Abstracts

Joint Meeting

Neurotoxicity Society and

International Neurotoxicology Association

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May 20

Jacob Hooisma Keynote Lecture: Parkinson disease: At the intersection of genes and environment

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About 10% of Parkinson disease (PD) cases have a monogenic cause, and although causative mutations or gene dupli- or triplications in the gene encoding α-synuclein are rare, it is clear that wildtype α-synuclein plays a central role in the pathogenesis of idiopathic (non-genetic) PD (iPD). It is the main component of Lewy pathology and it may travel transneuronally causing self-templating pathology similar to a prion. We have found that exposure to the pesticide, piscicide and mitochondrial toxin, rotenone, results in accumulation, oligomerization and aggregation of α-synuclein. At least in part, rotenone-induced α-synuclein oligomerization is dependent on activation of NAD(P)H oxidase 2 (NOX2). From an epidemiological perspective, it is important to note that even a brief, 'mild', temporally-remote exposure to rotenone causes – after a latency of months – progressive parkinsonism and α-synuclein pathology. Oligomers of α-synuclein bind specifically to the mitochondrial receptor, TOM20, which is critical for import of matrix-targeted proteins, such as components of the electron transport chain. In doing so, oligomeric α-synuclein produces senescent mitochondria that produce less energy and more ROS. Further emphasizing the importance of this gene-environment interaction, we found that therapeutic 'gene therapy' designed to reduce α-synuclein protein levels provides behavioral, anatomical and biochemical protection against rotenone.
Mutations in Leucine Rich Repeat Kinase 2 (LRRK2) are the most common genetic cause of PD, and causative mutations are believed to be associated with aberrantly increased kinase activity. There is great interest in developing LRRK2 kinase inhibitors for the small fraction of PD patients with these mutations, but the physiological regulators and the role of LRRK2 in iPD have been unknown. We developed a novel histological assay for LRRK2 activity and now show that in control human brain, there is very little WT LRRK2 activity at baseline. In contrast, in substantia nigra dopamine neurons from PD patients, there is dramatic activation of LRRK2. In vivo and in vitro experiments reveal that both rotenone treatment and α-synuclein overexpression activate LRRK2 in a NOX2-dependent fashion. Thus, LRRK2 is redox activated and is active in iPD (and models thereof), raising the possibility that LRRK2-targeted therapeutics may be useful for all patients with PD, even possibly when it is caused by environmental toxicants such as rotenone.

This research was supported NIEHS, NINDS, US Department of Veterans Affairs, the DSF Charitable Foundation, the Blechman Foundation and the American Parkinson Disease Association.

May 21

Workshop: Agilent Technologies Workshop on Mitochondrial Bioenergetics

Diets, Mitochondria and the Brain;
Alicia J. Kowaltowski, University of São Paulo, São Paulo, Brazil.

Symposium: Neuroinflammation and Neurodegeneration

Neuroinflammation in systemic inflammatory diseases

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Sepsis is associated with multiple organ dysfunction, including acute brain dysfunction, termed here sepsis-associated delirium (SAD), being an independent predictor of death. In addition, delirium in critically ill patients may predict long-term cognitive function after discharge from the intensive care unit. Thus it is possible that, differently from others organ dysfunctions, brain dysfunction can be not reversible, progressing from acute to

chronic brain dysfunction. Despite its importance, SAD has been neglected mainly because there are no precise clinical diagnoses of damage to the brain during sepsis. SAD phatophysiology is poorly understood but several mechanisms have been proposed, such as brain cellular death, mitochondrial and vascular dysfunction, neurotransmission disturbances, inflammation and oxidative stress. Sepsis-induced brain inflammation is dependent of several different mechanisms from blood brain barrier breakdown to microglia activation. Understanding these mechanisms will help to design new pharmacological tools to decrease SAD and long-term cognitive dysfunction that would impact in patients morbidity and mortality.

Influence of a Physiologically Relevant Brain Oxygen Tension on Mitochondrial Respiratory Impairment in Inflammatory Microglia

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Microglia are the innate immune cells of the central nervous system. Following brain injury, microglia shift to an 'activated' state characterized by upregulated production of proinflammatory factors. Sustained activation aggravates damage through the release of proinflammatory factors such as nitric oxide (NO). NO is thought to impair mitochondrial respiration by competing with O_2 at Complex IV. *In vitro* studies of microglial activation are generally performed at atmospheric oxygen tension (160mm Hg, 21% O_2) out of ease, although a far lower oxygen tension exists in the brain (~15-40mm Hg, ~2-5% O_2). Despite this O_2 competition model, the potential influence of pO_2 on respiratory inhibition has been largely overlooked in *in vitro* studies of microglial inflammation. This study tested the hypothesis that respiratory inhibition during proinflammatory microglial activation is mediated by NO at 3% O_2, but by peroxynitrite at 21% O_2. Oxygen consumption by rat microglia decreased by >50% at both oxygen tensions following activation triggered by 100ng/mL lipopolysaccharide and 10ng/mL interferon-gamma. Respiratory inhibition was first observed at 8 hours, in parallel with a significant increase in release of NO. Respiratory inhibition was acutely reversed by scavenging of NO with carboxy-PTIO at 3% O_2, but not at 21% O_2. Precluding NO production with the inducible nitric oxide synthase inhibitor 1400W entirely prevented the inhibition of respiration at 3% O_2, but only slightly at 21% O_2. Surprisingly, treatment with antioxidants during LPS/IFN-g exposure did not overcome respiratory inhibition at 21% O_2, inconsistent with the hypothesis that the deficiency was caused by peroxynitrite-mediated damage. Direct donation of electrons to Complexes III and IV using exogenous electron donors acutely rescued respiration at 21% O_2 but not at 3% O_2, suggesting that the impairment occurred at Complex IV at physiological oxygen but upstream of Complex III at atmospheric oxygen. Intriguingly, degradation of several mitochondrial Complex I subunits was observed at 21% O_2, beginning after 8 hours of LPS/IFN-g exposure, whereas the same Complex I subunits were more stable at 3% O_2. Our findings demonstrate that the oxygen tension used during assays influences the mechanism of bioenergetic impairment in inflammatory microglia, and suggest that oxygen tension is a consideration in future neuroinflammation models utilizing cultured microglia.

Research was supported by the National Institutes of Health R01NS085165.

Administration of branched-chain amino acids alters the balance between pro- and anti-inflammatory cytokines, and induces cerebral oedema, and blood-brain barrier breakdown

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Acute leucine intoxication and neurologic deterioration can develop rapidly at any age as a result of net protein degradation precipitated by infection or psychological stress in patients with maple syrup urine disease (MSUD). Here, we investigated the effects of acute and chronic branched-chain amino acids (BCAA) administration on pro- and anti-inflammatory cytokines in the brains of rats. For acute administration, Wistar rats (10 and 30 days) received three injections of BCAA pool (15.8 μL/g at 1 h intervals) or saline, subcutaneously. For chronic administration, Wistar rats (7 days) received of BCAA pool or saline twice a day for 21 days, subcutaneously. Our results showed that acute administration of BCAA increased IL-1β and TNF-α levels in the cerebral cortex but not in the hippocampus of infant rats. Moreover, IL-6 levels were increased in the hippocampus and cerebral cortex, whereas IL-10 levels were decreased only in the hippocampus.

However, repeated administration of decreased IL-1β, IL-6 and IFN-γ levels in the cerebral cortex, whereas the IL-6, IL-10 and IFN-γ levels were decreased in the hippocampus. These findings suggest that a better understanding of the inflammatory response in MSUD patients may be useful to develop therapeutic strategies to modulate the hyperinflammatory/hypoinflammatory axis. In another set of experiments, we investigated whether acute administration of BCAA causes cerebral oedema, modifies Na^+,K^+-ATPase activity, and affects the permeability of the blood-brain barrier (BBB). Additionally, we investigated the influence of concomitant administration of dexamethasone on the alterations caused by BCAA. Our results showed that the animals submitted to the model of MSUD exhibited an increase in the brain water content, both in the cerebral cortex and in the hippocampus. By investigating the mechanism of cerebral oedema, we discovered an association between BCAA and Na^+,K^+-ATPase activity and the permeability of the BBB to small molecules. Interestingly, we showed that the administration of dexamethasone successfully reduced cerebral oedema, preventing the inhibition of Na^+,K^+-ATPase activity, and BBB breakdown. In conclusion, these findings suggest that dexamethasone can improve the acute cerebral oedema and brain injury associated with high levels of BCAA, either through a direct effect on brain capillary Na^+,K^+-ATPase or through a generalized effect on the permeability of the BBB to all compounds.

Cognitive deficit and neuroinflammation in phenylketonuria: is there a link?
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Phenylketonuria (PKU) is a genetic disease caused by the deficiency of the enzyme phenylalanine hydroxylase activity, leading to the accumulation of phenylalanine in tissues and biological fluids of affected patients. The main characteristic of PKU is a severe intellectual disability, whose pathophysiology is still uncertain. It is proposed that phenylalanine exerts neurotoxic effects that contribute for the mental retardation presented by PKU patients. Recently, it has been shown that neuroinflammation exerts a role in the pathophysiology of this disease. The understanding of the link between neuroinflammation and cognitive deficit in PKU will help to design new therapeutic approaches to improve the intellectual disability found in phenylketonuric patients.

Financial Support: PKU Academy Fellowship, CNPq, and PKU Academy.

Symposium: Neuroinflammation: Reaction and Regulation

Increased neuroinflammation and neurodegenerative changes secondary to age-associated changes of Scavenger-A as a novel pathophysiological mechanism for Alzheimer's disease.
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Brain amyloid-β (Aβ) plaques, neurodegenerative changes and inflammatory activation of glial cells are hallmarks of Alzheimer's disease (AD) pathology. The "Amyloid Cascade hypothesis" considers Aβ aggregation as the cause of AD. However, increasing evidence show that Aβ accumulation can be a consequence of glial cell dysregulation in the brain, which results in an increased cytotoxicity, the impairment of Aβ clearance by glia, and neurodegenerative changes. Among several molecules that participate in the regulation of glial cell activation, scavenger receptor class A (SR-A), which participate both in Aβ phagocytosis and the inflammatory activation of glial cells, appears to be a potential target for AD. We have previously shown that in aging there is an increased inflammatory activation of glial cells as well as an increased basal activation of Smad2/3/4, the canonical pathway of the regulatory cytokine TGFβ, whereas Smad3 activation in response to inflammatory stimuli is reduced in aged animals. Here we show by both *in vitro* and in *ex vivo* experiments that SR-A expression is decreased in aged individuals as well as through a Smad3-dependent mechanism after stimulation with TGFβ. Expression of SR-A was low already in young APP/PS1 mice compared with WT mice. Furthermore, lack of SRA was associated with increased Aβ accumulation as observed in the APP/PS1/SR-$A^{-/-}$ congenic mouse generated in our lab. Lack of functional SR-A was associated with dysregulation of Glial cell activation, both at basal unstimulated conditions, with increased production of inflammatory cytokines and decreased levels of regulatory cytokines; and after inflammatory

stimulation with LPS. Those conditions of increased inflammatory activation are able to potentiate neurotoxicity, potentiating neurodegenerative changes. The APP/PS1/SR-A mouse, which accumulates Aβ and lacks SR-A, showed increased plasma and hippocampal levels of inflammatory cytokines, and increased numbers of Aβ plaques in the hippocampus. Our results show that aging is associated with increased activation of the TGFβ pathway, which reduces SR-A expression by glial cells, promoting the accumulation of Aβ plaques, and favoring the cytotoxic activation of microglia and astrocytes, which appear to led to neurodegenerative changes and the behavioral impairment observed in Alzheimer's disease.

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Inhibition of inflammasome activation by neopterin

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Neopterin, a well-established biomarker for immune system activation, is found at increased levels in the cerebrospinal fluid of individuals affected by neurological/neurodegenerative diseases. Here, it was investigated neopterin synthesis in neurons, astrocytes and/or microglia obtained from human and rodent and in the hippocampus under an inflammatory stimulus. It was also aimed to investigate whether neopterin preconditioning could modulate inflammasome activation, a component of the innate immune system, in human primary astrocytes. Increased neopterin levels was detected in the supernatants of human nerve cells (highest secretion in astrocytes) exposed to lipopolysaccharide (LPS) and interferon-gamma (INF-γ). It was also observed in the hippocampus of mice receiving LPS (0.33 mg / kg; intraperitoneal), in parallel to the hippocampal expression of the rate-limiting enzyme of neopterin biosynthetic pathway. Interestingly, both events occured before the inflammasome assembly. Moreover, a significant inhibition of the inflammasome activation was observed in neopterin pre-conditioned human astrocytes, when challenged with LPS, by reducing IL- 1β, caspase-1 and ASC expression or content, components of the NLRP3 inflammasome. Mechanistically, neopterin induced the nuclear translocation of the transcription factor Nrf-2, and the anti-inflammatory cytokines IL-10 and IL-1rα release, which would induce the inhibition of the inflammasome assembly. Altogether, this strongly suggests an essential role of neopterin during inflammatory processes.

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Can neurotoxic metals influence an inflammatory response or serve as an inflammasome trigger?

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<u>Symposium</u>: NeuroInflammation and Viral Infection in the Process of Neuropathology

Mechanisms of Zika infection, toxicity, and pathogenesis within the human nervous system.

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Zika virus (ZIKV) has become epidemic in several countries and now is a major public health issue. ZIK infection-induced fever related symptoms in most people, like Dengue and West Nile virus. However, the virus can be transmitted from the pregnant mother to the fetus resulting in severe brain malformation including microcephaly. Furthermore, in a small population of adults the virus results in a Guillain-Barre syndrome.

However, the mechanisms by which the virus compromise the developing CNS are not fully explored. Here, we identified that ZIKV from different regions of the world have differential infectivity in human fetal mixed cultures of astrocytes and neurons. We identified that cellular development is essential to support ZIKV replication and that ZIKV infects a small population of astrocytes compromising their Golgi, endoplasmic reticulum, and plasma membrane. Astrocyte infection results in changes in the cell to cell communication, gap junctions, and glutamatergic synapses, as well as adhesion molecules required for an efficient cortex formation. Furthermore, ZIK infection results in significant levels of apoptosis in astrocytes and neurons, supporting that hypothesis that ZIKV is highly neuropathogenic. In conclusion, our data indicates that ZIK infection of the developmental brain, especially astrocytes, could have profound consequences for cellular function, synaptic communication, neuronal migration, and cell death within the CNS. The examination of these mechanisms of toxicity elicited by ZIKV could provide new therapeutic approaches to prevent, treat and repair the damage produced by this devastating emerging virus.

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Neuroprotective Therapeutics for HAND

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There are severe neurological complications that arise from HIV infection, ranging from peripheral sensory neuropathy to cognitive decline and dementia for which no specific treatments are available. The HIV proteins secreted from infected macrophages, gp120 and Tat, are neurotoxic. A goal of this work was to screen, identify and develop neuroprotective compounds relevant to HIV-associated neurocognitive disorders (HAND). We screened more than 2000 compounds that included FDA approved drugs for protective efficacy against oxidative stress-mediated neurodegeneration and identified selective serotonin reuptake inhibitors (SSRIs) as potential neuroprotectants. Numerous SSRIs were then extensively evaluated as protectants against neurotoxicity as measured by changes in neuronal cell death, mitochondrial potential, and axodendritic degeneration elicited by HIV Tat and gp120 and other mitochondrial toxins. While many SSRIs demonstrated neuroprotective actions, paroxetine was potently neuroprotective (100 nM potency) against these toxins *in vitro* and *in vivo* following systemic administration in a gp120 neurotoxicity model. Paroxetine treatment also attenuated inflammatory cytokine production in mixed neuronal cultures. Subsequent studies in SIV infected non-human primates found similar neuroprotective activity of the paroxetine. Finally, in an initial clinical study in HIV infected patients with HAND, a positive effect on cognitive testing was found with paroxetine treatment. Thus, this approach may help to identify potential neuroprotective therapeutics to treat neurocognitive and neurodegenerative disorders resulting from HIV infection.

