.** The MSCs-CM seems to be protective against oxidative stress induced by H_2O_2 or 6-OHDA, in particular at lethal dose of 150μM or 100μM, respectively. **Conclusions.** The optimization of MSC-CM treatment can be used to explore novel cell-based therapies, as they have proved to be effective against oxidative stress without the need of a cell-to-cell contact.

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MDMA administration during adolescence exacerbates MPTP-induced cognitive impairment, neurodegeneration and neuroinflammation in motor, limbic and cortical areas

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Clinical observations report a higher propensity to develop Parkinson's disease (PD) in amphetamine users. 3,4-Methylenedioxymethamphetamine (MDMA) is an amphetamine-related drug which may have neuroinflammatory and neurotoxic effects. The present study was aimed at evaluating in mice whether administration of MDMA during adolescence might influence neurotoxicity towards dopaminergic neurons and neuroinflammatory effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a toxin known to induce PD in humans, in motor, limbic and cortical areas, and consequently affects cognitive performance.

Mice received MDMA (10 mg/kg, twice a day/a week) for 9 weeks, followed by MPTP (20 mg/kg × 4 administrations), starting 2 weeks after MDMA discontinuation. Activation of astroglia and microglia by GFAP and CD11b immunohistochemistry in motor areas, as substantia nigra compacta (SNc) and striatum, limbic and cortical areas, as hippocampus and medial prefrontal cortex (mPFC), was assessed. Degeneration of dopaminergic neurons by tyrosine hydroxylase (TH) immunohistochemistry in SNc and striatum was also evaluated. Neurochemical evaluations were paired with assessment of cognitive performance by means of the novel object recognition (NOR) and spontaneous alternation tests.

MPTP administration to MDMA-pretreated mice elicited a stronger increase in CD11b and GFAP levels in motor, limbic and cortical areas, and a stronger decrease of TH-positive neurons and fibers in motor areas, compared with either substance administered alone. Furthermore, NOR performance in the same group was lower, compared with mice that received either substance alone. Results demonstrate that MDMA administration during adolescence influence negatively MPTP effects on motor, limbic and cortical areas and result in cognitive impairment.

Metalloproteinases inhibition and ibuprofen treatments significantly decreased microglial activation and phagocytosis in MPTP-treated mice

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Inflammation is a predominant aspect of neurodegenerative diseases and many studies performed in experimental Parkinsonian models suggesting that sustained neuroinflammation exacerbates degeneration of the dopaminergic nigrostriatal pathway. Importantly, altered astrocyte and microglial functions could contribute to neuronal death in Parkinson's disease (PD) but insights into the inflammatory mechanisms in PD may help developing therapeutic strategies specifically targeting its harmful aspects.

The aim of this study was to investigate the time course and compare the potency of ibuprofen with and without the iminosugar 1-Deoxynojirimycin [1-dnj, which can attenuate the activity and expression of matrix metalloproteinases (MMPs)]. A total of 127 animals (4 m.o.) were treated with MPTP (80 mg/kg/day/i.p.) according to acute treatment plus saline or 1 dose of ibuprofen syrup (40 mg/kg/day) or 1 dose of 1-dnj (1 mg/kg/day), 1 hour or 30 min before MPTP administration, respectively, or both of them. Animals were sacrificed 72 or 48 hours after the first MPTP administration. Postmortem studies of microglia (Iba-1) and astroglia (GFAP) in the SNpc demonstrated the beneficial summatory effect of ibuprofen and 1-dnj. Our data suggest that inhibition of MMPs by dnj1 combined with ibuprofen treatment could decrease glia activation and microglial phagocytosis (even phagoptosis) and could be an strategy to ameliorate harmful inflammatory outcomes in Parkinsonism by blocking phagocytic signalling. Then, modulation of MMP9 activity combined with antiinflammatory drugs could provide promising research ways for therapeutic intervention in PD.

