FENS satellite symposium
Mechanisms of neurodegeneration and progression: From mechanisms to therapies in Parkinson’s disease

MENEDPRO II
Copenhagen
July 1st, 2016

Venue
Københavns Universitet Panum.
Nørre Alle 20. School of Dentistry.
Panum Building (building 29). Room 29.01.30

Organisers and contact persons:
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Please, enter via the main entrance at Nørre Alle 20.
Take the stairs down to floor 01 and follow the signs to 29.01.30.

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Description of the topic and purpose

Neurodegenerative disorders are relentless progressive diseases and produce care-dependency as well as important health-related public consumption. 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. Moreover, 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 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 misfolding and aggregation in different neurodegenerative diseases. In fact, the combination of genetic mutations and environmental 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 neurodegeneration in specific brain regions.

Then, this satellite event looks for discuss the multiple mechanisms of neurodegeneration in different diseases as well as to analyze the possible 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).
## PROGRAMME SCHEDULE

**July 1st, 2016**

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<td>09.30</td>
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<td>Maria Trinidad Herrero (President of NTS)</td>
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<td>Micaela Morelli (Member of the NTS Council)</td>
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<td>Harry Steinbusch (President-Elect of NTS)</td>
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<td>10.00</td>
<td>Neurobehavioural alterations after exposure to a glyphosate-based herbicide during pregnancy and lactation.</td>
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<td>Marta Antonelli. Buenos Aires, Argentina</td>
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<td>10.30</td>
<td>Glucocorticoids modify the differentiation potential of human neuroepithelial-like cells via long-term alterations of the redox state.</td>
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<td>Sandra Ceccatelli. Stockholm, Sweden</td>
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<td>Influence of administration setting and caffeine on the neuroinflammatory and neurotoxic effects of MDMA: relevance to dopaminergic neurodegeneration</td>
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<td>Micaela Morelli. Cagliari, Italy</td>
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<td>11.45</td>
<td>Electrical stimulation of the inferior colliculus: A promising animal model to study paradoxical kinesia</td>
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<td>Liana Melo-Thomas. Marburg, Germany</td>
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<td>No and glutamate interaction in treatment of L-dopa-induced dyskinesia.</td>
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<td>Elaine del Bel. riberao Preto, Brazil</td>
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<td>13.00</td>
<td>Striatal synaptic plasticity changes in L-dopa-induced dyskinesia.</td>
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<td>Lunch. Tables will be organised distributing sharing by speakers and students.</td>
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<td>15.30</td>
<td>Involvement of the kynurenine pathway in the pathogenesis of Parkinson’s disease.</td>
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<td>Gilles J. Guillemin. Sydney, Australia</td>
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16.00: Matrix metalloproteinases in aging and in Parkinsonism: friends and foes to the dopaminergic neurons.
   María Trinidad Herrero. Murcia, Spain

16.30: Discussion

16.45: Epigenetic changes and brainstem dysfunction in neuropsychiatric disorders - AD/PD/anx
   Harry WM Steinbusch. Maastricht, The Netherlands

17.30: High potency TFEB inducers as novel therapeutics for Parkinson’s disease.
   Julie K. Andersen. Novato, CA, USA

18.00: Discussion

18.15: Students presentation
   Titles TBC

18.45: Concluding remarks.
   María Trinidad Herrero, Micaela Morelli, Harry Steinbusch

20.00: Social Dinner.
   Venue TBC
Names and affiliations of speakers:

- Julie K. Andersen  
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NEUROBEHAVIOURAL ALTERATIONS AFTER EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE DURING PREGNANCY AND LACTATION

Cristina E. Gallegos ¹, Mariana Bartos ¹, Cristina Bras ¹, Fernanda Gumilar ¹, Marta C. Antonelli ², Alejandra Minetti ¹.