Chemokines and neurodegeneration in HIV-1 infection

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Chemokines and their receptors are expressed throughout the central nervous system (CNS) and the periphery, and contribute to many physiological processes, including decisions about cell death and survival in the brain. Two chemokine receptors, CCR5 and CXCR4, are the major co-receptors for HIV-1 infection in conjunction with CD4, and mediate the neurotoxicity of the viral envelope protein gp120. Infection with HIV-1 frequently causes degenerative brain injury. Viral proteins, such as the envelope protein gp120, initiate neuroinflammation and production of microglial neurotoxins. One model for brain damage seen in HIV/AIDS patients are transgenic (tg) mice expressing in their CNS the soluble gp120 of the CXCR4-utilizing HIV-1 isolate LAV under the control of a promoter for glial fibrillary acidic protein (GFAP) in astrocytes. HIVgp120tg mice develop key neuropathological features observed in AIDS brains, such as decreased synaptic and dendritic density, increased numbers of activated microglia and pronounced astrocytosis. HIVgp120tg mice and human AIDS brains also share a significant number of differentially regulated CNS genes. We recently observed in HIVgp120tg mice that the genetic knockout of CCR5 ameliorated microglial activation and

abrogated neuronal damage and behavioral impairment. A microarray analysis of CNS RNA expression showed that brains of CCR5 wild-type (WT) and CCR5KO gp120tg mice expressed markers of an innate immune response and inflammation. One of the most up-regulated factors was the acute phase protein lipocalin-2 (LCN2). *In vitro*, in cerebrocortical cell cultures comprising neurons, astrocytes and microglia, LCN2 was itself neurotoxic, but in a CCR5-dependent fashion. While inhibition of CCR5 alone failed to prevent neurotoxicity of the CXCR4-utilizing gp120, it surprisingly rescued neurons from gp120 toxicity in combination with LCN2, thus recapitulating the finding in CCR5-deficient gp120tg brain. Altogether, our study provided evidence for a coordinated pathological effect of CXCR4, CCR5 and LCN2 on microglial activation and HIVgp120-induced brain injury, and an unexpected protective effect of LCN2 that depended on the knockout or inhibition of CCR5.

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Neurotoxicity of HIV protein gp120

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The human immunodeficiency virus (HIV) envelope protein gp120 promotes axonal damage and neurite pruning, similar to that observed in HIV positive subjects with neurocognitive disorders. The goal of this study was to reveal novel mechanisms that explain why gp120 is neurotoxic. Gp120, after endocytosis into neurons, binds with high affinity (K_D ~30 nM) to tubulin beta III, a component of neuronal microtubules which is essential for retrograde and anterograde transport. Microtubule function, which modulates the homeostasis of neurons, is regulated by polymerization and post-translational modifications. Based on these considerations, we tested the hypothesis that gp120 induces dynamic instability of neuronal microtubules. We first observed that gp120 prevents the normal polymerization of tubulin *in vitro*. We then tested whether gp120 alters the post-translational modifications (PTM) of tubulin by examining the ability of gp120 to change the levels of acetylated tubulin in primary rat neuronal cultures. Gp120 elicited a time-dependent decrease in tubulin acetylation that was reversed by Helix-A peptide, a compound that competitively displaces the binding of gp120 to neuronal microtubules. Helix-A peptide was also able to prevent the neurotoxic effect of gp120 measured by mitochondrial damage, neurite pruning and activated caspase-3. To determine whether the PTM of tubulin also occurs *in vivo*, we measured acetylated tubulin in the cerebral cortex of HIV transgenic rats (HIV-tg) and in postmortem tissue of HIV positive subjects. We observed a decrease in tubulin acetylation in HIV-tg rats as well as in HIV positive subjects when compared to wild type and HIV negative controls. Our data suggest that endocytosis of gp120 and the subsequent binding to microtubules are two critical events for its neurotoxicity.

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<u>Symposium</u>: Antibiotics Action In Neurological Diseases: *In Vivo* and *In Vitro* Advancing Mechanistic Understanding

A study on the inflammatory reaction in the L-DOPA-induced dyskinesia in a Parkinson's disease rat model

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In Parkinson's disease (PD), L-DOPA is the most effective therapy, although leads to the emergence of motor complications including L-DOPA-induced dyskinesia. Among the mechanisms proposed to contribute to PD and more recently L-DOPA-induced dyskinesia considerable attention has been focused on L-DOPA-induced inflammatory responses. Our studies of L-DOPA-induced dyskinesia in rodents revealed in the dopamine-denervated striatum an increased expression of inflammatory markers, such as the enzymes COX2 in neurons and iNOS in glial cells. In the same brain region, dyskinetic animals exhibited an increased immunorreactivity

to glial fibrillary acidic protein in reactive astrocytes, an increased number of CD11b-positive microglial cells with activated morphology. All neuroinflammation indexes as well as L-DOPA-induced dyskinesia, were prevented by 7-nitroindazole (7NI, a neuronal NOS inhibitor) coadministration. The effect of 7NI in preventing this glial response, corroborates our hypothesis of an involvement of nitric oxide system. Indeed, Amantadine, the only pharmacological treatment used to deal with L-DOPA-induced dyskinesia in Parkinson's disease patients, among other action mechanisms, may act on glial cells. Our recent results showed that a treatment with amantadine plus 7NI, both in low doses, have a synergistic effect on L-DOPA-induced dyskinesia decrease. These results revealed a new strategy to reduce L-DOPA-induced dyskinesia, with lessen side effects and providing a superior therapeutic benefit. New approaches will be discussed.

A study on the protective role of doxycycline upon dopaminergic neuron of Parkinson's disease rodent model

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Objectives: The tetracycline-derivative doxycycline (α-6 deoxy-5-hydroxytetracycline) has been shown to be neuroprotective in *in vitro* and *in vivo* models of neurodegenerative diseases. The proven reliability and safety of the medication suggests its potential as an effective and inexpensive treatment to protect or at least mitigate the central nervous system from neurodegenerative diseases such as Parkinson's disease. It has been suggested that the modulation of astrocyte and microglial activation could prevent neuronal demise and thus the progression of neurodegeneration. We hypothesize that doxycline could exert a neuroprotective effect by suppressing astrocyte and microglial activation induced by the neurotoxin 6-hydroxydopamine (6-OHDA), a preparation with similarities to Parkinson's disease.
Methods: We investigated in striatal 6-OHDA lesioned mice the effects of a doxycycline given chronically either orally or subcutaneously at sub-antibiotic concentrations. To assess the protective mechanism conveyed by doxycycline we quantified using immunoreactive labeling tyrosine hydroxylase (neurons), glial fibrillary acid protein for astrocytes and cell surface marker macrophage antigen complex-1 for microglial cells. Brain regions containing cell bodies and fibers of dopamine in the nigrostriatal pathway were evaluated. Neuroinflammation indicators were sampled by Western blot analysis of metalloproteinase-3, cycloxygenase-2 and caspase-3 from the striatum. Results: Chronic treatment with doxycycline, either administered orally or injected subcutaneously at sub-antibiotic concentrations, mitigates the loss of dopaminergic neurons in the substantia nigra compacta and nerve terminals in the striatum. This protective effect was associated with: (1) a reduction of microglia in normal mice as a result of doxycycline administration *per se*; (2) a decrease in the astrocyte and microglia response to the neurotoxin 6-OHDA in the globus pallidus and substantia nigra compacta, and (3) decrease of the astrocyte reaction in the striatum. Conclusion: Our results suggest that doxycycline blocks 6-OHDA neurotoxicity *in vivo* by inhibiting microglial and astrocyte expression. This action of doxycycline in nigrostriatal dopaminergic neuron protection is consistent with a role of glial cells in Parkinson's disease neurodegeneration.

Role of Doxycline in neuroinflamation and protein aggregation

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Parkinson's disease is characterized by nigral dopaminergic neuronal death. The pathological role of several factors has been highlighted, namely oxidative stress, neuroinflammation, protein misfolding, and mitochondrial dysfunction, in addition to genetic predispositions. The current therapy with l-DOPA is mainly symptomatic, aiming to replace dopamine. However novel therapeutics are needed to influencing the different pathways participating to neuronal death. An attractive strategy to tackle this problem is discovering new uses for approved drugs to provide the quickest possible transition from bench to bedside. Our previous results supports the potential of doxycycline as a neuroprotective agent for dopaminergic neurons. The aim of this communication is to presents results concerning the anti-inflammatory effect of doxycycline and its ability to interfere with the pathologic cycle involved in synucleinopathies at the aggregation level. Primary microglial cells purified from post-natal day 1 C57BL/6J mouse pup brains were pre-treated or not with doxycycline, then challenged with LPS and incubated for 24 h. Conditioned media were collected to perform ELISA assays to measure levels of different cytokines. Adherent cells were either fixed for immunostaining procedures or lysed for western blot assays. We used a combination of biophysical techniques like fluorescence and infrared spectroscopy, electronic microscopy, small angle X Ray scattering and NMR together with cellular biology approaches to assess the impact of doxycycline on α-synuclein aggregation. Doxycycline attenuated the expression of key activation markers in LPS-treated microglial cultures in a concentration-dependent manner. More specifically, doxycycline treatment lowered the expression of the microglial activation marker IBA-1 as well as the production of ROS, NO, and proinflammatory cytokines (TNF-α and IL-1β). We also found that doxycycline inhibits LPS-induced p38 MAP kinase phosphorylation and NF-kB nuclear translocation. On the other side we proved that doxycycline interacts with α-synuclein early aggregation intermediates leading to the formation of off-pathway species, with parallel beta-sheet content that do not evolve into fibril formation. These aggregates are neither cytotoxic to dopaminergic cell lines, nor capable of disrupting the integrity of liposomes membrane. Doxycycline might act synergistically inhibiting neuroinflammation and the production of α-synuclein toxic species. These results place doxycycline as a pleiotropic drug becoming an attractive therapeutic strategy against Parkinson disease and others synucleinopathies.

Symposium: Regulated Necrosis and Associated Non-Apoptotic Mechanisms in Neurotoxicity

Binge alcohol and brain neuronal necrosis

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Unlike caspase-dependent apoptosis, necrosis was originally viewed as unregulated. However, with the discovery of a "necroptosis" pathway, it became evident that necrotic cell death is genetically controlled and highly regulated. With respect to neurons, regulated or programmed necrotic mechanisms provoked by neurodamaging insults or neurotoxins now encompass not only the prototype necroptosis, but also oxytosis, ferroptosis, pyroptosis, parthanatos, autophagy, and associated senescence mechanisms. While the toxicity of the neurotoxin, alcohol (ethanol), in adult brain is well-established, the underlying mechanisms are unsettled. Indeed, experimentally they appear to depend on whether alcohol exposure involves repeated high binges or more moderate, long-term treatments/intake. Our studies employing rat adult-age brain slice cultures demonstrate that high alcohol binges instigate oxidative stress-dependent neurodegeneration associated with potentiated intact poly-[ADP-ribose] polymerase-1 (PARP-1), which indicates the parthanatos pathway. Our PARP inhibitor experiments with both binged slices and repetitively binge-intoxicated adult rats establish PARP’s causative role in the resulting neurodegeneration. Furthermore, the studies reveal that binge alcohol-induced elevations in calcium (Ca+2)-dependent and secreted phospholipase A2 (PLA2) isoforms (mobilizing neuroinflammatory ω-6 arachidonic acid) along with increases in proinflammatory danger signaling protein, HMGB1, a brain TLR4 agonist, are PARP-dependent. In contrast, the alcohol binges reduce brain ω-3 docosahexaenoic acid (DHA) and Ca+2-independent PLA2, believed to regulate endogenous DHA turnover.

Consequently, DHA supplementation of the slice cultures during binges potently reverses increases in PARP-1 and the above neuroinflammatory players, while suppressing neuronal necrosis.

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Programmed necrosis in photoreceptors: Implications for AMD and other retinal degenerations
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In age-related macular degeneration (AMD) -the leading cause of blindness in the developed world-, toxic tissue debris accumulates as drusen beneath the retinal pigment epithelium (RPE), followed by eventual cell death and vision loss. Although the formation of drusen constitutes an early characteristic of AMD, the mechanisms accounting for its generation and eventual toxicity remain elusive. Studies from our group have elucidated that there are two complementary redundant cell death pathways that affect photoreceptors and RPE. Namely caspase dependent apoptosis and receptor interacting protein kinase (RIPK) regulated necrosis. Induction of cell death to photoreceptors by separation from the RPE, or via genetic mutations or via chemical injury or via autophagic machinery inhibition and drusen deposit formation appears to be mediated predominantly by apoptosis, whereas RPE (and cones in some genetic models) appear to die by necrosis. RIPK pathway also regulated inflammasome activation of infiltrating immune cells. Suppression of apoptosis shifted cell death to RIPK necrosis necessitating combination therapies for effective cell survival.

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Cellular senescence as an alternative programmed pathway in brain cells under stress: Potential role in Parkinson's disease (PD)
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In age-related neurodegenerative disorders including PD, neurodegeneration has historically been considered to be primarily mediated via caspase-associated apoptosis. Findings from our laboratory have recently suggested however that in addition to apoptosis, an alternative cell fate may drive neurodegenerative phenotypes associated with PD--a process known as cellular senescence. Stress-induced modulation of autophagy within non-neuronal brain cells such as astrocytes can drive cells into this quiescent non-dividing state which prevents tumor formation, but also elicits the release of pro-inflammatory cytokines and other factors that may damage neighboring neurons. New data from the laboratory indeed suggests that ablation of senescent brain cells prevents neurodegeneration associated with both various mouse models of PD and in human iPSC-derived astrocytic-culture models. Suppression of senescence therefore may constitute a novel therapeutic target for PD and other related neurodegenerative diseases.

Symposium: Advances in the Neurotoxicology of Abused Drugs

Behavioral and neurobiological effects of low and high alcohol dose
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Alcoholism or alcohol used disorders (AUD) are major health concerns worldwide due to substantial mortality (approximately 6% of global death) as well significant social and financial burdens. On the other hand, moderate use of alcohol (one drink for women and 2 for men) may result in health benefits such as reducing

the risk of heart disease and ischemic stroke. The progression from moderate to heavy use is influenced by genetic and environmental (e.g. epigenetic) factors that ultimately result in disturbances in certain brain circuits. Interestingly, the euphoric or mood elevating effects of initial alcohol exposure, particularly in women, may be an important factor in continuation and eventual escalation of alcohol use, leading to behavioral and pathological conditions. In this presentation, evidence of antidepressant and "depressogenic" effects of low and high alcohol dose, respectively, in animal models will be provided. Moreover, some of the underlying neurochemical substrates including involvement of central alpha-2 adrenergic system as well as neurotrophic factors (e.g. brain-derived neurotrophic factor BDNF), in the frontal cortex and the hippocampus, areas involved in executive function and mood regulation, respectively, will be presented. Finally, results of pharmacological manipulations (e.g. tricyclic antidepressants and ketamine) with implications in prevention of AUD or relapse will be discussed.