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Free d-aspartate exerts neurotrophic effects in the mammalian brain

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D-Aspartate (D-Asp) is an atypical amino acid which is especially abundant in the developing mammalian brain. After birth, the endogenous levels of this atypical amino acid rapidly decrease, due to the concomitant expression of the catabolic enzyme D-Aspartate Oxidase (DDO). Recent evidence has shown that D-Asp activates the N-methyl-D-Aspartate subclass of glutamate receptors (NMDAR) through the binding to the glutamate site of GluN2 subunits. In line with its pharmacological features, we find that adult mice chronically treated with D-Asp show enhanced NMDAR-mediated miniature excitatory postsynaptic currents and basal cerebral blood volume in fronto-hippocampal areas. In addition, we show that both chronic administration of D-Asp and deletion of the *Ddo* gene trigger plastic modifications of neuronal cytoarchitecture in the prefrontal cortex and CA1 subfield of the hippocampus and promote a cytochalasin D-sensitive form of synaptic plasticity in the adult mouse brains. To translate these findings to humans and consistent with the experiments using *Ddo* gene targeting in animals, we performed a hierarchical stepwise translational genetic approach. Specifically, we investigated the association of variation in the gene coding for DDO with complex human prefrontal phenotypes. We demonstrate that genetic variation predicting reduced expression of *DDO* in *post-mortem* human prefrontal cortex is mapped on greater prefrontal grey matter and activity during working memory as measured with Magnetic Resonance Imaging. In conclusion our results identify novel NMDAR-dependent effects of D-Asp on plasticity and physiology in rodents, which also map to prefrontal phenotypes in humans.

Multiple Mechanisms of Neurodegeneration and Progression

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Neuromelanin (NM) is a dark pigment present in neurons of several brain areas, particularly in substantia nigra and locus coeruleus, which selectively degenerate in Parkinson's Disease. NM structure is very complex and contains melanic, peptide, lipid moieties and metal ions; all these components are linked together by covalent bonds. Interestingly, powder X-ray diffraction studies showed that isolated NM bears a structural motif different from the typical aromatic-stacking interaction of other natural and synthetic melanins. Its stacking distance is instead similar to that observed in the cross-β-sheets structure of amyloid protein aggregates. In order to understand if this motif is due to the presence of fibrillar protein in NM core, we have synthesized NM models containing fibrillated β-lactoglobulin. We also studied the interaction of these conjugates with iron(III) ion, which is the most abundant metal ion in NM. The melanin-fibrils conjugates have been characterized with different techniques (NMR, LC-MS, ICP-MS, EPR). Our results suggest that melanin can bind to fibrillar protein and that the protein retains its fibrillar configuration when bound to the melanic component. These well characterized models will be used for cell culture experiments, in order to establish a structure/effect relationship for the components present in NM structure. In particular we will study the activation of microglia by extracellular NM, which leads to neuroinflammation and neuronal death.

A role of adenosine recepors in caffeine effect on da and 5-ht release induced by mdma in the mouse striatum

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MDMA (3,4-methylenedioxymethamphetamine) used recreationally is the main component of ecstasy tablets which also contain another psychostimulants such as caffeine (CAF) to gain stronger effect. Amphetamines cause an increase in motor activity, anxiety, aggression. Their chronic use can lead to mental disorders like schizophrenia or depression and may influence progression of neurodegenerative diseases. Pharmacological mechanism of CAF is based on blockade of adenosine A_1 and A_{2A} receptors which may affect neurotransmitter release. Our earlier data have shown that CAF (10 mg/kg) given together with MDMA (20 or 40 mg/kg) potentiated stimulatory effect of MDMA on extracellular level of dopamine (DA) and serotonin (5-HT) in the mouse striatum. In a present study we aimed to understand if potentiating effect of CAF on DA and 5-HT release produced by MDMA occurs via blockade of adenosine A_1 and A_{2A} receptors. Mice were treated with selective adenosine A_1 and A_{2A} receptor antagonists, DPCPX and KW-6002 (1.25 or 2.5 mg/kg) alone or in combination with MDMA (40 mg/kg) and the release of DA and 5-HT was assayed using microdialysis in freely moving mice. The extracellular level of DA and 5-HT was determined by HPLC with coulochemical detection. Both adenosine A_1 and A_{2A} receptor antagonists enhanced DA and 5-HT release increased by MDMA, but DPCPX was only effective in the higher dose. It can be speculated that CAF effect on DA and 5-HT release may be caused by blockade of A_1 and A_{2A} adenosine receptors.

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Neuroprotective effect of early postnatal environmental enrichment in a rat model of Parkinson's disease

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Environmental enrichment is a popular strategy of neuroprotection. The aim of our study was to investigate the effect of early postnatal environmental enrichment in a rat model of Parkinson's disease in adulthood.