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²Instituto de Biología Celular y Neurociencias “Prof. Eduardo De Robertis”, Universidad de Buenos Aires, Buenos Aires, Argentina

The massive influx of genetically modified crops resistant to glyphosate (Gly) in Argentina is the main reason why the most widely marketed herbicides within this country are those containing Gly in their formula. Pesticides are postulated as the main environmental factor associated with the etiology of neurodegenerative disorders, such as Parkinson’s and Alzheimer’s disease. However, the impact of sub-lethal doses of herbicides on human health and the environment is a matter of controversy. Due to the fact that evidence particularly of the effects of Gly on the central nervous system of rat offspring by in utero exposure is scarce, the purpose of the present study was to assess the neurobehavioral effects of chronic exposure to a glyphosate-containing herbicide during pregnancy and lactation. To this end, pregnant Wistar rats were exposed through drinking water to 0.2% or 0.4% of a commercial formulation of Gly (corresponding to a concentration of 0.65 or 1.30 g/L of Gly, respectively) during pregnancy and lactation and neurobehavioral alterations in offspring were analyzed. The postnatal day on which each pup acquired neonatal reflexes (righting, cliff aversion and negative geotaxis) and that on which eyes and auditory canals were fully opened were recorded for the assessment of sensorimotor development. Locomotor activity and anxiety levels were monitored via open field test and plus maze test, respectively, in 45- and 90-day-old offspring. Pups exposed to a Gly-based herbicide showed early onset of cliff aversion reflex and early auditory canal opening. A decrease in locomotor activity and in anxiety levels was also observed in the groups exposed to a Gly-containing herbicide. Findings from the present study reveal that early exposure to a Gly-based herbicide affects the central nervous system in rat offspring probably by altering mechanisms or neurotransmitter systems that regulate locomotor activity and anxiety, such as GABAergic, dopaminergic and/or serotonergic pathways.
Glucocorticoids modify the differentiation potential of human neuroepithelial-like cells via long-term alterations of the redox state

Sandra Ceccatelli, Edoff K, Weis L, Ong J, Battagli C, Falk A, Raciti M.
Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden

High levels of glucocorticoids are known to exert detrimental effects on the developing nervous system, including alterations in neural stem cell proliferation, differentiation as well as increased susceptibility to oxidative stress. In this study, we have used the induced pluripotent stem cells (iPSC)-derived lt-NES AF22 cell line to investigate the heritable effects of the synthetic glucocorticoid dexamethasone (Dex) on neuronal differentiation in relation to the changes in reactive oxygen species (ROS) balance. Parent cells (P) directly exposed to Dex, were kept in a proliferating state to obtain daughter cells (D), never directly exposed to Dex, which were used for experimental purposes (see the figure below).

By analyzing gene expression in D cells we observed an increase in intracellular ROS concentration, as well as a downregulation of four antioxidant enzymes, namely Catalase, superoxide dismutase 1, superoxide dismutase 2 and glutathione peroxidase7. The altered intracellular REDOX state was concomitant to a significant downregulation of major neuronal markers and an increased number of glial cells. Interestingly, treatment with the antioxidant N-acetyl-cysteine (NAC) restored the expression of both neuronal and glial markers to control levels. These results indicate that the increased ROS concentration plays a critical role in the heritable alterations of the differentiation potential induced by Dex exposure. In addition, the data support the hypothesis that early insults can have negative long-lasting consequences on neurogenesis. Based on the positive effects exerted by NAC, it is conceivable that therapeutic approaches including antioxidants may be beneficial in the treatment of neuropsychiatric disorders that have been associated to increased ROS and impaired neurogenesis, such as depression.
Influence of Administration Setting and Caffeine on the Neuroinflammatory and Neurotoxic Effects of MDMA: Relevance to Dopaminergic Neurodegeneration

Giulia Costa, Lucia Frau, Nicola Simola, Micaela Morelli

Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Via Ospedale 72, 09124, Cagliari, Italy.