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Leading to drug addiction: participation of ethanol metabolizing enzymes in the increased voluntary ethanol intake in perinatally-lead-exposed rats

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Developmental exposure to environmental neurotoxicants induces differential reactivity to challenging events later in life, including drug addiction. We have demonstrated that low level lead (Pb) exposure (220 ppm in drinking water) during gestation and lactation enhances the motivational responses to ethanol (Etoh) in periadolescent male pups in both, a voluntary Etoh consumption test and oral self-administration task. In search of the neurobiological basis that may account for these differences, the focus of these studies was on central Etoh metabolism and the enzymes involved in brain acetaldehyde (ACD) modulation. In the brain, catalase (CAT) mediates central Etoh oxidation to ACD, whereas ALDH favors ACD oxidation to acetate. At this respect, we report here that both, pharmacological CAT activation or brain ALDH inhibition was able to increase Etoh intake in both, control and Pb-exposed animals, while CAT blockade reduced the drug consumption selectively in the Pb group. Moreover, an intra VTA shRNA antiCAT vector microinfusion reduced Etoh intake only in the Pb group. At the neurochemical level, on the last session of the Etoh intake test, blood CAT activity and midbrain CAT expression were increased as result of Pb exposure, difference that disappeared with pharmacological CAT inhibition and resurged with CAT activation. On the opposite, brain ALDH2 activity and expression were reduced in the Pb-exposed group, a fact that may have prevented the expected ALDH2 activity inhibition after pharmacological blockade. These results contrast with peripheral ACD accumulation induced by systemic ALDH inhibition (a therapeutic approach employed in clinical practice to dissuade Etoh consumption) that resulted in a decrease in Etoh intake predominantly in the Pb-exposed animals. Overall, these results indicate that Etoh metabolizing enzymes are modulated by developmental Pb exposure with resultant central ACD accumulation, suggesting a prevalence of the reinforcing effects of brain ACD against the aversive peripheral ACD accumulation.

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3,4-methylenedioxymethamphetamine: Abuse potential and neurodegenerative effects

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3,4-methylenedyoxymethamphetamine (MDMA or "ecstasy") is a recreational drug mostly consumed by adolescents and young adults. Although both preclinical and clinical investigations have extensively studied MDMA, several questions on the behavioral and neurochemical effects of this amphetamine-related drug are still unanswered. To gain further insight into the neurodegenerative effects and abuse potential of MDMA, we investigated how repeated MDMA administration affected behavior and markers of both neurotoxicity and glia activation in mice and rats and how administration conditions influenced these effects. Thus, we first studied

how crowding and exposure to high environmental temperature, which often feature MDMA intake, influenced glia activation and dopaminergic neurotoxicity induced by MDMA in mice. C57BL/6J mice received MDMA (4 × 20 mg/kg) while kept 1, 5, or 10 × cage at room temperature (21° C) or while kept 5 × cage at either room (21° C) or high (27° C) temperature. Crowding amplified MDMA-induced glia activation and dopaminergic neurotoxicity, whereas exposure to a high environmental temperature potentiated MDMA-induced glia activation only. Moreover, we evaluated, in rats, how MDMA affected the emotional state by measuring 50-kHz ultrasonic vocalizations (USVs), a behavior indicative of positive affect, and how MDMA modified markers of serotonergic and dopaminergic toxicity. Furthermore, to clarify the similarities between MDMA and other recreational drugs, we evaluated, in rats, the behavioral and neurochemical effects of methoxamine (MXE), a ketamine-like derivative that is increasingly being abused by youngsters and adults. Sprague-Dawley rats received five MDMA (7.5 mg/kg, i.p.) or MXE (0.5, 2.5, 5 mg/kg, i.p.) administrations on alternate days and then an acute drug challenge seven days later. MDMA and MXE did not induce acute and long-term changes in 50-kHz USVs, suggesting that these drugs have similar effects on the emotional state. Moreover, both MDMA and MXE induced changes in markers of neurotoxicity in the rat brain, although with different outcomes that depended on the specific markers and brain regions considered. Taken together, these results shed further light on the behavioral and neurochemical effects of MDMA and on the conditions that may exacerbate these effects, and may help to clarify the detrimental consequences associated with the long-term intake of this amphetamine-related drug.

Neurobiology of compulsive behaviors: Translational tools in addiction

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It has become clear that drug addiction is not a moral failure but a complex neuroadaptive process and chronic relapsing brain disease characterized by compulsive drug use despite adverse consequences to the individual and society. It is also clear that not everyone who tries drugs become addicted and some are more vulnerable, while some are more resilient. Addiction is further characterized by entry at multiple stages: binge-intoxication, withdrawal-negative affect and preoccupation-anticipation ("craving") that overlap and provides a heuristic basis for vulnerability and resilience in some individuals. Therefore drug addiction research explores a number of molecules in the CNS and many processes associated with the development of compulsive drug. Thus, developmental, genetic, epigenetic and environmental risk factors have been linked to molecular neurobiological mechanisms in drug addiction. Significant progress and advances have been made in understanding the neurobiology of drug addiction but major gaps and lack of specific tools in probing the mechanisms associated with brain function and disorders, including drug addiction remains. Understanding the triggers for compulsive drug seeking and taking and relapse from quitting may provide biomarkers in substance use disorders and new therapeutic targets. In this presentation we update and highlight new development and strategies including optogenetic, epigenetic, neuroimaging and pharmacotherapeutic strategies in substance abuse therapy. Some of these concepts like immunotherapeutics, nanotherapeutics, or gene-editing technologies (CRISPR or RNAi) still require more development and studies for efficacy and safety to treat drug addiction. This may open new therapeutic approaches in the era of pharmacogenomics to individualize drug addiction treatment.

<u>Symposium</u>: Neurotoxicity of New Psychoactive Substances: From molecular target to clinical toxicology

Introduction on New Psychoactive Substances (NPS): Occurrence, prevalence of use and their primary mode of action

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Currently, over 600 different new psychoactive substances (NPS) exist on the drug market and their number is increasing annually. NPS are becoming 'drugs of choice' and the prevalence of use of specific NPS is even close to that of common drugs like cocaine. Also, the number of poisonings with NPS is increasing, while limited data is available on the possible adverse health effects. For most NPS, the primary mode of action is inhibition and/or reversal of monoamine reuptake transporters (DAT, NET and SERT), thereby increasing monoamine levels in the brain. This is often investigated using labour-intensive radiometric assays without kinetic measurements. We examined the applicability of a high-throughput fluorescent assay that is less laborious and allows for kinetic measurements. Inhibition of human monoamine transporters was determined by comparing the uptake of a fluorescent substrate that mimics the biogenic monoamine neurotransmitters between exposed and unexposed human embryonic kidney (HEK293) cells that were stably transfected with hDAT, hNET and hSERT. Cells were exposed to common drugs (cocaine, DL-amphetamine and MDMA), NPS (4-fluoroamphetamine, PMMA, α-PVP, 5-APB, 2C-B, 25B-NBOMe, 25I-NBOMe, methoxetamine) and the antidepressant fluoxetine. Fluorescence was measured using a microplate reader. Exposure to NPS and commonly used illicit drugs concentration-dependently inhibited uptake of the fluorescent substrate by monoamine transporters, mostly at concentrations relevant for human exposure. The phenethylamines amphetamine, 4-fluoramphetamine and PMMA potently inhibited hNET and to a lesser extent hDAT. Cocaine potently inhibited all three transporters, though α-PVP was over ten times more potent on hNET and hDAT. In contrast, hallucinogens like 2C-B, NBOMEs and MXE were more potent on hSERT compared to hDAT and hNET. The obtained IC_{50} values were mostly comparable with radiometric assays, although we observed higher IC_{50} values for phenethylamines on hSERT, likely due to experimental differences. Compared to radiometric assays, this fluorescent assay is less laborious, requires no specific laboratory facilities and allows for kinetic measurements at physiological conditions. This assay is therefore a good alternative for radiometric assays for future screening of transporter inhibition of the annually increasing large number of NPS.

Neurotoxicity screening for acute effects of new psychoactive substances (NPS) on neuronal activity using cortical neurons and human iPSC-derived neurons grown on microelectrode array (mwMEA) plates

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The number of available recreational drugs is continuously increasing. In recent years, over 600 new psychoactive substances (NPS) have entered the drug market. However, the hazard and health risks associated with these compounds are poorly studied. Given the large and increasing number of NPS, there is an urgent need for (high-throughput) *in vitro* neurotoxicity drug screening.
We therefore used rat cortical cultures grown on multi-well microelectrode arrays (mwMEAs) to measure changes in neuronal activity following acute drug exposure. Our data demonstrate these cultures develop into spontaneously active networks consisting of astrocytes, glutamatergic and GABAergic neurons. Neuronal activity is concentration-dependently affected by common neurotransmitters (glutamate, GABA, serotonin, dopamine, acetylcholine and nicotine) and receptor (ant)agonists (bicuculline, diazepam, CNQX and MK-801). Further, we demonstrate that exposure to common illicit drugs (3,4-methylenedioxymethamphetamine (MDMA) and amphetamine) and NPS (1-(3-chlorophenyl)piperazine (mCPP), 4-fluoroamphetamine (4-FA) and methoxetamine (MXE)) decreases neuronal activity. MXE most potently inhibits neuronal activity with an IC50 of ~0.5 μM, whereas MDMA, amphetamine and 4-FA are least potent with IC50s of ~100 μM.
While our data demonstrate the suitability of rat cortical cultures to investigate the effects of different (illicit) drugs on neuronal activity, using human cells could further increase relevance and eliminate the need for interspecies translation. We therefore also used human induced pluripotent stem cell (iPSC)-derived neurons to screen for the neurotoxicity of selected drugs (amphetamine and MXE). These human iPSC-derived cultures consist of excitatory and inhibitory neurons that also develop spontaneously active networks, which can be pharmacologically modulated. Interestingly, the IC50 of amphetamine is comparable between rat cortical cultures and human iPSC-derived neurons. However, human iPSC-derived cultures are considerably less

sensitive to MXE, illustrating the need to further characterize and validate human iPSC-derived cultures for future neurotoxicity testing.

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Overview of desired and unwanted (acute toxicity) effects following NPS exposure in humans.

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Globally there is increasing evidence of the availability and use of novel psychoactive substances (NPS) (also known by users and others as "legal highs"). Currently there remains limited understanding and awareness of the desired and unwanted effects related to the use of NPS. For the majority of NPS, there has been no previous pre-clinical *in vitro*, animal testing or human clinical trials to be able to fully document the desired and unwanted effects related to their use. There are a number of different sources of information, some of these are more scientific and robust whereas some are more "grey" and anecdotal. Each of these sources of information have their own distinct limitations, however it is possible to combine information from a number of these different sources through a process of 'data triangulation' to minimise the limitations of any one source and build a more complete understanding of the desired and unwanted effects of an individual NPS or class of NPS. These sources of information include: i) user self-reports in on-line discussion forums; ii) sub-population surveys of users of NPS; iii) information obtained from calls to regional or national poisons information centres/services (including information from on-line poisons information services such as TOXBASE provided by the UK National Poisons Information Service); iv) published case reports / series (preferably with analytical confirmation of the drug(s) and NPS used by the individual(s) involved; and v) data from emergency department (ED) presentations. The latter of these can be collated through national or international networks of EDs, such as done by the European Drug Emergencies Network (Euro-DEN) Plus project which has been running across Europe since October 2013 [now collecting data from 22 Emergency Departments across 15 European and neighbouring countries] or combined with information from regional poisons information centres and analysis of biological samples from cases [as undertaken by the STRIDA project in Sweden].

This process is undertaken formally by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) and the United Nations when they undertake a risk assessment of an individual NPS, and on the basis of the information gained by this process a decisions is made as to whether there should be a recommendation for control of that NPS.

Platform Session 1

The place of epigenetics in the effect of alcohol on brain plasticity

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Chronic and excessive ethanol consumption induces neurodegeneration responsible for the cognitive deficits observed in alcohol abuser and triggers adaptive mechanisms linked to epigenetic regulations leading to functional and structural changes. Whether epigenetic modulations could account for the changes in brain plasticity and the behavioural consequences induced by chronic ethanol consumption in C57BL/6J mice was the purpose of this work. In the hippocampus, chronic ethanol consumption induced global epigenetic modulations that were correlated with chromatin remodelling at the BDNF gene level. These effects involved post-translational histone modifications and DNA methylation. Epigenetic changes at the BDNF gene level probably allowed the increase in BDNF protein expression observed within the hippocampal dentate gyrus in mice having consumed ethanol for 3 weeks. Upregulation of BDNF expression was linked to both the

stimulation of intracellular cascades downstream BDNF/TrkB receptor activation, and the increase in neurogenesis within the dentate gyrus. Using a specific TrkB antagonist, ANA-12, we demonstrated that the hippocampal neurogenesis induced by chronic ethanol intake was under the control of BDNF. Behavioural analysis evidenced learning and memory impairments after ethanol consumption without any synaptic plasticity alterations within the hippocampus, suggesting the involvement of others mechanisms in the observed cognitive deficits. Altogether, these data bring new elements for understanding the hippocampal neurogenesis stimulation observed under chronic and voluntary ethanol consumption in C57BL/6J mice. Moreover, this apparent increase in plasticity might be probably considered as an adaptive and compensatory mechanism in response to the cognitive deficits induced by ethanol consumption.

Cholesterol metabolism in adult neural stem cells: Impaired adult hippocampal neurogenesis in an animal model of familial hypercholesterolemia

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Familial hypercholesterolemia is a disorder of lipoprotein metabolism caused by genetic abnormalities of the low-density lipoprotein (LDL) receptor. This condition is characterized by defective catabolism of LDL which results in increased plasma cholesterol concentrations from the time of birth and premature atherosclerosis development. Recently, clinical and preclinical studies have demonstrated an association between familial hypercholesterolemia and cognitive and mood impairments. We assessed the hypothesis that the well described cognitive impairments in LDLr -/- mice, a widely used rodent model of familial hypercholesterolemia, may be related to impairment in hippocampal region-dependent behaviors and reduced neurogenesis. Additionally, to dissociate the effects of hypercholesterolemia and the LDLr absence, we proceeded LDLr gene knockdown and isolated LDL treatment in dentate gyrus (DG) neural stem cells (NSCs) in vitro. Firstly, C57BL/6 and LDLr-/- mice, 12 weeks old, were evaluated in the DG-dependent metric change and cornu ammonis -dependent temporal order behavioral tasks (n = 10-15/group). To assess overall DG cell proliferation and survival C57Bl/6 and LDL-/- mice received BrdU injections (100 mg/kg i.p.; 12/12h for 3 days) and were sacrificed 24 h (proliferation group) or 28 days later (survival group; n = 7/group). Following, cerebral serial coronal sections were obtained and immunohistochemistry performed for BrdU, Ki67 and NeuN markers. In addition, NSCs from DG of 8 weeks old C57BL/6 mice were cultured as adherent monolayers and cholesterol metabolism dynamics was evaluated during proliferation and differentiation phases (n = 5). Moreover, the monolayers were treated with LDL (0 - 200 µg/mL) or transfected with LDLr siRNA and 24 or 48h after treatments several assays were run (n = 3). Our results show that the LDLr -/- mice presented impairment in dentate gyrus (DG)-dependent cognitive task, corroborating the reduction in DG cell proliferation and neurogenesis. Primary culture of NSCs isolated from the DG of adult C57BL/6 mice demonstrated that the expression of enzymes involved in cholesterol synthesis (HMG-CoA reductase and squalene synthase) and of LDLr peaks during the proliferation stage of the neurogenic process. On the other hand, the expression of LRP1, cholesterol 24-hydroxylase, and sterol 27-hydroxylase is up-regulated during the differentiation stage. In agreement with the observations from the LDLr-/- mice, exposure to both human LDL as well as silencing of the LDLr (using an LDLr siRNA) reduced cell proliferation and/or neuronal differentiation of aNSCs monolayers. LDL treatment was also associated with an increase in the number of lipid droplets and a down-regulation of mRNA levels of the LDLr and enzymes involved in cholesterol synthesis, whereas LDLr siRNA also reduced LRP1 gene expression. Microarray analysis showed that LDL down-regulated cholesterol metabolism. Overall, these data indicate DG-dependent impairment in spatial processing associated to decreased neurogenesis in LDLr-/- hypercholesterolemic mice. Furthermore, according to our in vitro study both the high cholesterol levels and the LDLr absence might have a role in this outcome.