We used adult Wistar rats in the experiment. The animals of the standard group were placed under regular circumstances. For environmental enrichment, we placed rats in larger cages, supplemented with different toys during the first five postnatal weeks. Two moths later the rats were treated with unilateral injections of 2 µl 6-OHDA (5 µg/ 1 µl) into the left substantia nigra, contol animals received 2 µl physiological saline. Behavioral experiments were done preinjury, and 1 & 10 days after the operation. Tyrosine-hydoxylase immunohistolochemistry was performed after the behavioral testing to label dopaminergic cells of the substantia nigra.

We found that physiological saline did not make significant difference between the treated and non-treated side of the brain. The 6-OHDA treatment made significant cell loss in the standard group: more than 40% of dopaminergic cells died, while in rats which were kept in enriched environment the cell loss was significantly lower.

Our experiments provided evidence for protective effect of early postnatal environmental enrichment in adulthood, because rats under regular circumstances showed more severe acute neurological sings and dopaminergic cell loss after 6-OHDA lesion of the substantia nigra compared to animals grew up in environmental enrichment.

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Diffusion kurtosis imaging detects the microstructural changes in basal ganglia induced by alpha synuclein overexpression in tnwt-61 transgenic mouse model of parkinson's disease

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Purpose: Compelling evidence suggests that accumulation and aggregation of α-synuclein contribute to the pathogenesis of Parkinson's disease. The aim of this study was to evaluate changes in grey matter microstructure induced by α-synuclein aggregation in *TNWT-61* mice by MRI diffusion weighted imaging (both diffusion kurtosis – DKI and tensor imaging – DTI) and correlate the results with behavioural data. We hypothesized that the presence of α-synuclein aggregates in the transgenic mouse model would create more water diffusion barriers resulting in higher kurtosis and no changes in diffusion metrics.

Methods: *Thy1aSyn* mice and wild type littermates at the age of 9 months underwent behavioural studies to detect the motor impairment and 9.4 Tesla DKI and DTI MRI scanning to detect structural changes in the brains in vivo. Kurtosis and diffusion metrics were obtained for multiple regions (substantia nigra, striatum, hippocampus, sensorimotor cortex and thalamus).

Results: *TNWT-61* mice showed significant progressive impairment of motor coordination compared to their wild type littermates. Values of mean, radial and axial diffusion kurtosis were significantly elevated in the *TNWT-61* group as compared to the wild type control group. For the diffusion parameters (mean, radial and axial diffusivity) no inter-groups differences were observed in any of the analysed regions.

Conclusion: The current study provides evidence that DKI is sensitive for *in vivo* detection of the microstructural changes in basal ganglia induced by α-synuclein accumulation. Thus DKI has the potential to improve the clinical diagnosis of Parkinson's disease compared to conventional diffusion-tensor imaging.

Modulation of microglia cytokines by PPAR-gamma agonist in the MPTPp model of Parkinson disease

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Aims: Dysregulated cytokines production and prevalence of neurotoxic over neuroprotective microglia is a pathological event in Parkinson's disease (PD). The peroxisome proliferator-activated receptor (PPAR)-gamma is expressed in CNS immune cells, and receptor agonists mediate neuroprotection in PD models. we sought if the chronic administration of MPTP/probenecid (MPTPp), a recognized rodent model of PD, and stimulation of PPAR-gamma with rosiglitazone modulated cytokines production by microglia.

Methods: Mice received 10 MPTPp injections over 5 weeks. Rosiglitazone was administered daily, starting after the 7th MPTPp injection. The colocalization of pro- and anti-inflammatory cytokines such as TNF-alpha and TGF-beta, with IBA-I positive microglia was measured by immunofluorescence.

Results: As previously shown,chronic MPTPp induced a progressive nigrostriatal degeneration, while rosiglitazone stopped neurodegeneration. In vehicle-treated mice, microglia expressed low levels of pro- and of anti-inflammatory cytokines. Across the chronic MPTPp treatment microglia acquired a highly activated morphology and expressed increasing levels of TNF-alpha. In contrast, the expression of TGF-beta was increased significantly after 3 MPTPp injections, to be reverted to control levels after 10 injections. Rosiglitazone administration did not suppress microglia activation, however it reverted TNF-alpha to control levels while induced an overproduction of TGF-beta, which exceeded control levels.

Conclusion: Results suggest that MPTPp treatment polarized microglia activation toward a pro-inflammatory phenotype, while rosiglitazone induced a switching in microglia polarization from pro- to anti-inflammatory. Stimulating the production of anti-inflammatory over pro-inflammatory cytokines by PPAR-gamma agonists may drive microglia activity toward a neuroprotective phenotype and may underlie the neuroprotective activity of these drugs.