Preclinical studies in mice have reported that 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”), a recreational drug of abuse, can induce neuroinflammation and dopaminergic neurotoxicity. Moreover, MDMA is often consumed in crowded environments featuring high temperature and together with other psychoactive substances (e.g., caffeine): all these conditions have been suggested to influence the noxious effects of MDMA in the brain. Building on this evidence, we studied dopamine neuron degeneration in the substantia nigra pars compacta (SNc) and glial activation in the caudate-putamen (CPu) of adolescent and adult mice treated with MDMA. In a first set of experiments, mice received MDMA (4 x 20 mg/kg), alone or in combination with caffeine (10 mg/kg). In a second set of experiments, mice received MDMA (4 x 20 mg/kg) in different conditions: 1) while kept 1, 5, or 10 x cage at room temperature (21° C); 2) while grouped 5 x cage at either room (21° C) or high (27° C) temperature. At the end of the experiments, immunohistochemistry was performed in the CPu to mark interleukin (IL)-1β, tumor necrosis factor (TNF)-α, glial fibrillary acidic protein (GFAP), and CD11b as index of neuroinflammation, together with neuronal nitric oxide synthase (nNOS), and in the SNc to mark tyrosine hydroxylase (TH), as index of dopamine neuron degeneration. MDMA stimulated glial activation in the CPu and decreased TH in the SNc of both adolescent and adult mice. In the CPu of adolescent mice, caffeine potentiated MDMA-induced GFAP without altering CD11b, whereas in the SNc caffeine did not influence MDMA-induced glial activation. IL-1β, TNF-α and nNOS were increased by MDMA in CPu of adults, whereas in adolescents, levels were only elevated after combined MDMA plus caffeine. Moreover, crowding (5 or 10 mice x cage) amplified glial activation (in adult and adolescent mice) and dopaminergic neurotoxicity (in adolescent mice) induced by MDMA. Conversely, high environmental temperature (27° C) potentiated MDMA-induced glia activation in adult and adolescent mice grouped 5 x cage, but not dopaminergic neurotoxicity. Finally, crowding and exposure to high environmental temperature amplified MDMA-induced hyperthermia, and a positive correlation between body temperature and activation of either microglia or astroglia was found in adult and adolescent mice. Taken together, these results demonstrate that combined use of MDMA plus caffeine during adolescence may worsen the neurotoxicity and neuroinflammation elicited by MDMA, and that the administration setting influences these noxious effects of MDMA in the mouse brain, although crowding and high environmental showed a divergent influence on MDMA effects.
ELECTRICAL STIMULATION OF THE INFERIOR COLLICULUS: A PROMISING ANIMAL MODEL TO STUDY PARADOXICAL KINESIA

Liana Melo-Thomas ¹, Ana Luisa Gil-Martínez², Lorena Cuenca², Ana González-Cuello², Francisco J Fernández-Gómez², Cristina Estrada², Rainer K. Schwarting¹, María Trinidad Herrero²

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² Clinical and Experimental Neuroscience (NiCE-IMIB), Institute of Aging Research. School of Medicine, University of Murcia, Spain.

The inferior colliculus (IC) is an important midbrain relay station for the integration of descending and ascending auditory information. Additionally, IC has also been implicated in the modulation of motor responses as demonstrated in haloperidol-induced catalepsy and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal models of parkinsonism. We previously showed that the intracollicular administration of glutamate receptor antagonists (AP7 and MK-801) and agonist (NMDA) alters haloperidol-induced catalepsy in rats, which is reduced by the antagonists and increased by the agonist. In addition, a motor improvement was demonstrated after either intracollicular electrical stimulation or MK-801 microinjection in mice (C57BL/6 strain) chronically treated with MPTP. Furthermore, electrical stimulation of the IC can elicit motor responses in anesthetized rats, as it does for classical motor structures like the striatum, and reduce the catalepsy time induced by systemic haloperidol in awake rats. Taking into account that (i) paradoxical kinesia, observed in some parkinsonian patients, seems to be dependent of their emotional state and (ii) the IC plays a role in the sensorimotor gating activated by emotional stimuli, we can suggest that the electrical stimulation of the IC can be a promising animal model of paradoxical kinesia. Glutamatergic neural substrate in the IC can play an important role in the modulation of this phenomenon. Based on these and other important data from our group, three relevant points will be discussed: (1) the IC plays a role in the sensorimotor gating activated by emotional stimuli; (2) electrical stimulation at the IC can be a promising new animal model to study paradoxical kinesia in rats; (3) the IC can be a new therapeutic non-conventional target in the treatment of motor impairment.
NO AND GLUTAMATE INTERACTION IN TREATMENT OF L-DOPA-INDUCED DYSKINESIA

Elaine Del Bel
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and Center for Interdisciplinary Research on Applied Neurosciences (NAPNA), USP, Ribeirao Preto, Brazil