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Role of Hypothalamic HIF-1 Complex in the Regulation of Energy Homeostasis

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The balance between food intake and energy expenditure is mainly regulated by the hypothalamus. Dysregulation of this process is critical for the development of obesity. Hypoxia-inducible factor-1 (HIF1) is a transcription factor that activates several genes in response to hypoxia or other harmful conditions. Besides its importance in hypoxia conditions, HIF1 complex also plays a role in the regulation of glucose and energy homeostasis. Dysregulation of HIF1 complex could also be involved in the development of obesity and type 2 diabetes. Previously, it was reported that HIF1 can regulate the expression of POMC modulating food intake. In this work, we hypothesize that the consumption of high-fat diet alters the expression of HIF1 in the hypothalamus with changes in the expression of neuronal POMC, and consequently altering the neuronal pathways that control food intake. Our main purpose was to analyze whether high-fat diet feeding changes the expression of HIF1 in hypothalamus. We also inhibited HIF1 in the arcuate nucleus to evaluate changes on high-fat diet consumption and body composition. We used male, 8 week old C57Bl6 mice, fed on chow or a high-fat diet for 1, 3, 7, 14 or 28 days. The expressions of HIF-1 proteins were measured by PCR and western blot and their hypothalamic distribution were evaluated by fluorescence microscopy. Inhibition of HIF1β in arcuate nucleus of hypothalamus was performed using stereotaxic injection of shRNA lentiviral particles and animals were grouped under chow or high-fat diet for 14 days. Body mass, food intake and glycemia were evaluated throughout the experiments. HIF-1 proteins were mainly localized in the arcuate nucleus of the hypothalamus, and they were colocalized with microglia and glial cell markers, and also with POMC and with ACTH. The expression of mRNA of HIF-1 proteins significantly decreased after 3 and 7 days of high-fat feeding, returning to baseline levels after 14 and 28 days. However, the protein levels of HIF-1alpha significantly increased after 7, 14 and 28 days on high-fat diet consumption, with a decreased in the protein levels of VHL (E3ligase) which indicates an increase in HIF-1α stabilization. Mice with inhibition of HIF-1β in the arcuate nucleus and fed on chow, had an increase in body mass compared with control animals. This effect was more pronounced when animals were maintained on high-fat diet. These animals have also an increase in glycemia. However, food intake was not affected by the inhibition of HIF-1β. The inflammatory markers in hypothalamus were also increased in animals with inhibition of HIF-1β. Conclusion: In summary, HIF1 complex is mainly expressed in POMC neurons in the arcuate nucleus of the hypothalamus. Although the mRNA expression of HIF-1 proteins decreased with high-fat diet feeding, its protein levels are increased indicating an increase in HIF-1α stabilization. These results point to a dysregulation of hypothalamic HIF1 complex associated with the consumption of high-fat diet, which can, at least in part, impact on changes in body mass. The increase in body mass without change in caloric intake in mice with hypothalamic inhibition of HIF complex, suggest that HIF1 complex could be involved in the regulation of energy expenditure. The role of HIF1 complex in the regulation of energy expenditure will be addressed in future studies.

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Isovitexin rich fraction of Passiflora actinia extract provides cognitive improvement and neuroprotection after cerebral ischemia in mice

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Excessive release of glutamate leading to excitotoxicity is responsible for neuronal injury during brain ischaemia. *Passiflora. actinia* Hook. (vernacular name: 'maracuja-do-mato') is a native species widely distributed through South Brazil which has been associated to sedative, anxiolytic and neuroprotective effects. The main bioactive constituents described for the *Passiflora* species are C-glycosyl flavonoid derivatives such as isoxitexin. The aim of study work was to evaluate the neuroprotective effects of an isovitexin rich ethyl

acetate fraction (EAF) of *Passiflora actinia* against learning and memory impairment in a cerebral ischemia mouse model by bilateral common carotid artery occlusion (BCCAO) and on neurotoxicity induced by glutamate or oxygen glucose deprivation (OGD) in hippocampal slices. Oral administration of EAF at 600 mg/kg/day (N=15) for 7 days (2 days before BCCAO and 5 days after BCCAO) significantly prevented the cognitive deficit induced by BCCAO in the Morris water maze test. Hippocampal brain slices from 7 days EAF-treated mice were subjected to *in vitro* OGD or to glutamate-induced neurotoxicity and cellular viability was evaluated by the MTT reduction test. EAF (300 and 600 mg/kg/day; N=6) was also able to prevented hippocampal cell viability reduction induced by OGD or glutamate. The present study provides evidence that EAF results in cognitive benefits and neuroprotection and may be of potential interest for the treatment of neurological conditions associated with cerebral ischemia and/or neurotoxicity associated with excessive glutamate release.

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Restoration of cognitive performance in mice carrying a deficient allele of 8-oxoGuanine DNA glycosylase by x-ray irradiation

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Environmental stressors like ionizing radiation may influence cognitive function, and reprogramming of brain development. Recent studies have shown that transgenic mice deficient of DNA glycosylases display unexpected cognitive deficiencies due to changes in gene expression in the mouse hippocampus genome. The main objectives of the present study were to investigate learning and memory performance of C57BL/6NTac $Ogg1^{+/-}$ mice compared to wt, and to study whether a single x-ray challenge (0.5 Gy, dose rate 0.457 ± 0.009 Gy/min) influenced the cognitive performance. We found that the $Ogg1^{+/-}$ mice had a poorer learning performance compared to the wt mice. Surprisingly, exposure of the F2 $Ogg1^{+/-}$ animals to the x-ray challenge improved the learning performance in the Barnes maze compared to the unexposed wt and the $Ogg1^{+/-}$ group while no persistent effects on memory one or six weeks after the x-ray exposure were observed. Gene expressional analyses of the hippocampus were performed to investigate if $Ogg1^{+/-}$ mice differ at the transcriptome level from wt and why x-ray irradiation improved learning performance in the $Ogg1^{+/-}$ mice.

<u>Symposium</u>: Different Aspects of Adverse Effects on the CNS of Stress-Related Factors Such as Glutamate and Ammonia

Hepatic encephalopathy and hyperammonemia lead to altered brain energy metabolism, GABAergic neurotransmission and behavioral deficits in the animal model of bile duct-ligation

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Hepatic encephalopathy is a neurological disease that arises as a consequence of acute or chronic liver diseases. It is a condition that has a broad range of clinical symptoms, affecting patients at psychological, motoric and cognitive levels. The severity of these manifestations will depend upon the degree of liver metabolic dysfunction and the rate by which it evolves. It is well accepted that one of the pivotal pathophysiological mechanisms related to HE is the increased level of ammonia. Due to absence of adequate mechanism of ammonia detoxification in the CNS, HE causes impairment of different neurotransmitter systems, increased oxidative stress and inflammation and importantly altered brain energy

metabolism. When co-cultures of neurons and astrocytes were exposed to high concentrations of ammonia increases in glycolysis and in oxidative metabolism in the neurons were observed. Astrocytes exhibited increased glycolysis and anaplerosis, leading to an augmented synthesis of glutamine, the main process for ammonia detoxification in the brain. In addition, HE/hyperammonemia altered the pathway by which GABA is synthetized, being favored through the mechanisms that involve the TCA cycle relative to the pathway that occurs through direct decarboxylation of glutamate. This could be a result of altered expression of the GABA synthesizing enzyme, glutamate decarboxylase (GAD), which is expressed in two isoforms in the brain, GAD67 and GAD65. GAD65 has been linked to the GABA neurotransmitter pool originating from metabolism involving the TCA cycle and driven towards neurotransmission, while GAD67 is related to the pool synthetized without the involvement of TCA cycle. No differences in the isoforms of GAD were observed during HE. Since GABA synthesis is dependent on the transfer of glutamine from the astrocytic compartment to the neuronal compartment, altered expression of glutamine transporters, i.e. SNAT3 and SNAT5, could also play an important role in this mechanism. However, these transporters were expressed at normal protein levels. Therefore, taken together these results suggest that altered biosynthesis of GABA might be a consequence of dysfunctions of neuronal energy metabolism. Interestingly, the neurotransmitter alterations could be related to the impairment in locomotor and exploratory activities and deficit in short-term memory observed in a rat model of this neurological disease.

Metabotropic glutamate receptors and neurodegeneration

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Maternal behavior, glutamate and oxytocin balance in the perinatal stress rat model

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Perinatal stress in rats triggers an epigenetic program that makes the adult offspring more vulnerable to stress-related disorders. These changes depend, at least in part, to a reduced maternal care, which can be corrected by pharmacological activation of oxytocin receptors in lactating dams. An important question is whether the (mal)adaptive programming induced by perinatal stress extends to the old age influencing emotional behavior and cognitive functions and which are the molecular mechanisms associated with this altered programming. We report that the adult (3-4 month old) and aged (17-month old) male progeny of dams exposed to repeated episodes of restraint stress during pregnancy showed a reduced exploration of the open arm in the elevated plus maze, a reduced time spent in exploring the noval arm in the Y maze, a defect in social interaction towards a juvenile intruder of the same sex, an impairment in spatial memory in the Morris water maze, and an increased activation of the hypothalamic-pituitary-adrenal axis associated with an altered transcription of the genes involved in the response to stress including oxytocin system. All these changes could be corrected by treating lactating dams with an oxytocin receptor agonist (carbetocin), which reversed the defect in maternal behavior induced by gestational stress. Carbetocin administration to the mothers did not correct the decrease in body weight shown by perinatally stressed rats in the first week of postnatal age. In addition, carbetocin could not act directly on pups because the drug is a peptidergic analogue of oxytocin and, even if transfered to the milk, it is not absorbed by the pups. Thus, the protective effect of early carbetocin treatment against behavioral and endocrinological abnormalities shown by perinatally stressed aged rats was due to an increased maternal care. Interestingly, all these altered parameters described in the PRS progeny are regulated by the activity of the hippocampus and hippocampal glutamate seems play an interesting role, too. Indeed, we observed that in adult rats an in vitro acute administration of carbetocin enhanced glutamate release in the synaptosomes of ventral hippocampus and also an in vivo chronic treatment with carbetocin was able to increased glutamate release in the ventral hippocampus. These findings demonstrate that perinatal stress cause cognitive dysfunction and an altered emotional phenotype during ageing presumably as a result of a defective maternal care associated with both an altered gene transcription and a reduced glutamate release in the hippocampus, key brain region sensible to the effects of maternal behavior. This raises the possibility that in humans early life stress may accelerate cognitive dysfunction associated with brain ageing,

thereby increasing the vulnerability to the development of dementia. Remarkably, this could be prevented by therapeutic strategies that improve the quality of maternal behavior in a critical time window after birth.

Glutamate metabolism impairment in a rat model of prenatal restraint stress

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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 brain neurotransmitter systems. Glutamate is the principal excitatory neurotransmitter in the mammalian central nervous system, participating in the integration of brain function and in synaptic plasticity, memory and learning processes. The glutamatergic normal function depends on the interactions between neurons and surrounding astroglia. Previous studies from our laboratory have shown that male adult offspring of stressed mothers exhibited higher levels of ionotropic and metabotropic glutamate receptors than control rats. These offspring also showed long lasting astroglial hypertrophy and a reduced dendritic arborization with synaptic loss. Since metabolism of glutamate is dependent on interactions between neurons and surrounding astroglia, our results suggest that glutamate neurotransmitter pathways might be impaired in the brain of prenatally stressed rats. To study the effect of prenatal stress on the metabolism and neurotransmitter function of glutamate, pregnant rats were subjected to restrain stress during the last week of gestation. Brains of the adult offspring were used to assess glutamate metabolism, uptake and release as well as expression of glutamate receptors and transporters. While glutamate metabolism was not affected it was found that prenatal stress (PS) changed the expression of the transporters, thus, producing a higher level of vesicular vGluT-1 in the frontal cortex (FCx) and elevated levels of GLT1 protein and messenger RNA in the hippocampus (HPC) of adult male PS offspring. We also observed increased uptake capacity for glutamate in the FCx of PS male offspring while no such changes were observed in the HPC. The results show that changes mediated by PS on the adult glutamatergic system are brain region specific. Overall, PS produces long-term changes in the glutamatergic system modulating the expression of glutamate transporters and altering synaptic transmission of the adult brain.

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May 22

Symposium: Emerging Neurotoxicological Mechanisms in Parkinsonian Neurodegeneration: Advances in Models and Concepts

Mechanisms of α-synuclein aggregation and cell-to-cell transmission of neurotoxic protein oligomers through the exosomal pathway

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The aggregation of α-synuclein (αSyn) is considered a key pathophysiological feature of certain neurodegenerative disorders collectively termed synucleinopathies. Recent studies suggest that a prion-like cell-to-cell transfer of misfolded αSyn contributes to the spreading of αSyn pathology in synucleinopathies. The biological mechanisms underlying the propagation of the disease with respect to environmental neurotoxic chemical exposures, however, are not well understood. Considering the potential role of the divalent metal manganese (Mn) in synucleinopathies, we characterized its effect on αSyn misfolding and transmission in experimental Parkinsonian models. Using dopaminergic neuronal cells stably expressing wild-type human αSyn, we show that misfolded αSyn is secreted through exosomes into extracellular media following Mn exposure. In functional studies, we demonstrate that these exosomes are e*ndocytosed via caveolae* into microglial cells, thereby inducing neuroinflammatory responses. Furthermore, Mn-triggered exosomes exert a

neurotoxic effect in differentiated human dopaminergic (LUHMES) cells as seen by caspase-3 activation. Moreover, our bimolecular fluorescence complementation (*BiFC)* assay shows that Mn elevated the cell-to-cell transmission of αSyn, resulting in dopaminergic neurotoxicity in a mouse model of Mn neurotoxicity. Importantly, we report that welders exposed to Mn have higher misfolded αSyn content in their serum exosomes. We also show for the first time that stereotaxically delivering αSyn-containing exosomes, isolated from Mn-treated αSyn-expressing cells, into the striatum can initiate Parkinsonian-like pathological features in mice. Collectively, these results demonstrate that Mn exposure promotes αSyn secretion via exosomal vesicles, which subsequently evokes pro-inflammatory and neurodegenerative responses in both cell culture and animal models
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Blocking mitochondrial fission is protective against dopaminergic neurodegeneration
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Mitochondrial dysfunction is a pathogenic mechanism in both familial and sporadic Parkinson's disease (PD). However, effective therapy targeting this pathway is currently inadequate. Mitochondria undergo frequent changes in shape, size, number and location. These alterations are affected by mitochondrial morphology, which in turn, is controlled mainly by mitochondrial fission and fusion proteins. Targeting these proteins to manipulate mitochondrial dynamics (fission, fusion and movement of mitochondria) has emerged as a potential novel therapeutic approach for Parkinson's disease and other neurodegenerative diseases. We have evaluated the protective effects of blocking the mitochondrial fission dynamin-related protein-1 (Drp1) in genetic and toxicant-induced cell and animal models of PD. In this presentation, data obtained from four complementary animal models will be presented: 1) Pink1-null (*Pink1*$^{-/-}$) mice represent a genetic model with age-related impairments in mitochondrial function and evoked striatal DA release; 2) Human α-synuclein-A53T overexpression in the rat nigrostriatal pathway produces protein aggregation, neurodegeneration and motor impairment; 3) MPTP-treated mice provide a model of complex I inhibition with nigrostriatal neurotoxicity; 4) The parkinsonism-linked herbicide paraquat (PQ) generates superoxide which also induces mitochondrial dysfunction. To enhance PQ neurotoxicity, we used novel mutant mice with deletion of the organic cation transporter-3 (*OCT3*$^{-/-}$) to create a PQ animal model with a damage in both the nigra and striatum as we previously described (Rappold et al., PNAS, 2011). Together, our strategies of blocking Drp1 using gene therapy and a small molecule inhibitor attenuate mitochondrial abnormalities, oxidative stress, striatal dopamine release deficits, neurodegeneration, protein aggregation, and motor impairment. These results suggest Drp1 inhibition as a potential treatment for PD.