A critical role for rhes in the striatal physiology

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Ras homolog enriched in striatum (Rhes) is a small GTP-binding protein developmentally modulated by thyroid hormones and highly expressed in the striatum of rodents. In the present study, we explored the expression and the role of Rhes in distinct striatal cell-populations. Notably, *in situ* hybridization analysis showed that Rhes mRNA, like in rodents, is localized in human striatal medium spiny neurons (MSNs) and, most remarkably, it is also contained in striatal cholinergic interneurons (ChIs), both in mouse and humans. In support of a physiological role of Rhes in regulating dopaminergic signaling in ChIs, we found reduced dopamine (DA) D1R/D5R- and, most strikingly, aberrant D2R-dependent responses in *Rhes* mutant mice. Moreover, electrophysiological studies indicated also an involvement of Rhes in controlling inhibitory GABAergic inputs in MSNs. On the other hand, in line with a robust negative modulatory role of Rhes in adenosine A2AR-dependent cAMP/PKA-signaling, administration of the DA D2R-antagonist haloperidol in knockouts resulted in higher striatal levels of GluR1 phosphorylation at Ser845, with an exaggerated cataleptic behavior and immediate early-gene mRNA-expression. We also showed that Rhes affects striatal DA D1R-related behavior and long-term potentiation in a gender-sensitive manner. In conclusion, our data indicate the concomitant striatal expression of Rhes both in MSNs and ChIs, showing that *Rhes*, in a tight interaction with sex-specific factors, is important for influencing striatal dopaminergic-mediated functions. These data considerably enlarge the complexity related to the specific role of this small GTP-binding protein in controlling striatal physiology.

Overexpression of the astrocyte glutamate transporter, GLT1, exacerbates phrenic motor neuron degeneration, diaphragm compromise and forelimb motor dysfunction following cervical contusion spinal cord injury

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A major portion of spinal cord injury (SCI) cases affect mid-cervical levels, the location of the phrenic motor neuron (PhMN) pool that innervates the diaphragm. While initial trauma is uncontrollable, a valuable opportunity exists in the hours-to-days following SCI for preventing PhMN loss and consequent respiratory dysfunction that occurs during secondary degeneration. One of the primary causes of secondary injury is excitotoxic cell death due to dysregulation of extracellular glutamate homeostasis. GLT1, mainly expressed by astrocytes, is responsible for the vast majority of functional uptake of extracellular glutamate in the CNS, particularly in spinal cord. We found that, in BAC-GLT1-eGFP reporter mice following unilateral mid-cervical (C_4) contusion SCI, numbers of GLT1-expressing astrocytes in ventral horn and total intraspinal GLT1 protein expression were reduced early after injury and the decrease persisted for at least 6 weeks. We employed intraspinal delivery of adeno-associated virus type 8 (AAV8)-Gfa2 vector to rat cervical spinal cord ventral horn for targeting focal astrocyte GLT1 overexpression in areas of PhMN loss. Intraspinal delivery of AAV8-Gfa2-GLT1 resulted in transduction primarily of $GFAP^+$ astrocytes that persisted for at least 6 weeks post-injury, as well as increased intraspinal GLT1 protein expression. Surprisingly, we found that astrocyte-targeted GLT1 overexpression increased lesion size, PhMN loss, phrenic nerve axonal degeneration, and diaphragm neuromuscular junction denervation, and resulted in reduced functional diaphragm innervation as assessed by phrenic nerve-diaphragm compound muscle action potential (CMAP) recordings. These results demonstrate that GLT1 overexpression via intraspinal AAV-Gfa2-GLT1 delivery exacerbates neuronal damage and increases respiratory impairment following cervical SCI.

Novel therapeutic strategy for the control of dyskinesia in the therapy of Parkinson's disease

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Recent report demonstrated that mixed serotonin 5-$HT_{1A/B}$ receptor agonist, eltoprazine, produces a near to full suppression of dyskinetic-like behaviors in animal models of Parkinson's disease (PD). However, eltoprazine resulted in a partial reduction of motility induced by L-DOPA, both in rats and in monkeys. Moreover, in a recent clinical trial, the partial 5-HT_{1A} agonist sarizotan has been found to be only partially effective. Preclinical and clinical studies showed that adenosine A_{2A} receptor antagonists significantly increase L-DOPA efficacy in PD, without exacerbating dyskinetic-like behaviors. Preladenant, an A_{2A} receptor antagonist utilized in clinical trials, is an excellent tool for assessing the role of A_{2A} receptors in movement disorders.