Amantadine is the noncompetitive antagonist of N-methyl-D-aspartate, receptor activated by the excitatory neurotransmitter glutamate. It is the only effective medication used to alleviate dyskinesia induced by L-3,4-dihydroxyphenylalanine (L-DOPA) in Parkinson’s disease patients. Unfortunately, adverse effects as abnormal involuntary movements (AIMs) known as L-DOPA-induced dyskinesia limit its clinical utility. Combined effective symptomatic treatment modalities may lessen the liability to undesirable events. Likewise drugs known to interfere with nitrergic system reduce AIMs in animal models of Parkinson’s disease. We aimed to analyze an interaction between amantadine, neuronal nitric oxide synthase inhibitor (7-nitroindazol, 7NI), and nitric oxide donor (sodium nitroprusside, SNP) in 6-hydroxydopamine- (6 OHDA)-lesioned rats (microinjection in the medial forebrain bundle) presenting L-DOPA-induced dyskinesia (20 mg/kg, gavage, during 21 days). We confirm that 7NI - 30 mg/kg, SNP-2/4 mg/kg and amantadine-40 mg/kg, individually reduced AIMs. Our results revealed that coadministration of sub-effective dose of amantadine (10 mg/kg) plus sub-effective dose of 7NI (20 mg/kg) potentiates the effect of reducing AIMs scores when compared to the effect of the drugs individually. No superior benefit on LDOPA-induced AIMs was observed with the combination of amantadine and SNP. The results revealed that combination of ineffective doses of amantadine and 7NI represents a new strategy to increase antidyskinetic effect in L-DOPA-induced AIMs. It may provide additional therapeutic benefits to Parkinson’s disease patients from these disabling complications at lower and thus safer and more tolerable doses than required when either drug is used alone. To close, we discuss the paradox of both nitric oxide synthase inhibitor and/or donor produced AIMs reduction by targeting nitric oxide synthase.
In Parkinson’s Disease (PD) decreased striatal dopamine produces dendritic spine loss in medium spiny neurons (MSNs) and increases their excitability. However, the synaptic changes in MSNs, in particular those induced by L-DOPA in parkinsonian animals, are still poorly understood. BAC-transgenic D1-tomato or D2-eGFP mice were lesioned with 6-OHDA and treated with L-DOPA. Synaptic changes were assessed with intracellular-sharp electrode recordings and structural changes with electron microscopy and single-cell injection for 3-D reconstructions. Dopamine depletion induced spine pruning in both types of MSNs, affecting mushroom and thin spines equally. It also increased firing rate in D1- and D2-MSNs, but evoked-EPSP amplitude was reduced only in D2-MSNs. L-DOPA treatment in dyskinetic animals differentially affects plasticity in D1- and D2-MSNs. In D1-MSNs, spine density remained reduced but the remaining spines are enlarged and have bigger heads. Curiously, the synaptic efficacy is maintained despite the morphological changes. By contrast, in D2-MSNs although L-DOPA restores spine density, it does not restore the synaptic efficacy. L-DOPA increases the number of spines and these spines have shorter post-synaptic densities correlating with a decrease of the evoked-EPSP amplitude. These findings indicate that abnormal spine morphology and opposite synaptic efficacy in D1- and D2-MSNs correlates with dyskinesia.

Funded by grants #SAF2013-48532-R, CIBERNED, ISCIII from the Spanish Ministries of Ciencia e Innovación and Sanidad y Política Social.
Involvement of the Kynurenine Pathway in the Pathogenesis of Parkinson’s Disease

Chai K. Lim¹, Francisco J. Fernández-Gomez², Nady Braidy³, Cristina Estrada³, Cristina Costa³, Silvia Costa⁵, Alban Bessede⁴, Emiliano Fernandez-Villalba¹, Maria Trinidad Herrero², Gilles J. Guillemin¹,⁶

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² Clinical & Experimental Neuroscience, School of Medicine, University of Murcia, Murcia, Spain
³ Centre for Healthy Brain Ageing, University of New South Wales, Sydney, Australia
⁴ ImmuSmol, Pessac, France.
⁵ Laboratório de Neuroquímica e Biologia Celular, Universidade Federal da Bahia, Brasil.
⁶ St Vincent’s Centre for Applied Medical Research, Sydney, Australia