Mechanisms of alpha-synuclein protein aggregates induced neuroinflammation in neurotoxicity models
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Protein aggregation is emerging as a major pathophysiological process of chronic neurodegenerative processes but cellular mechanism underlying protein aggregates induced degenerative process is not well understood. Although recent studies implicate microglia mediated neuroinflammatory response in neurodegeneration, signaling mechanisms underlying chronic microglial action remain to be identified. We link PKCδ to heightened microglial activation and neuroinflammation following alpha-Synuclein (αSyn) aggregates exposure. Exposure to α-synuclein aggregates dramatically upregulated PKCδ and concomitantly increased its kinase activity and nuclear translocation in both microglial cell line and primary microglia. The known inflammogens tumor necrosis factor α and lipopolysaccharide (LPS) recapitulated the effect of αSyn aggregates. Notably, exosomes containing αSyn aggregates released during manganese exposure induced neuroinflammatory responses in primary murine microglial cultures as determined by increased levels of IBA-1 and iNOS expression and the production of pro-inflammatory cytokines. In further mechanistic studies, shRNA-mediated knockdown and genetic ablation of PKCδ in primary microglia blunted the microglial proinflammatory response elicited by αSyn aggregates, including ROS generation, nitric oxide production, and proinflammatory

cytokine and chemokine release. PKCδ activated NFκB, a key mediator of inflammatory signaling events, after challenge with inflammatory stressors. NFκB transactivation led to nuclear translocation of the p65 subunit, IκBα degradation and phosphorylation of p65 at Ser536. Both genetic ablation and siRNA-mediated knockdown of PKCδ attenuated NFκB activation, suggesting that PKCδ regulates NFκB activation following microglial activation. We also identified that PKCδ regulates autophagy in cell models. To further validate the proinflammatory role of PKCδ, we used PKCδ knockout mice. We found that a PKCδ deficiency attenuated the proinflammatory response in the mouse substantia nigra, reduced locomotor deficits and recovered sick mice from LPS-induced neuroinflammation. Collectively, our results demonstrate that sustained PKCδ activation drives chronic mediated proinflammatory process during protein aggregation.

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Impaired mitochondrial transport and proteasome function in human dopaminergic neurodegeneration

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The underlying mechanisms of neurodegenerative diseases such as Parkinson's disease (PD) are not completely understood. One key event in PD, amongst others, is disturbed protreostasis, which comprises the disturbance of the ubiquitin proteasome system (UPS) and altered organellar transport. Various attempts in the past to treat these diseases and to slow down the progressive neuronal loss failed at different clinical stages. Most of these attempts followed the concept of blocking specific stress pathways (e.g. caspase activation). An alternative therapeutic approach would be to identify and harness endogenous cellular defense mechanisms and thereby to prevent the activation of deleterious cascades. In this study, we used a proteasome inhibitors and mitochondrial inhibitors to model key eents in PD pathogenesis. In a first step, we monitored the stress responses orchestrating the neurodegeneration in human dopaminergic neurons (LUHMES cells). Neurons, exposed to MG-132 (nanomolar concentrations), underwent rapid apoptotic cell death. Prior to caspase activation, we observed an increase in AKT and p38 phosphorylation. Moreover, the ATF4 stress response was induced, and this lead to an increase in GSH synthesis capacity. In parallel mitochondrial motility was rapidly altered. In a second step, we identified cysteine supplementation as a protective intervention. This intervention prevented ATF4 activation, AKT and p38 phosphorylation and cell death, while other amino acids showed no effect. Since glia cells are known to support neurons with thiols, we wondered, whether they might have protective properties in a co-culture model. When astrocytes were added to neurons, the latter were protected against MG-132. Under these conditions, the neuronal GSH increase was not observed and ATF4 activation was not detected. The major part of the protective effect of astrocytes was mediated by thiol supply to neurons and this indicates that elevation of cysteine levels within neurons in the brain might have a protective effect against disturbances of the UPS system in neurons. The ATF4-mediated induction of DDIT4, NOXA and PUMA, and the mechanism of thiol-transfer from astrocytes to neurons may provide new pharmacological targets to prevent neurodegeneration.

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Dysregulation of Metal Transporters in Dopaminergic Neurodegeneration

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Manganese (Mn), is a trace metal required for normal physiological processes in humans. Mn levels are tightly regulated, as high levels of Mn result in accumulation in the brain and cause a neurological disease known as manganism. Manganism shares many similarities with Parkinson's disease (PD), both at the physiological level and the cellular level. Exposure to high Mn-containing environments increases the risk of developing manganism. Combining genetics and biochemical assays, we established in the nematode (*C. elegans*) and other experimental models that dopamine (DA) is responsible for Mn-induced DAergic neurodegeneration, and that this process (1) requires functional DA-reuptake transporter (DAT-1), (2) is associated with oxidative stress and lifespan reduction, (3) and is enhanced by iron deficiency. The presentation will focus on the

mechanisms of Mn uptake and efflux into the brain, genetic susceptibility to Mn-induced damage, and molecular mechanisms of neurotoxicity.

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Symposium: Mechanisms for Neurodegeneration, Neuroprotection and Treatment in Parkinson´s Disease: 1

Aminochrome as preclinical model for Parkinson´s disease

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L-Dopa continues to be the most effective drug in Parkinson´s disease therapy despite the severe side effects observed after 4-6 years of treatment. Fifty years of intensive worldwide research has not been enough to discover new therapies to halt the progression of this disease to replace L-dopa use. One of the most promising attempts to find a new therapy to revert the progression of the disease was GDNF. GDNF was able to regenerate dopaminergic neurons and it was also possible to treat dyskinesia in different preclinical models such as 6-hyroxydopamine, MPTP and rotenone. However, the translation of successful preclinical models clinical studies failed. There is a long list of failed clinical studies based on successful preclinical studies with MPTP, 6-hydroxydopamine or rotenone. The question is why. First, exogenous neurotoxins, such as 6-hyroxydopamine, MPTP and rotenone, induce a very rapid and extensive degeneration. For example MPTP induces a severe Parkinsonism in just 3 days, contrasting with the very slow degenerative process in Parkinson´s disease where take years for the apparition of motor symptoms. Second, exogenous neurotoxins don't exist in human brain and therefore they cannot replicate what happen in the disease.
We have proposed that aminochrome as a new preclinical model based on the facts that (i) aminochrome is a metabolite of dopamine oxidation to neuromelanin (dopamine → dopamine *o*-quinone → aminochrome → 5,6-indole-quinone → neuromelanin) [3]. Aminochrome is formed inside of the dopaminergic neurons containing neuromelanin lost during the disease; (ii) aminochrome induces mitochondrial dysfunction, aggregation of alpha synuclein to neurotoxic oligomers, protein degradation dysfunction of both lysosomal and proteasomal systems, oxidative and endoplasmic reticulum stress [3]. Therefore, we have proposed that aminochrome is the molecule that triggers the mechanisms involved in the degeneration of the nigrostriatal neurons in Parkinson´s disease; (iii) intracerebral injection of aminochrome into striatum induces a neuronal dysfunction of dopaminergic neurons. The level of dopamine was significantly decreased while GABA level was increased. A significant decrease in dopamine release induces a progressive contralateral behavior. The low release of dopamine can be explained by a significant reduction in the number of monoaminergic vesicles in the terminals. Aminochrome induces mitochondrial dysfunction resulting in lower ATP level required for both axonal transport of vesicles to the terminals and dopamine release.

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Epigenetic changes and brainstem dysfunction in neuropsychiatric disorders – AD/PD/Anx

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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 Alzheimer's disease (AD), Parkinson's disease (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 Alzheimer's (AD) and Parkinson's (PD) disease 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.

Mechanisms of neuroprotection and immunomodulation of fitoestrogens: Application for Parkinson disease in perspective

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Flavonoids are bioactive compounds have been known to be beneficial for human health and for improving cognitive function since ancient times. Many studies have shown that neuroinflammation and glutamate-mediated excitotoxicity are the major causes of neurodegeneration. In this context, there is evidence that steroid hormones including estrogens may delay the onset and ameliorate the severity of neurodegenerative disorders. The synthetic estrogen estradiol is used therapeutically in humans, but its therapeutic use in controlling neurodegeneration is limited because of the increased risk of some estrogen-dependent tumors. An alternative is selective estrogen receptor modulators (SERMs), a class of compounds that act on the estrogen receptor (ER), which includes some flavonoids. The aim of this study was to evaluate the neurogenic effects of the Agathisflavone (FAB) and to investigate its immunommodulatory and neuroprotective potential against glutamate-mediated neurotoxicity in neuron-glial co-cultures from postnatal rat cerebral cortex. Compared to controls, treatment with FAB significantly increased the number of cells expressing doublecortin (DCX), a

marker for neuronal precursors and immature neurons. FAB also induced the expression of markers for mature neurons, including β-tubulin-III (β-tub III), microtubule-associated protein 2 (MAP2), and vesicular glutamate transporter 2 (VGLUT2). Together, these results indicate FAB promoted neurogenesis and differentiation. These effects of FAB were suppressed by antagonists of estrogen receptors (ERα and ERβ), indicating this was a primary mechanism of action of FAB. In addition, FAB reduced cell death induced by glutamate and this effect was associated with increased expression of glutamate regulatory proteins in astrocytes, namely glutamine synthetase (GS) and Excitatory Amino Acid Transporter 1 (EAAT1). Our data revealed that FAB reduced the expression levels of the microglial pro-inflammatory cytokines TNFα, IL1β and IL6, which are induced by glutamate-induced neurotoxicity, and reduced cytokines IL6 and IL18 after inflammatory stimulation with LPS. On the other hand, FAB increased the expression of IL10 and Arginase 1, which are associated with anti-inflammatory microglia. We also observed that FAB increased neuroprotective trophic factors such as BDNF, NGF, NT4 and GDNF. These findings show that FAB promotes neurogenesis and is protective against glutamate-mediated neurotoxicity and neuroinflammation, in part by modulating the neuroprotective properties of astrocytes and microglia point this flavonoid as a possible adjuvant for neurodegenerative disorders as Parkinson Disease.

Superimposed control by ATP and adenosine of behavioral dysfunction in animal models of Parkinson's disease

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Adenosine A_{2A} receptor ($A_{2A}R$) antagonists are considered a leading non-dopaminergic therapy to manage motor symptoms of Parkinson's disease (PD). We have described a particular ability of $A_{2A}R$ to control mood and memory dysfunction, which broadens the therapeutic interest of $A_{2A}R$ antagonists in PD. The activation of $A_{2A}R$ is ensured by ATP-derived adenosine, mediated by ecto-nucleotidases. Accordingly, we have defined the ability of ecto-nucleotidase inhibitors to mimic the neuroprotection afforded by $A_{2A}R$ antagonists, thus unraveling ecto-5'-nucleotidase as a new target in PD. ATP is released as a danger signal upon brain dysfunction: it not only provides a source for adenosine, but it can also activate 15 different P2 receptors. Notably, we found that both P2X7R and P2Y1R antagonists attenuate motor and non-motor symptoms in the 6-OHDA model of PD. Exploring the action of P2R in cell lines indicated a prominent control of mitochondria function. Overall, these results show superimposed and parallel roles of different purinergic systems in the control of motor and non-motor symptoms of PD.

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Symposium: Mechanisms for Neurodegeneration, Neuroprotection and Treatment in Parkinson´s Disease: 2

The solution structure and dynamics of full-length human CDNF and its neuroprotective role against α-synuclein oligomers

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Cerebral dopamine neurotrophic factor (CDNF) is a promising therapeutic agent for Parkinson disease. As such, there has been great interest in studying its mode of action, which remains unknown. The three-dimensional crystal structure of the N terminus (residues 9 –107) of CDNF has been determined, but there have been no published structural studies on the full length protein due to proteolysis of its C-terminal domain, which is considered intrinsically disordered. An improved purification protocol enabled us to obtain active full-

length CDNF and to determine its three-dimensional structure in solution. CDNF contains two well-folded domains (residues 10 –100 and 111–157) that are linked by a loop of intermediate flexibility. We identified two surface patches on the N-terminal domain that were characterized by increased conformational dynamics that should allow them to embrace active sites. One of these patches is formed by residues Ser-33, Leu-34, Ala-66, Lys-68, Ile-69, Leu-70, Ser-71, and Glu-72. The other includes a flexibly disordered N-terminal tail (residues 1–9), followed by the N-terminal portion of-helix 1 (residues Cys-11, Glu-12, Val-13, Lys-15, and Glu-16) and residue Glu-88. The surface of the C-terminal domain contains two conserved active sites, which have previously been identified in mesencephalic astrocyte-derived neurotrophic factor, a CDNF paralog, which corresponds to its intracellular mode of action. We also showed that CDNF was able to protect dopaminergic neurons against injury caused by α-synuclein oligomers.

Effect of physical exercise and anti-oxidant treatment on dopaminergic neuronal death in MPTP-treated mice

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Neuroprotection is becoming relevant to slow down dopaminergic cell death and inflammatory processes related to progressive degeneration in Parkinson's Disease (PD). Interestingly, among others, physical exercise and anti-oxidant treatments (such as *N*-acetyl-L-cysteine, NAC) are common therapeutic strategies. Therefore, this study aims to analyze the synergistic effect of physical activity and NAC treatment on inflammatory activation and dopaminergic degeneration in the Parkinsonism model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). To ascertain this possibility, 48 eight-weeks-old mice (C57BL/6 strain) were used. 24 of them were individually placed in cages where voluntary physical exercise was monitored during four weeks and divided into groups: i) control; ii) NAC treatment; iii) MPTP and iv) MPTP + NAC. The other 24 mice were divided into the same four groups, described above, without physical exercise. To analyze motor function we perform rotarod test to study motor coordination and balance. We found an increase in motor condition in exercise MPTP+NAC group compared with MPTP groups. *Post-mortem* studies with midbrain cells (Substantia Nigra, SNpc) and striatum show that the physical exercise significantly decreases GFAP and Iba-1 expression; enhanced with NAC administration compared with no-exercise groups. Moreover, voluntary exercise in Parkinsonian mice treated with NAC induced a significant decrease of the pro-inflammatory response evaluated by detection of pro-inflammatory cytokines (TNF-α and IFN-γ) and activation of Toll-like receptor 2 (TLR-2). These results suggest that the combination of physical exercise with an anti-oxidant drug has a synergistic effect improving motor condition as well as reducing inflammatory processes that protects dopaminergic neurons against neurodegeneration.