On this basis, we hypothesize that combination of eltoprazine with preladenant may produce suppression of L-DOPA-induced dyskinesia, without impairing the efficacy of L-DOPA in relieving motor symptoms. Unilateral 6-hydroxydopamine-lesioned rats, rendered dyskinetic by repeated-L-DOPA-treatment, were administered with eltoprazine (0.3 or 0.6 mg/kg) and preladenant (0.3 or 1 mg/kg), singularly or in combination with L-DOPA (4 or 6 mg/kg), and rotational behavior, as index of motility, and abnormal involuntary movements (AIMs) as index of dyskinesia, were evaluated.

Results show that combined administration of L-DOPA (4mg/kg) plus eltoprazine (0.6mg/kg) plus preladenant (0.3mg/kg) significantly reduced dyskinetic-like behaviors, as revealed by AIMs test without impairing the motor activity, as revealed by similar number of contralateral and ipsilateral rotations. Overall these data suggest that combination of L-DOPA (4mg/kg) with eltoprazine (0.6mg/kg) and preladenant (0.3mg/kg) could be a new therapeutic strategy for treating motor symptoms and dyskinesia in PD.

Efficacy of rasagiline on progressive dopamine neurondegeneration in a chronic MPTPp model of Parkinson's Disease

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Aims: Parkinson's Disease (PD) is characterized by a chronic progressive loss of nigrostriatal dopaminergic neurons, associated to chronic neuroinflammation. The MAO-B inhibitors have neuroprotective activity in cellular and animal models of neurodegenerative disorders, as PD. In particular, rasagiline has a neurorestorative activity in degeneration of nigrostriatal dopamine neurons induced by acute MPTP treatment. In the present study we have evaluated the efficacy of rasagiline on neurodegeneration in a chronic mice model of MPTP administration which induce a progressive loss of nigrostriatal dopaminergic neurons.

Methods: Mice were treated with vehicle, or MPTP plus probenecid administered twice a week for 5 weeks, alone or in the presence of rasagiline administered 18 hrs before each MPTP administration. After treatment, the motor performance of mice was evaluated by the beam-walking test. Dopamine neuron degeneration was measured by immunohistochemical evaluation of tyrosine hydroxylase (TH)-positive neurons in striatum and in substantia nigra pars-compacta (SNc).

Results: Chronic MPTP induced an impairment of motor performance, a decrease in dopamine neurons in striatum and SNc. Previous administration of rasagiline decreased MPTP-induced motor deficits, as well as dopamine neurondegeneration.

Conclusions: The inhibition of MAO-B with rasagiline showed neuroprotective efficacy in a model that more closely reproduce neurodegeneration in PD in which the dopamine neuron degeneration takes place over time.

Dietary polyphenols as a neuroprotective strategy against neurodegeneration in experimental models of parkinson's disease

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Although Parkinson's disease (PD) has been at the center of intensive research, prevention of neuronal loss has not yet been addressed by existing symptomatic treatments. Among the proposed underlying mechanisms, oxidative stress, neuroinflammation and impaired autophagy have been credited as major pathways of neurodegeneration[1,2]. Neuroprotection by dietary polyphenols may be an interesting avenue in current attempts to overcome the multifaceted causes of dopaminergic (DAergic) neuron apoptosis[3]. In order to evaluate the neuroprotective potential of polyphenols in a PD context, we have studied the anti-oxidative, anti-inflammatory and pro-autophagic effects of resveratrol, quercetin and oleuropein *in vitro* as well as *in vivo*. Our results show that quercetin and resveratrol protect DAergic neuronal PC12 cells from oxidative and pro-apoptotic insults from MPP+ and high-glucose treatment by modulating gene expression and reactive oxygen species production. These polyphenols could also offset the secretion of pro-inflammatory cytokines by N9 microglia activated by LPS or MPP+ consequently protecting co-cultured DAergic neuronal PC12 cells from a cytotoxic inflammatory effect. Oleuropein showed pro-autophagic properties in DAergic PC12 cells treated with 6-OHDA, which was accompanied by increased survival of the neurons and mitigation of the apoptotic cascade. Finally, resveratrol was tested *in vivo* where it was able to rescue DAergic neuron loss in the midbrains of mice treated with MPTP. Our results demonstrate a powerful neuroprotective role for polyphenols that should be considered in the development of complementary therapeutic strategies in PD.