Parkinson’s disease (PD) is a common neurodegenerative disorder characterized by loss of dopaminergic neurons and localized neuroinflammation occurring in the midbrain several years before the actual onset of symptoms. Neuroinflammation leads to microglia activation and release of a large number of proinflammatory mediators. The kynurenine pathway (KP) of tryptophan catabolism is one of the major regulators of the immune response and is also likely to be implicated in the inflammatory and neurotoxic events in Parkinsonism. Several neuroactive compounds are produced through the KP that can be either a neurotoxic, neuroprotective or immunomodulator. Among these metabolites kynurenic acid (KYNA), produced by astrocytes, is considered as neuroprotective whereas quinolinic acid (QUIN), released by activated microglia, can activate the N-methyl-D-aspartate (NMDA) receptor-signalling pathway, leading to excitotoxicity and amplify the inflammatory response. Previous studies have shown that NMDA antagonists can ease symptoms and exert a neuroprotective effect in PD both in vivo and in vitro. There are to date several lines of evidence linking some of the KP intermediates and the neuropathogenesis of PD. Moreover, it is likely that some of the KP metabolites could be used as prognostic biomarkers and that pharmacological modulators of the KP enzymes represent a new therapeutic strategy for PD.
Matrix metalloproteinases (MMPs) are ubiquitous extracellular calcium-dependent zinc-containing endopeptidases responsible for degradation of the extracellular matrix (ECM) and which play significant roles in both physiological and pathological conditions. The expression of MMPs in normal physiological conditions is low in order to maintain homeostasis. Then, increase activity of MMPs could result in neuroinflammation being important actors in a variety of brain disorders related with either acute or chronic neuroinflammation as in Parkinson’s disease. In fact, MMP-3 has been localized within the Lewy bodies and there are increased levels of active MMPs (MMP-1, MMP-2, MMP-3 and MMP-9) and tissue inhibitors of MMPs (TIMPS) in the substantia nigra (SN) and in the striatum of post-mortem brains of PD patients and Parkinsonian animals. In MMP-9 KO mice, there is a decrease of microglia and astrocytes activation after MPTP intoxication as well as BBB disruption. Additionally, in Parkinsonism MMP-3 (unlike MMP-2 and MMP-9) is activated not only inside the cells but as well in the ECM, leading: i) to increased dopaminergic cell death by intracellular active MMP-3 which drives lipid peroxidation and apoptosis; and ii) to a vicious circle between oxidative stress and inflammation by MMP-3 in the ECM, which promotes microglia activation with increased release of different pro-inflammatory cytokines. Then, the initial protective and beneficial function of the MMPs could turn into a harmful one maintaining (and increasing) the progression of the pathological state.

Funded by Fundación Séneca (FS/19540/PI/14), the Spanish Ministry of Science and Innovation (FIS PI13 01293), and Istituto Pasteur-Fondazione Cenci Bolognetti, ASI (Agenzia Spaziale Italiana) and MIUR.
Despite the fundamental role of the brainstem in regulating vital functional abilities such as arousal, breathing, autonomic nervous system activity as well as regulating all higher cerebral functions via neurotransmitter projections systems originating in the brainstem, the role of the brainstem has received relatively little attention in most neuropsychiatric disorders. Besides the dorsal and median raphe nuclei complex comprising mainly serotonin-producing neurons, the brainstem also contains noradrenalin, dopamine and histamine-producing nuclei, i.e. resp. the locus coeruleus, the substantia nigra and the mamillary bodies. The brainstem is furthermore the relay station of afferent and efferent projections between the autonomic nervous system in the peripheral body and higher cerebral brain regions. The current presentation aims to review the neuroanatomy of the brainstem as well as the current status on findings, derived from a wide range of studies using molecular, cellular and imaging technologies, of brainstem involvement in neurodevelopmental (i.e. autism, schizophrenia) and neurodegenerative disorders (Alzheimer’s and Parkinson’s disease).

Over the past decades, the incidence of age-related, neurological and psychiatric disorders such as AD, PD, but also depression has considerably increased. Mood disorders are strongly related to the exposure to stress. The hippocampus and other forebrain structures are the apex of the stress hormone control mechanism and damage to them may be one way in which stress hormone secretion escapes from inhibitory control in depression. In turn, stress, probably through toxic effects of glucocorticoids, decreases neurogenesis and cell survival while antidepressants enhance these processes in experimental animals. Therefore, since treatment strategies are not yet available, primary prevention in these age-related and stress related neurological disorders is of importance. As mentioned before most of the focus on neurobiological questions on above mentioned disease are related to forebrain structures since they are often associated with cognitive dysfunction. The brainstem is a highly neglected brain area in neurodegenerative diseases, including AD and PD and frontotemporal lobar degeneration. Likewise, despite a long-standing recognition of brainstem involvement, relatively few studies have addressed the exact mechanisms that underlie brainstem autonomic dysfunction. Improved insight in the cellular and molecular characteristics of brainstem function is pivotal to study the developmental origins. As brainstem dysfunction also poses
health issues in several other, neurodegenerative, disorders (like AD and PD), progress in these neurological fields will benefit from scientific advancement in the current proposal as well. In the area of depression, several observations have been made in relation to changes in one particular brain structure: the Dorsal Raphe Nucleus (DRN). In addition dysfunction of the cerebellum is also observed in AD and associated with pulmonary deregulation. The DRN is also related in the circuit of stress regulated processes and cognitive events.