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Norepinephrine upregulates the expression of tyrosine hydroxylase and protection against 6-hydrodopamine toxicity in dopamine neurons

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The locus coeruleus (LC) is the primary source of brain norepinephrine (NE), with its projections innervating the whole central nervous system, and is one of the first structures affected in Parkinson's Disease (PD). Many studies showed that the disturbance, and/or a functional enhancement of the LC-NE system influence both the onset and progression of neuronal damage to the dopamine (DA) nigrostriatal tract, indicating that there is a close correlation between the LC and DA systems in the brain and a functional LC-NE system may facilitate the survival of the DA neurons. Therefore, as a main neurotransmitter in the LC-NE system, NE may play an important role in this correlation. In the present study, using primary cultured dopaminergic neurons, effects of NE on expression of tyrosine hydroxylase (TH) and its neuronal protection against 6-hydrodopamine toxicity are examined. The results showed that NE dose- and time-dependently upregulated TH protein levels. To

investigate the potential mechanisms underlying this regulation, further experiments demonstrated that exposure of the cultured DA neurons to NE also markedly increased protein levels of the brain-derived neurotrophic factor (BDNF) in a concentration- and time-dependent manner, as well as protein levels of the phosphorylated extracellular signal-regulated protein kinase 1 (pERK1) and pERK2, indicating the involvement of these proteins. Administration of K252, an antagonist of the tropomyosin-related kinase B (TrkB), the cognate receptor for BDNF, completely blocked NE's effects on TH expression. Similarly, administration of U0126, a dual MEK1 & MEK2 inhibitor, or PD098059, a MEK1 inhibitor, also reversed NE-induced upregulation of TH, indicating that both BDNF and MAPK pathways are involved in NE-induced upregulation of TH. Moreover, administration of NE can attenuate 6-hydrodopamine-induced neuronal death. These results suggest that NE can activate TrkB and MAPK pathways to upregulate TH expression and that NE also has a neuronal protective role for dopaminergic neurons, which may have implications for the improvement of therapeutic strategies for PD.

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Depression in Parkinson's disease: Neurobiology and new therapeutic agents
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Parkinson's disease (PD) is an increasingly prevalent neurodegenerative disorder currently diagnosed solely on the presence of motor symptoms (i.e., tremor, rigidity, bradykinesia, and postural instability) and their response to dopamine replacement therapy. A major unmet need is the limited number and failure of clinical trials with putative neuroprotective agents intended to halt or delay disease progression. Depression is a frequent non-motor symptom in PD, affecting up to 40% of patients over time, and it adversely impacts quality of life and is associated with more rapid motor and cognitive decline. It can be a prodromal or preclinical feature up to 10 years before diagnosis, is more common in patients at the time of diagnosis than in healthy controls, and increases in frequency throughout the course of the illness. In the present presentation we will discuss recent data from our laboratory and other groups showing the role of prefrontal cortex and striatum in the neurobiology of depression in PD. We will also highlight recent results showing the antidepressant-like effects of agmatine in different animal models of PD and possible molecular mechanisms associated with these effects.

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Platform Session 2

Molecular signatures of Parkinson's, Alzheimer's and bipolar diseases: Altered regulons, molecular targets and innovative therapeutics interventions
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We investigated regulatory units in *postmortem* brain samples from Parkinson's, Alzheimer's and Bipolar Disease (PD, AD and BD) patients to identify transcription factors (so called *Master Regulators* – MR) that control large groups of differentially expressed genes. Data from large-scale microarray studies were used to reconstruct transcriptional associations in the human *substantia nigra*, frontal cortex, hippocampus and prefrontal cortex, and results from several independent case/control datasets were used to generate PD, AD and BD diseases signatures. Using this network-based approach with high stringency threshold, the consensus across tests were achieved only for *ATF2*, *SLC30A9*, and *ZFP69B* in PD; *MTA3*, *TSC22D1*, *ZBTB18*, *FBXW7*, *CTBP1*, *SREBF2*, *ZNF483*, *ZFP69B* and *ATF2* in AD; and *EGR3*, *TSC22D4*, *ILF2*, *YBX1* and *MADD* in BD. Then, we compared the obtained disease signature to a panel of drug signatures using the *Connectivity Map Approach*. Briefly, the similarity of a signature-test (in this case Diseases Signatures) with an expression profile of a reference cell lines databank is evaluated using a non-parametric ranking strategy

based on the Kolmogorov-Smirnov statistic. The expression profiles of the reference lines are from cell bank microarrays treated with over 1309 different compounds. Using this approach, we obtained a set of drugs that were able to revert the diseases signatures *in vitro*. We now are clinically validating *in house* our diseases signatures by immunohistochemistry (IHC) of molecular targets using human brain biopsies obtained from the *Queens Square Brain Bank for Neurological Diseases* (UCL/UK) and pre-clinically evaluating the efficacy of the selected drugs to protect or restore degenerating and/or dysfunctional neurons in established cellular and animal models of PD, AD and BD. We believe that our network-based approaches could help to elucidate the molecular pathways governing the pathophysiology of PD, AD and BD, revealing targets and new drug candidates for potential therapeutic intervention.

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Genetic susceptibility to neurodegeneration in Amazon: apolipoprotein E genotyping in vulnerable populations exposed to mercury

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Apoliprotein E is a key component of lipid metabolism. It modulates the delivery of cholesterol to neurons and plays a major role in axonal growth, integrity and neuronal repair. In humans, there are three major isoforms (ApoE2, ApoE3 and ApoE4) with six possible combinations of genotype (□2/□2, □2/□3, □2/□4, □3/□3, □3/□4, □4/□4). The presence of apolipoprotein E4 (ApoE4) is the only genetic risk factor confirmed to play a role in the development of neurodegenerative diseases such as Alzheimer's disease. Recent studies demonstrated a strong association in humans between the different isoforms of ApoE and the individual susceptibility to mercury intoxication, with individuals containing one or two copies of the □4 allele being more susceptible to damage. Mercury exposure is a serious problem of public health in the Amazon. Major symptoms of human intoxication include altered motor coordination, visual and tactile dysfunction and paralysis. Moreover, chronic exposure to relatively low levels of methylmercury causes long-term deleterious consequences as genotoxicity and cardiovascular alterations. To face this problem, in addition to reduce mercury levels in the environment, efforts must be dedicated for the early detection of individuals at high risk. This work aimed to analyze ApoE genotyping (by real time PCR using TaqMan assay) and mercury content in hair (with a GC-pyro-AFS system) in Amazonian riverside populations. A total of 623 participants showed frequencies of 0.045, 0.777 and 0.178 for the alleles □2, □3 and □4, respectively. Only □2/□2 genotype was not detected and the most frequent genotype was □3/□3 (60.5%). Still, about 32% of participants showed the presence of ApoE4, being 23 individuals considered at high risk with □4/□4 genotype. Two hundred and thirteen individuals had its mercury content in hair evaluated ranging from 0.18 to 75.8 ppm with a median value of 4.26 ppm. Forty five individuals of them showed mercury content >10 ppm (the safety limit designed by World Health Organization). To date, we already identified 14 individuals with maximum risk showing mercury content in hair above 10 ppm and the presence of ApoE4. For the first time, it was carried out an epidemiological study to analyze ApoE genotypes in Amazonian populations exposed to mercury. This knowledge is essential to improve prevention strategies and adequately manage intoxicated patients.

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Color vision impairments at low-level methylmercury exposure of an Amazonian population at the Madeira River - Brazil

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Land exploitation that follows deforestation and mining can result in soil erosion and the release of mercury to the waters of rivers in the Amazon Basin. Inorganic mercury is methylated by bacteria present in the environment and serves as a source of human contamination through fish consumption in the form of methyl mercury. Long-term exposure to low-level methyl mercury in the riverside populations can lead to nervous system alterations, some of which are visual impairments such as loss of luminance contrast sensitivity, restricted visual fields and color vision defects. The present study sought to examine the color vision in a group of adults living in the Brazilian Amazon at the Madeira River and exposed to low levels of methyl mercury. Such results are needed in order to pressure the authorities to ensure a safer environment for the Amazonian population. The color vision arrangement tests D-15d and FM-100 were applied in a population of 36 (22 males) and 42 (25 males), respectively. Controls were healthy volunteers from the cities of São Paulo for the D-15d and Belem for the FM-100. Total Hg concentration was also measured from hair collected at the time of tests for both tested population and controls. There was a statistically significant difference in performance between the tested population and controls in both D-15d and FM-100 tests ($p < 0.01$ and $p < 0.0001$, respectively, Mann-Whitney *U*-test), meaning that adults living in the Brazilian Amazon at the Madeira River made more mistakes in both tests when compared to controls. A linear regression was performed using Hg concentration and test scores, having Hg concentration accounting for 22% of color arrangement test performance distribution (R= 0.22). Although other studies have previously found color vision impairment in the Amazon, they were found in the region of the Tapajos River at the east side of Amazon while this study was conducted in the central Amazon. These results suggest that long-term exposure to low-level methyl mercury in riverside populations is wider spread in the Amazon Basin than previously verified.

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Advances in Understanding the Mechanism of Dioxin Induced Neurotoxicity

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Exposure to and bioaccumulation of dioxins produce a wide variety of toxic and health effects, such as tumor promotion, teratogenicity, endocrine disruption and tissue- and organ-specific toxicities. The study of mechanisms of dioxins’ neurotoxicity is a hot area in the past years since significant deficits in cognitive functioning have been reported in humans exposed to dioxins and dioxin-like compounds. Studies demonstrated that dioxin produced its neurotoxicities by disrupting neuronal differentiation and the function of neuro-transmission systems.
We recently found novel mechanisms whereby dioxin may produce its biological or toxicological effects by decreasing neuronal AChE activity through a transcriptional down-regulation mechanism via the AhR-dependent signaling pathway. Meanwhile, dioxin was also found to exert the disturbance on neuronal differentiation by up-regulating the expression of neurofilaments, the main component of neurites. Furthermore, cAMP pathway, as a vital pathway for the function of nervous system, was found to be involved in dioxin induced neuronal disorder.

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Symposium: Developing an Integrated Testing Strategy Using *In Vitro* Data to Screen and Prioritize Compounds with High Throughput / High Content Methods: 1

Analytical methods to distill useful information from high-throughput screening of chemicals
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Recent advancements in high-throughput screening (HTS) technologies have the potential to significantly speed the pace of chemical testing by generating huge volumes of data. However, making use of such data for complex health effects diagnosable only at a systemic level, such as developmental neurotoxicity, will require integrative analytical methods. We describe the development and application of such analytical methods to distill useful information from combinations of *in vitro* molecular screening assays, rapid *in vivo* developmental morphology and behavior assays, plus biomedical literature mining. These methods aim to overcome statistical challenges presented by individual data sources by leveraging massive data volume to robustly quantify results across data sources for a given chemical set. For example, chemicals eliciting *in vivo* behavioral responses can be mapped against external, HTS results to identify specific molecular targets and neurosignalling pathways. We can also map across assays at different life-stages to identify early responses that are predictive of observable adverse effects later in life. Thus, Big Data generated in separate systems can be harnessed toward development of a truly integrated testing strategy.

Screening the ToxCast Phase II library for acute neurotoxicity using cortical neurons grown on multi-well microelectrode array (mwMEA) plates
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We have used primary cortical neurons grown in multi-well microelectrode array (mwMEA) plates to screen the ToxCast Phase II library of 1055 unique compounds for the ability to cause acute neurotoxicity. Each compound was screened at a single high concentration of 40 μM in triplicate. Following normalization of mean firing rate (nMFR), hit calls were made based on exceeding 2x the standard deviation of DMSO-treated control wells. Overall, 325 compounds exceeded the nMFR threshold. Of these, 308 compounds reduced nMFR past the hit threshold, while 17 compounds increased it beyond the threshold. Compounds classified as pesticides, pharmaceuticals, fungicides, chemical intermediates and herbicides accounted for ~78% of the hits. Changes in nMFR occurred largely in the absence of cytotoxicity, as only 8 compounds decreased viability following exposure. Further, based on hits in the single point screen, 384 compounds were identified for concentration-response evaluation. While complete analyses of these data are still ongoing, preliminary analysis indicates that pyrethroid insecticides cause both concentration and time-dependent changes in network function that aligns with the presence or absence of an α-cyano group. This is consistent with known structure-activity relationships for interactions of these compounds with voltage-gated sodium channels as well as acute neurotoxicity of these compounds. Overall, these results demonstrate that neural networks grown on MEAs can be a useful approach to screen compounds for the potential to cause acute neurotoxicity (This abstract was supported by a Cooperative Research and Development Agreement between the US EPA and Axion Biosystems (EPA CRADA #644-11). This abstract does not reflect EPA policy).

Using developing cortical cultures on microelectrode arrays to identify and prioritize compounds based on changes in network formation
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Characterization of the potential adverse effects is lacking for tens of thousands of chemicals that are present in the environment, and characterization of developmental neurotoxicity (DNT) hazard lags behind that of other adverse outcomes (e.g. hepatotoxicity). This is due in part to the high cost and large number of animals needed to characterize DNT hazard. Thus, faster, less expensive approaches for DNT testing are needed. To address this need, we have developed a suite of assays to screen compounds for effects on critical neurodevelopmental processes, including network formation. Primary cortical neurons grown on microelectrode arrays (MEAs) spontaneously form connected networks and begin communicating. MEAs allow the spatial and temporal measurement of action potential spikes and bursts in these developing networks, and allow the assessment of chemical effects on network formation. To date, we have screened over 200 compounds using this assay, including a set of 60 compounds known to cause developmental neurotoxicity in vivo. Of these compounds, 49/60 altered at least one parameter of network development. By comparing the potency of compounds on network function to the potency of effects on cell viability, the specificity of effects can be determined and compounds can be prioritized for additional testing based on the specificity of effects on network formation. In addition, data from the MEA network formation assay can be combined with data from assays for neurite outgrowth, proliferation and other neurodevelopmental effects to develop a prioritization scheme based on multiple assays. This presentation will provide an overview of the assay, present examples of compound effects and show how the data can be used to provide information to regulatory decision-makers.

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Methodological approaches for in vitro-based high-throughput toxicity assessment using NT2 cells

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Human pluripotent embryonal carcinoma stem (NT2) cells are increasingly considered as a suitable model for in vitro developmental toxicity (DT) and developmental neurotoxicity (DNT) studies, as they differentiate into human neuronal (NT2-N) cells upon treatment with retinoic acid (RA) and allow toxicity testing at different developmental stages. For in vitro-based toxicity evaluation, we created first an optimized and robust protocol for differentiation of NT2 cells based on three different differentiation protocols from literature, that reproducibly reveals differentiated cells with high yield. Using the optimized differentiation method, we established a cell fitness screening assay, based on the analysis of intracellular ATP levels, and applied the assay in a large-scale drug screening experiment in NT2 stem cells and early differentiating NT2 cells. Subsequent analysis of ranked fitness phenotypes revealed 19 chemicals with differential toxicity profile in early differentiating NT2 cells. To evaluate whether any of the identified drugs have previously been associated with DT/DNT, we conducted a literature search on the identified molecules and quantified the fraction of chemicals assigned to the FDA (Food and Drug Administration) pregnancy risk categories (PRC) N, A, B, C, D, and X in the hit list and the small molecule library. While the fractions of the categories N and B were decreased (0.81 and 0.35-fold), the classes C, D and X were increased (1.35, 1.47 and 3.27-fold) in the hit list compared to the chemical library. From these data as well as from the literature review, identifying large fractions of chemicals being directly (~42%) and indirectly associated with DT/DNT (~32%), we conclude that our method may be beneficial to systematic in vitro-based primary screening for developmental toxicants and neurotoxicants and we propose cell fitness screening in early differentiating NT2 cells as a strategy for evaluating chemical susceptibility at different stages of differentiation to reduce animal testing in the context of the 3Rs.