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Effect of perinatal asphyxia on hippocampus functioning evaluated in vitro and in vivo: neuroprotection by hif1-alfa inhibition

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Perinatal asphyxia can affect the hippocampus, inducing inflammation and astrocyte reactivity impairing neuronal branching and synaptogenesis. HIF-1alpha is a first molecule in the signalling reacting to hypoxia, triggering neuroprotective or deleterious cascades depending upon the intensity of the insult. Here we show alterations in the hippocampus following asphyxia and protection by HIF-1alpha inhibition. Asphyxia was induced by immersing G22 foetuses into a water bath at 37°C for 21 min. Rats were treated with YC-1 (HIF-1alpha inhibitor) 2mg/kg i.p. 30 min after delivery. Hippocampi from control and asphyxia-exposed rats were dissected out short after delivery for (i) preparing primary cultures (6h after delivery); or given to surrogate dams pending further experiments, including: (ii) intra-cardial formalin-fixation at P7 and P22; (iii) electrophysiological recording at P24, or (iv) behavioural studies performed approximately 3 months after birth. We found: (i) At DIV-2-7, a reduction in the number of neurons, neurite length, and pre-synaptic dots in primary cultures from asphyxia-exposed rats. (ii) At P7-22 in vivo, a loss of synaptic structure and a decrease in the number of synapsis in CA3. (iii) At P24, a reduction of LTP. (iv) Finally, at 3 months, asphyxia-exposed animals showed increased anxiety and loss of non-spatial memory, compared to the controls. HIF-1alpha inhibition prevented several of the effects associated to perinatal asphyxia, providing a promising neuroprotective strategy.

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Multiple neuroprotective mechanisms of mesenchymal stem cells

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Neurodegenerative diseases are different and many-sided disorders affecting both the Central and the Peripheral Nervous System. Despite the very different peculiar features, all the neurodegenerative diseases are characterized by the neuronal degeneration, which may be the consequence of different processes, such as an altered protein accumulation, an axonal damage, or the exposure to toxic agents. The progressive neuronal death leads to disease progression, which is not effectively counteracted by the current symptomatic therapies. Among the newly proposed therapeutic approaches, encouraging results have been obtained with Mesenchymal Stem Cells (MSCs), adult stem cells initially proposed for their differentiation potential and for their immune-modulatory abilities.

Here we first verified in vivo the protective potential of MSCs into an in vivo model of Multiple Sclerosis (MS), represented by Experimental Autoimmune Encephalomyelitis (EAE), demonstrating that intravenous administration of MSCs are able to ameliorate the clinical score and the functional skills, and to reduce demyelinated lesions. We then investigated in vitro the possible molecular mechanisms of MSC protective action, thus demonstrating that, besides immunomodulation, MSCs are able to support neuronal survival after toxic stimuli exposure by reducing the apoptosis and by inhibiting the Metalloprotease pathway, which is supposed to be involved in neurodegenerative disease progression. Moreover, MSCs are able to promote the axonal myelination through the modulation of p75 receptor.

For all these abilities, MSCs can represent a promising therapeutic approach for the treatment of neurodegenerative disorders.

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Complexity and regularity of spontaneous locomotion in freely moving animals – changes in relation to ageing

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Exploration is a purposeful activity, defined as behavioural acts and postures that allow the collection of information about objects or parts of the environment. Environmental and social stimuli, but also internal physiological processes contribute to the modulation of spontaneous activity in circadian and ultradian rhythms. The aims of this study were (1) to characterize the regularity and the complexity of circadian and ultradian rhythms in group-housed mice; (2) to characterize the dynamics of these parameters in relation to the process of ageing; and (3) to characterize the effects of well-established interventions in ageing mice. Adult male C57Bl/6 mice were implanted with subcutaneous radio frequency identification (RFID) tags, and the activity in the homecage was monitored using a TraffiCage™ system. The raw data were exported and analysed offline for assessing the underlying regularity (approximate entropy), fractal-like structure (detrended fluctuation analysis), and circadian rhythms (cosinor). We found that spontaneous locomotion in group-housed adult mice displays strong underlying regularity and a scale-invariant complexity (fractal-like) as found in humans. The evolution of these parameters with the age is described in healthy control animals followed for up to 27 months. We then investigated the effects of dietary restriction (75% of normal food intake) from the age of 18 months. The mice were sacrificed at the age of 27 months, and the expression of selected genes in the hippocampus was analysed in relation to alterations in locomotor activity.

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