In order to gain more information about the underlying mechanisms that may govern the neurodegeneration, e.g. amyloid plaques, neurofibrillary tangles, and impaired synaptic transmission in AD, a rat dissociation culture model was established that allows mimicking certain aspects of our autopsy findings. We observed a similar phenomenon in brains from patients suffering from neurodegenerative disease since this also related to changes in BDNF levels. The ascending projections and multitransmitter nature of the DRN in particular and the brainstem in general stress its role as a key target for AD/PD research and autonomic dysfunction. It also points towards the increased importance and focus of the brainstem as key area in various neurodevelopmental and age-related diseases.
HIGH POTENCY TFEB INDUCERS AS NOVEL THERAPEUTICS FOR PARKINSON’S DISEASE.

Shankar Chinta, Mannish Chamoli, Almas Siddiqui, Anand Rane, Subramanian Rajagopalan, Dipa Bhaumik, Gordon Lithgow, Julie K. Andersen
Buck Institute for Research on Aging, 8001 Redwood Blvd, Novato, CA 94945. USA

Parkinson’s disease (PD) is characterized by a progressive loss of dopaminergic (DAergic) neurons within the substantia nigra pars compacta (SNpc) and presence of proteinaceous inclusions called Lewy bodies in affected brain regions. It has been proposed that Lewy body formation may be due in part to defects in lysosomal autophagy resulting from reduced expression of the master regulator of lysosomal biogenesis, transcription factor EB (TFEB). Recent studies from our own laboratory assessing the neuroprotective effects of the autophagic inducing agent rapamycin in a genetic parkin mutant mouse model showed that rapamycin significantly abrogated alpha-synuclein accumulation and DAergic SNpc cell loss via its ability to up-regulate expression of TFEB. The efficacy of TFEB activation in mediating clearance of toxic protein aggregates makes it an attractive potential disease-modifying target for treating PD. In order to identify novel pharmacological agents capable of eliciting potent induction of TFEB, we recently carried out a small molecule screen in cultured neuronal cell lines and were successful in identifying a series of compounds with similar structural motifs displaying much higher induction than rapamycin. Subsequent qPCR analysis of a subset of compounds showing the greatest levels of TFEB induction revealed that not only do they elicit elevations in expression of TFEB itself, but also of several downstream TFEB target genes within cultured neuronal human SY5Y cells and in human iPSC-derived neurons. The lead compound C1, which displayed a nearly 10-fold induction of TFEB in our original assay, was additionally found to induce TFEB expression in vivo in C. elegans using a strain expressing the worm orthologue of human TFEB, HLH-30 protein, fused to GFP from the hlh-4 transcriptional promotor. Significantly, C1 administration was found to result in dosage-dependent dopaminergic neuroprotection and reversal of loss of motility in C. elegans models expressing human alpha-synuclein, suggesting that the compound is bioavailable and able to suppress alpha-synuclein-related proteotoxicity. This was found to be TFEB-dependent, as TFEB siRNA was able to prevent these neuroprotective effects. Further bioinformatics analysis revealed that not only do these compounds give substantial TFEB induction, but they were also found have favorable characteristics for CNS-acting drugs based on several factors including their predicted blood brain barrier (BBB) permeability, lipophilicity, and polar surface area. These derivatives are part of a family of natural benzopyrone...
compounds found in many edible plants. Closely related derivatives demonstrate low toxicity and good blood-brain-barrier (BBB) permeability, making this class of compounds promising candidates for use in PD. Preliminary pharmokinetic studies suggest TFEB induction in the SNpc following systemic administration. Based on these encouraging preliminary results, we propose to move these compounds forward into preclinical testing for efficacy of these newly identified TFEB inducers in order to move these (and possibly other related analogued compounds) forward towards the clinical trial phase. We hypothesize that these agents are likely to be superior to already established TFEB-inducing compounds.