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Symposium: Developing an Integrated Testing Strategy Using *In Vitro* Data to Screen and Prioritize Compounds with High Throughput / High Content Methods: 2

Applications of human induced pluripotent stem cell (3D) models for neurotoxicity testing

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Present neurotoxicity testing still relies heavily on ethically debated, costly and time consuming *in vivo* experiments, which are unsuitable for high-throughput screening of chemicals. Consequently, there is an urgent need for rapid *in vitro* test strategies, preferably using human cells to eliminate interspecies translation. Therefore, the current study explores the applicability of commercially available human induced pluripotent stem cell (iPSC)-derived neurons and astrocytes as (3D) models for neurotoxicity assessment.
Several cell models with different ratios of astrocytes and excitatory/ inhibitory neurons were developed. Using immunofluorescent stainings we demonstrate that these different models form mixed neuronal cultures consisting of different types of neurons in the presence and absence of astrocytes. Further single cell measurements of the intracellular calcium concentration indicated the presence of functional GABA, glutamate and acetylcholine receptors as well as voltage-gated calcium channels. Applying multiwell microelectrode arrays (mwMEAs) we show that these human iPSC-derived cultures rapidly develop spontaneous network activity and bursting. Our data indicate that the presence or absence of astrocytes alters the activity pattern of the human iPSC-derived culture. Additionally, the ratio of excitatory and inhibitory neurons affects spiking patterns and bursting behaviour. Finally, spontaneous network activity and bursting can be modulated by different physiological, pharmacological and toxicological compounds. We demonstrate with mwMEA that our model is sensitive for a number of reference compounds such as GABA, glutamate, endosulfan and picrotoxin. While our research shows that human iPSC-derived neurons are physiologically relevant, further characterisation and validation is urgently required to expedite acceptance of this animal-free model. Nevertheless, this model is already suitable for prioritisation and effect screening studies. These human iPSC-derived cell models combined with the current effort to culture them in 3D have the potential to change the future neurotoxicity testing landscape.

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A 3D human brain organotypic model to study developmental neurotoxicology

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We have limited understanding of the function of the CNS and the complexity of the brain, especially during development and neuronal plasticity. Simple *in vitro* systems do not represent physiology and function of the brain. Development of 3D organoid systems has generated more complex *in vitro* human models that better simulate the organ's biology and function. To accurately assess risk, a brain model should preferable recapitulate the complex interactions between different types of glial cells and neurons in a 3D platform. Moreover, human cells are favored over cells from rodents to eliminate cross-species differences in sensitivity to chemicals. The use of iPSC allows us to address gene environment interactions of different donors and makes it possible to evaluate inter-individual sensitivities to chemical exposure. Our human iPSC derived 3D model has been able to recapitulate some early *in vivo* human neurodevelopment. It displays the emergence of different kinds of neurons and glial cells, induction of genes that play important roles in neurodevelopment as well as presence of active glutamate receptors by functional calcium live imaging. Moreover, very few *in vitro*

models have shown *de novo* myelination, and in most of the cases these models are either animal-based or single cell type human cultures. In our model, quantitative assessment of the myelination process (MBP immunostaining) along axons showed an increase in differentiation over time reaching 42% of myelinated axons at eight weeks. These findings are of particular relevance since myelin is a critical element for proper neuronal function and development, and the covering of axons by myelin allows faster action potential transmission, reduces axonal energy consumption and protects the axons from degeneration. Our model has been able to recapitulate known neurotoxic effects by using a pesticide that inhibits mitochondrial complex I (Rotenone) and 6 different dys-myelination chemicals (Bisphenol A, Cuprizone, Methymercury(II) chloride, DE-71, Nicotine and TDCPP). In addition, our model has been able to reproduce Zika pathology and observed various effects by different strains of the virus. Thus, the use of a 3D human *in vitro* model has the potential to give new mechanistic information about Zika induced developmental neurotoxicity. This also adds confidence to the assessment of neurodevelopmental effects of chemicals studied in parallel.

Creating a developmental neurotoxicity (DNT) testing roadmap for regulatory purposes

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Current approaches for generating data relevant to developmental neurotoxicity hazard evaluation according to the OECD TG 426 are entirely based on complex animal testing that is time- and resource-consuming, therefore rarely performed. As a result, there is a lack of information concerning the developmental neurotoxicity (DNT) hazard posed by industrial and environmental chemicals. New testing approaches based on batteries of alternative and complementary (non-animal) tests are badly needed to identify chemicals with DNT potential. The need for more effective DNT screening is driven by the scientific fact that the developing nervous system is more sensitive to exposures of some chemical classes of hazardous substances. In addition, recent societal concerns have been raised linking the rise in children's developmental learning disabilities to chemical exposures. To facilitate the use of alternative methods in DNT regulatory decision making process the Adverse Outcome Pathway (AOP)-informed and key neuro-develop-mental processes-driven an Integrated Approaches to Testing and Assessment (IATA) will presented. IATA should be customised for the chemical/class of chemicals and the specific regulatory need, using various sources of information (non-testing methods, in vitro approaches, in vivo animal and human data). For generation of new data the proposed IATA framework should be based on a set of in vitro test methods that can be used in a flexible combination (fit-for-purpose), anchoring the assays against molecular initiating events, the selected set of key events identified in the existing DNT AOPs and key neurodevelopmental processes (including neural precursor cell proliferation, migration, neuronal differentiation, neuronal network formation etc). The advantage of such types of assays is that they capture toxicants with multiple targets and modes-of-action. Such IATA would facilitate an application of mechanistic knowledge into DNT evaluation produced by in vitro methods, increasing scientific confidence in decision making process, delivering data that could contribute to screening for prioritization, hazard identification and characterization and possibly safety assessment of chemicals, speeding up evaluation of thousands of compounds present in industrial, agricultural and consumer products that lack safety data on DNT potential. The AOP concept relies on understanding causal relationship between the Molecular Initiating Event (MIE), in which a chemical interacts with a biological target, resulting in a sequential series of measurable key events (KEs), which are triggered at different biological levels (cellular, tissue, organ) ultimately resulting in adverse outcome (AO) manifesting in an individual organisms and/or a population. DNT AOPs hold great potential to impact the manner in which in vitro DNT data can be interpreted since the causative links between MIEs, KEs and AO are based on empirical, mechanistic data and biologically relevant knowledge, providing more certainty for regulatory use. Moreover, AOPs provide a strong biological/pathophysiological rationale to compound classification, which is usually based on chemical structures correlated to apical endpoints from animal experiments. It is an important tool that facilitates generation of the data needed for formation of chemical biological categories: chemicals can be grouped according to their MIEs, and common KEs. AOP-based biological chemical grouping has the potential to add a value for DNT testing due to the complex nature of the underlying biology that is currently inadequately captured by chemical category formation (structure or reactivity). Furthermore, read-across and toxicity

classification models can be vastly improved when large amounts of in vitro data are available from high-throughput testing. However, currently the limited number of the developed DNT AOPs has hampered both judgement of the predictive ability, as well as regulatory use of high-throughput in vitro DNT data. The concept that underlies the AOP framework can also guide more effective selection of existing in vitro DNT data and can advise on the most relevant in vitro tests to be included in Integrated Approaches to Testing and Assessment (IATA) for generation of new data reflecting appropriate coverage of MIEs and KEs. In this presentation possible AOP applications in regulatory context will be discussed based on examples of the existing DNT AOPs.

Platform Session 3

The role of chemical speciation for manganese disrupting cross-talking pathways in cerebellar granule cells and its implication for a putative adverse outcome pathways (AOP) relevant for manganese-induced neurotoxicity and neurodegeneration

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Manganese (Mn) is essential for living organisms, playing an important role in nervous system function, bone mineralization, protein and energy metabolism, metabolic regulation and cellular protection. Nevertheless, chronic and/or acute exposure to this metal, specially during early life stages, can lead to neurotoxicity and dementia (cognitive and neurobehavioral impairment) by unclear mechanisms. For that reason, we hypothesized that the complexity and unsolved mechanism of the neurotoxicity induced by manganese can be associated with the alteration of simultaneous and/or concurrent pathways, which can be dependent of chemical speciation too. Therefore, this study investigated the mechanisms mediating the toxic effects of manganese in primary cultures of cerebellar granular cells neurons (CGN). The cells from 1, 2 and 5 days in vitro (div) were exposure to chemical species of manganese, during 1, 2, 5 and 10 div and cell viability determined through MTT-assay. Full mechanistic study was conducted through integrated approaches such as semi-quantitative proteomics - Tandem Mass Tag (TMT), quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR), metallomics (metal bioaccumulation, metal homeostasis and its potential link with the proteome) and bioinformatics (protein-protein interaction and gene ontology analysis, using the string-database). Peptides in databases were identified using the Protein Discovery of Thermo-Instruments software. Results were expressed as mean ± SEM of at least 3 experiments; LC50 and statistically significant differences were estimated by ANOVA (analysis of variance followed by Bonferroni's tests) and, alternative t-student, using the GraphPad Prism (GraphPad 4.0 Software Inc, San Diego, CA, USA). The study identified that Maneb and Mancozeb induced similar high cytotoxicity for cell exposed between 2-7 days and 2-12 div (LC_{50} ~ 7-10 µM) as well as both Mn species were more cytotoxic than $MnCl_2$ (LC_{50} ~ 27 µM). However; for non-cytotoxic concentrations (0.3 – 3 µM) the $MnCl_2$ is more effective than Maneb for alteration of metal homeostasis, myelin sheath and multiple signaling pathways (MSP). Nevertheless; both Mn-species shared mode of actions, including impairment of extracellular exosome, vesicles, nucleus, ribosome, and protein

complexes. This lead to alteration of MSP, which can elicit or silence the protein biosynthesis, a process energetically dependent and associated with the proteasome. Altogether, these findings contribute to a putative adverse outcome pathways (AOP) relevant for understanding the role of manganese neurotoxicity and neurodegenerative disorder such as Alzheimer´s Parkinson´s and Huntinton´s.

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Biosafety assessment of nanostructured materials by using co-cultures of neurons and astrocytes

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Implants of neural electrodes in soft tissues are mostly made of inert materials; however side effects consisting of inflammation and radical formation have been described after their use. We have synthesized hybrid nanocomposites of iridium oxide—IrOx—and carbon nanotubes—CNT, polyethylenedioxythiophene—PEDOT—and polypyrrole—PPY—polymers, which confer mechanical flexibility and avoid the undesirable radical formation. We have previously reported that nanocomposites of IrOx and CNT are compatible with neuronal growth; however nanocomposites containing polymers have variable compatibility with neuronal growth in primary enriched neuronal cultures. Since astrocytes increase neuronal growth we aimed to test the biocompatibility of these materials in neuron-astrocyte co-cultures as a neuronal model closer to the real system. In this work we tested the safety of several nanocomposites for their use in neural repair by determining: i) the adhesion, growth and differentiation of neural cells in primary cultures of astrocytes and of neurons and in neuron-astrocyte co-cultures, and ii) the response of cultured astrocytes against an inflammatory insult. All nanostructured materials allowed the adhesion and proliferation of astrocytes at different degrees with similar morphology. GFAP mRNA expression was not different among materials suggesting the absence of reactive astrocytes. Furthermore, Pt and IROx-CNT-PEDOT that did not allow neuronal growth in enriched neuronal cultures, showed full compatibility with neuron-astrocyte co-cultures. The other nanostructured materials tested were compatible with neuron-astrocyte co-cultures. Functional assays were performed by determining glutamate uptake and NMDAR response. The uptake of glutamate in neuron-astrocyte co-cultures was significantly higher than the sum of the uptake in separately cultured astrocytes and neurons, this increase being significantly reduced by the EAAT2 inhibitor 2,7-dihydrokainate. In co-cultures grown on IrOx, IrOx-CNT and IrOx-CNT-PEDOT, glutamate induced a significant increase of intracellular calcium supporting the expression of functional NMDA receptors. LPS- induced inflammatory response in astrocytes measured by the expression of the enzymes COX2 and NOS2 showed a significant increase of NOS2 mRNA for all the materials except for IrOx-CNT-PEDOT, and of COX2 mRNA levels for Pt and IrOx but not for IrOx-CNT, IrOx-CNT-PEDOT and PEDOT-PSS. In summary, our results support the use of neuron-astrocyte co-cultures as an improved model assay for assessing the biocompatibility and safety of nanostructured materials. They also suggest that IrOx-CNT-PEDOT nanocomposites can afford resistance to inflammatory insults in astrocytes.

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<u>Symposium</u>: Physiological Assessment of Sensory Toxicity and the Role in Human Risk Assessment in the 21st Century

Vestibular function assessment in humans and in animals exposed to ototoxic compounds

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The vestibular system in the inner ear detects linear and rotational accelerations to maintain equilibrium and gaze control. Vestibular toxicity causes loss of vestibulo-spinal and vestibulo-ocular reflexes, with a profound impact on motor behavior and vision competences. Vestibular deficiency also has autonomic, hormonal, and cognitive consequences. Symptoms of vestibular dysfunction include vertigo, blurred vision, dizziness, and feeling of unsteadiness. These are reported by humans, but likely remain undetected in animal studies. Nevertheless, rodents bearing significant loss of vestibular function suffer a characteristic syndrome of alterations in motor behavior. This syndrome has been successfully assessed by a semi-quantitative test battery to characterize the effects of several ototoxic compounds, but more sensitive and quantitative measures of vestibular dysfunction are needed to improve risk assessment and to evaluate candidate drugs for vestibular therapy. The identification of functional measures with good translational value is eased by the fact the vestibular system is highly conserved across species, and similar vestibular reflexes can be defined in humans and animals that are based on similar neuronal pathways. Traditionally, assessment of vestibulo-ocular reflexes has been a keystone in human vestibular evaluation. In recent years, the video head impulse test (vHIT) has been spreading as a sensitive and reliable measure of the vestibulo-ocular reflex and hence of semi-circular canal function. In rodents, video-nystagmography and vestibulo-ocular reflex measures are available, but these are not frequently obtained in vestibular toxicity studies. Ongoing animal studies in our laboratory and others provide new measures of vestibulo-spinal function during either spontaneous or reflex movements. Establishing the relationship between these rodent measures and human postural and vestibulo-spinal reflex measures will open new paths for robust animal-to-human extrapolation in vestibular toxicity.

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Membrane fluidity does not explain how solvents act on the middle-ear reflex Physiological consequences for exposures to noise

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The middle-ear reflex (MER) reduces the acoustic energy penetrating the cochlea and therefore the cochlea-traumatic impact of the noise on the organ of Corti. As a result, it can be considered as a protective reflex against high intensity noises. Disruption, or simple disturbances of the reflex by solvents, can indirectly increase the risk of occupational deafness encountered by workers exposed to noise and pollutants. Because there are a multitude of volatile solvents in working environments, the main objective of this study was to develop a screening test capable of evaluating how much these substances can modify the middle-ear reflex. The aromatic solvents were chosen first to better understand the mode of action of solvents on the nervous centers involved in the reflex circuit. For this purpose, Brown Norway rats were anesthetized (Ketamine + xylazine) and then intratracheally exposed to aromatic solvents selected according to their lipophilicity (log Kow), or their chemical structure. The MER amplitude was determined by measuring the amplitude variations of the cubic distortion product oto-acoustic emissions (DPOAES, 2f1-f2, primaries f2/f1=1.2 at f2=9600 Hz) in the ipsilateral ear, while an acoustic stimulation was delivered in the contralateral ear (95 dB at 4400 Hz). It clearly appeared that the lipophilic characteristic of aromatic solvents such as benzene, toluene, ethylbenzene, styrene, o-, m-, p-xylene … did not play a key role on the MER. Moreover, solid-state Nuclear Magnetic Resonance spectra for brain microsomes confirmed that brain lipid fluidity was unaffected by toluene exposure, except at high concentrations (>0.01%; >12.2µg/g). Such a concentration was unrealistic with exposures by inhalation. On the contrary, the stereospecific parameter of the molecules seems to be a more pertinent parameter to explain their effect on the MER. Volatile substances would be capable of acting directly on the neuronal targets involved in the acoustic reflex circuit. The affinity of this interaction would be determined by stereospecific rather than lipophilocity. In conclusion, the MER and the particular set up can be used to detect potential hazardous volatile substances for hearing when associated with noise. By disturbing the MER, certain volatile substances could potentiate the noise effects. This study revealed that aromatic solvents have two distinct actions: an acute neuropharmacological impact on the nervous central system and/or a long-term

cochleotoxic action that can act separately on the hearing of workers exposed to noise and solvents simultaneously.

Visual impact of occupational exposure to mercury

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Mercury intoxication has been known to affect vision. However several questions remained as to the sensitivity, nature and duration of the effect. Are safety limits sufficient to prevent toxicity? Are the losses restricted to peripheral vision as first thought? Is the damage reversible after cessation of exposure? Psychophysical and electrophysiological techniques were used to assess visual functions of mercury intoxicated subjects, whose neuropsychological functions were also assessed. We studied color vision, grating contrast sensitivity, contrast sensitivity to a homogeneous field, visual perimetry, using psychophysical procedures. We used electrophysiological methods to study contrast sensitivity measured in the VEP, to assess retinal activity using the electroretinogram and to obtain a functional map of the retina with the multifocal electroretinogram. These studies were conducted over more than a decade in a single cohort, a group of former workers that were placed on disability retirement due to mercury intoxication. As expected from their history of mercury exposure, their neuropsychological functions were altered. Our studies led to the conclusions that the psychophysical and electrophysiological functions mediated by the central retina, in addition to the peripheral retina, were altered in the mercury exposed subjects and that the alterations were irreversible. Relative to controls these subjects showed poorer color discrimination, and lower temporal and spatial contrast sensitivity for luminance and for chromatic stimuli. Signs of impaired function were present in retinal activity. Finally, the impairments we assessed behaviorally and electrophysiologically were detected in individuals that had been exposed to mercury below the current safety limits. These findings, obtained under rigorous laboratory conditions, confirm and extend previous field work, showing the profound neurotoxic impact of mercury on human health.

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Evaluating visual system toxicity in relation to human risk assessments in the 21st century.

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Traditional approaches to toxicity testing have been inadequate to keep up with the demand for health and safety information on the large numbers of existing and newly developed chemicals and materials. Recent changes to toxicity testing involve increased emphasis on computational modeling and high-throughput screening (NRC, 2007). The development of Adverse Outcome Pathways has been proposed as a conceptual framework through which molecular initiating events can produce adverse effects across levels of biological organization spanning molecular, cellular, systemic, organism and population levels. What is the value of sensory function assessment in such a structure? Physiological methods may help bridge between molecular screening targets and adverse outcomes to better enable interpretation of screening level testing. Examples can be found in tests of thyroid dysfunction, exposure to volatile organic solvents and engineered nanomaterials. Substantial development will be required, however, before it is possible to confidently develop predictive links between screening level assays and performance in systems as complex as vision or other sensory processes.

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May 23

Symposium: Mitochondria-related Metabolism Leading to Neurodegeneration and Neuroprotection

Mechanisms of neurotoxicity at physiological O_2

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Physiological oxygen tension in the brain typically ranges from 15 to 40 mm Hg, or 2 to 5% O_2. However, *in vitro* studies of neurotoxicity have been performed almost exclusively at atmospheric O_2 (160 mm Hg, 21%). Oxygen tension regulates many biochemical processes, including superoxide and nitric oxide (NO) formation, the stability of oxygen-sensitive transcription factors such as HIF-1a, and the activity of oxygen-dependent enzymes. Microglia, the innate immune cells of the brain, produce reactive oxygen and nitrogen species during proinflammatory activation. We tested the hypothesis that proinflammatory microglial cells inhibit the respiration of co-cultured neurons by either nitric oxide or peroxynitrite-dependent mechanisms at physiological 3% O_2 or atmospheric 21% O_2, respectively. The Seahorse XF24 microplate-based cell respirometer was used to measure O_2 consumption rate. A combination of lipopolysaccharide and interferon-g was used to activate rat HAPI immortalized microglia, which were subsequent plated onto a monolayer of primary rat cortical neurons. Activated microglia dose-dependently impaired the respiration of co-cultured neurons at brain physiological 3% oxygen. Strikingly, the same numbers of activated microglia had little effect on the neuronal oxygen consumption at atmospheric 21% O_2. The NO scavenger carboxy-PTIO acutely reversed the respiratory inhibition of neurons exposed to activated microglia at 3% O_2, consistent with the hypothesis that the impairment of oxygen consumption is mediated by nitric oxide. The NO donor DETA-NO recapitulated the effect of activated microglia on neuronal respiration at 3% O_2 but required >10-fold higher doses to elicit the same effect at 21% O_2. SIN-1 breaks down to form NO and superoxide, which then react to form peroxynitrite. The ability of SIN-1 to inhibit neuronal respiration at physiological O_2 was markedly enhanced by the addition of superoxide dismutase to remove superoxide, increasing the effective NO concentration. Collectively, results demonstrate that NO is a more potent inhibitor of neuronal respiration compared to peroxynitrite at 3% O_2 and likely mediates the suppression of neuronal respiration by activated microglia at brain physiological oxygen tension.

Disruption of mitochondrial functions caused by the major fatty acids accumulating in long-chain fatty acid oxidation defects

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Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency is the most frequent disease of long-chain fatty acids β-oxidation (14 to 20 carbons). The disorder is biochemically characterized by predominant tissue accumulation of myristic (Myr - C14:0) and cis-5-tetradecenoic (Cis5 - C14:1) acids and clinically by episodes of metabolic decompensation, hypoketotic hypoglycemia, liver dysfunction and cardiomyopathy. Considering that the pathophysiology of this disease is poorly understood, we investigated the effects of Myr and Cis-5 on important mitochondrial functions in rat brain, liver and heart mitochondria. Regarding to the parameters of mitochondrial respiration measured by oxygen consumption, Myr markedly increased state 4 respiration in heart, liver and brain mitochondria, whereas Cis5 only altered this parameter in the brain. Myr also clearly decreased state 3 and uncoupled respiration as well as mitochondrial membrane potential ($\Delta\psi m$) in mitochondria from all tissues, with smaller effects evidenced with Cis-5. Furthermore, Myr and Cis-5 severely decreased NAD(P)H content in the heart, with less intense decrease in the liver and brain. We also observed

that these effects were enhanced after Ca^{2+} addition, particularly in the heart. Ca^{2+}-induced brain mitochondrial swelling was also elicited by Myr but not by Cis-5. Noteworthy, Myr-induced decrease of Δψm and swelling was abolished by cyclosporine A (CsA) plus ADP or ruthenium red (RR) in brain, liver and heart, implying the involvement of mitochondrial permeability transition (mPT). Finally, we found that Ca^{2+} retention capacity was reduced by Myr and Cis-5 in heart and liver mitochondria, but not in brain. Taken together, our data indicate that Cis-5 and particularly Myr severely compromise important mitochondrial functions, behaving as uncouplers and metabolic inhibitors of oxidative phosphorylation, as well as inducing mPT. It is presumed that disturbance of mitochondrial homeostasis through these mechanisms of lipotoxicity may be involved in the liver dysfunction and cardiomyopathy characteristic of VLCAD deficient patients.

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Carnosine, a possible suppressor of neurotoxicity?

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There is a strong association between methylglyoxal (MG)-mediated protein glycation, which eventually generate advanced glycation end-products (protein-AGEs), and neurotoxicity. There are two likely origins of protein-AGEs, (i) those arising endogenously via metabolic generation of excessive amounts of MG, and (ii) those originating exogenously via dietary intake of glycated proteins in cooked food, which, following binding to AGE receptors (RAGEs) on enteric neurones, could (controversially) enter the CNS i.e. behave like prions. Endogenous MG generation is likely to occur as a result of excessive/persistent glycolysis which can result in a "wear and tear"–induced decline in triose-phosphate isomerase (TPI) activity due to the catalytic activity-induced deamidation of certain asparagine residues in TPI. Insufficient TPI activity will result in accumulation of dihydroxyacetone phosphate which spontaneously decomposes into MG. Excessive MG formation may occur especially in cells unable to re-synthesize TPI, e.g. erythrocytes. Additionally, accumulation of beta-amyloid protein can cause a decline of TPI activity via protein nitration. Studies in model systems have revealed that the endogenous dipeptide carnosine (beta-alanyl-L-histidine) can scavenge a range of deleterious aldehydes including MG, formaldehyde, acetaldehyde, dihydroxyacetone, acrolein and hydroxynonenal and thereby prevent protein modifications such as glycation and protein–protein and protein-DNA crosslinking, as well as protect cells against aldehyde-induced toxicity. Other studies have shown that carnosine can exert inhibitory effects upon glycolysis but can stimulate mitochondrial function, resulting in delayed cell senescence; carnosine can also inhibit growth of tumour cells. Whilst the precise mechanisms responsible for these effects remain obscure, possibilities include enhanced proteolysis and effects on the mTOR regulatory apparatus. Animal studies suggest that carnosine may be ameliorative against diabetic complications, stroke, Alzheimer's disease and Parkinson's disease (PD). By scavenging MG, carnosine also could prevent formation of ADTIQ, a neurotoxin formed by the spontaneous reaction of dopamine with MG, and which accumulates in the brains of PD patients and diabetic animals. Although there is no evidence to either support or refute the speculation that carnosine could be beneficial following intake of dietary protein-AGEs, studies reveal that carnosine supplementation has beneficial effects in cases of schizophrenia, Gulf War illness, chronic heart failure and autism, which suggests that the dipeptide's efficacy in not completely negated by serum carnosinase.

Astrocyte dysfunction in experimental models of glutaric acidemia type I

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Glutaric acidemia type I (GAI) is a rare inherited neurometabolic disorder characterized by accumulation of glutaric (GA) acid and related metabolites in brain and tissue fluids. GA-I shows a paradigmatic postnatal neuropathology characterized by massive degeneration of neurons in the striatum, myelin defects and vascular abnormalities. While the disorder is caused by genetic mutations on the enzyme glutaryl-CoA dehydrogenase (GCDH), neurological defects usually start months after birth and seemed dependent on the occurrence of precipitating encephalopatic crises. Pathogenesis of GAI has remained largely unknown. Specifically it is unknown whether GA toxicity is due to direct effects on vulnerable neurons or mediated by GA-intoxicated astrocytes that fail to support neuron function and survival. By using a pharmacological model of GA-I we have shown that a primary defective astrocyte maturation leads to a co-morbid spectrum of neurologic symptoms similar to those of GA-I patients. In our hands, astrocytes are not only vulnerable to GAI metabolites but also suffer long-lasting phenotypic changes leading to striatal neuron degeneration as well as defective myelination and blood brain barrier maturation. By using astrocytes obtained from the Gcdh-/- mouse we have also demonstrated that damaged astrocytes not only can induce neuronal death when challenged with Lys but also contribute to sustain high GA levels. Upon Lys treatment, Gcdh-/- astrocytes synthetized and released GA and 3-hydroxyglutaric acid (3HGA) to the culture medium. Lys and GA treatments also increased oxidative stress and proliferation in Gcdh-/- astrocytes. Pretreatment with Lys also caused Gcdh-/- astrocytes to induce extensive death of striatal and cortical neurons when compared with milder effects caused by wild type (WT) astrocytes. Antioxidants abrogated astrocyte response to Lys and GA as well as the neuronal death induced by astrocytes exposed to Lys or GA. In contrast, Lys or GA direct exposure on Gcdh-/- or WT striatal neurons cultured in the absence of astrocytes was not toxic, indicating that neuronal death is mediated by astrocytes. In summary, GCDH-defective astrocytes actively contribute to produce and accumulate GA and 3HGA when Lys catabolism is stressed. In turn, astrocytic GA production induces a neurotoxic phenotype that kills striatal and cortical neurons by an oxidative stress-dependent mechanism. Targeting astrocytes in GA-I may prompt the development of new antioxidant-based therapeutic approaches.

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Mitochondrial DNA damage as a peripheral biomarker for human rotenone exposure

S.M. Goldman, E.H. Howlett, C.M. Tanner, J.T. Greenamyre and L.H. Sanders

Mitochondria are the target of a large number of environmental toxicants. Rotenone, an environmental toxicant, inhibits complex I of the mitochondrial electron transport chain. Exposure to rotenone has been associated with an increased risk of developing Parkinson's disease (PD). Rats exposed to rotenone mimic many of the clinical and pathological features of PD. We recently found that mitochondrial DNA (mtDNA) damage in peripheral tissues was a biomarker of past or ongoing systemic complex I inhibition via rotenone exposure in the rat PD model. We were then interested in applying our rat model experimental findings into translational investigations in humans. To do this, we took advantage of extensive epidemiologic and clinical data sets, linked to stored DNA, as part of the Farming and Movement Evaluation Study (FAME). FAME is a nested case-control study within the Agricultural Health Study (AHS) that is investigating the relationship between PD and pesticide exposure, farm-related exposures and other putative PD risk factors. The 84,000-member AHS population is comprised of professional pesticide applicators (mostly farmers) and their spouses in NC and IA. For FAME, we screened AHS members for self-identified PD, and verified diagnosis by in-person neurological evaluations. Random controls were matched to cases on gender, age and state. We interviewed participants about having mixed or applied rotenone, and obtained peripheral blood samples. 82 men (37 cases, 45 controls) were included in the current analysis, 19 (23.2%) of whom reported using of rotenone. DNA derived from blood buffy-coat samples was analyzed in a blinded manner using a quantitative polymerase chain reaction (QPCR)-based assay that simultaneously measures multiple forms of mtDNA damage. Mitochondrial DNA damage was higher in rotenone exposed subjects (exposed median 0.16, unexposed 0.015; non-parametric p=0.02). Differences remained significant in models adjusted for case status, age, and smoking (p=0.03). No difference in mtDNA copy number was detected between groups. Our data suggests that mtDNA damage may be a useful biomarker for human rotenone exposure.

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Symposium: Neuroprotective and/or Neurotoxic Roles of SUMOylation in Neurodegenerative Diseases

Mechanisms and consequences of neuronal protein SUMOylation in health and disease

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Post-translational protein modifications are integral components of signalling cascades that enable cells to efficiently, rapidly and reversibly respond to extracellular stimuli. These modifications play crucial roles in the CNS where the communication between neurons is particularly complex. Protein SUMOylation is a critically important post-translational protein modification that participates in the regulation of nearly all aspects of cellular physiology. SUMO modification is a highly dynamic and transient process that, depending on the target protein, either enhances or hinders protein-protein interactions to alter substrate localisation, function and/or stability. Hundreds of different proteins are SUMO substrates and the mechanisms and protein targets of SUMOylation are activity-dependently controlled and highly sensitive to cell stress. In neurons, SUMOylation is involved in processes ranging from neuronal differentiation and synapse formation to regulation of synaptic transmission and mitochondrial function. We are particularly interested in how SUMOylation of proteins outside the nucleus impacts on mitochondrial integrity, synaptic function and plasticity, and neuroprotective responses to cell stress. Unsurprisingly given the core pathways that are regulated, dysfunction of protein SUMOylation is implicated in many different diseases including Alzheimer's disease. I will outline the SUMO system and discuss recent discoveries that illustrate some of the roles of SUMOylation in healthy and diseased neurons.

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SUMOylation and mitochondrial dysfunction in neurodegenerative diseases

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SUMOylation acts as a biochemical switch in many pathways through regulating the function of a vast array of proteins, and is thus crucial in all eukaryotic cells. It has emerged recently that SUMOylation is involved in multiple neuronal signalling cascades and is implicated in many neurodegenerative diseases, including Alzheimer and Parkinson's diseases. We are currently investigating the global SUMOylation levels and the effects of manipulating SUMOylation and deSUMOylation pathways in cultured neurons and animal models of Alzheimer and Parkinson's diseases. In particular, we are focusing on the role of potential SUMO targets relevant to mitochondrial dysfunction, such as dynamin-related protein 1, a GTPase that regulates mitochondrial fission. This work will reveal if SUMOylation represents a potentially tractable target for therapeutic intervention and may also identify novel SUMO substrates as targets for drug development.

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SUMOylation, aging and autophagy in neurodegeneration

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SUMO, the Small Ubiquitin-like MOdifier, covalently conjugates to lysine residues similarly to ubiquitin in a wide range of substrate proteins, modulating the functional properties of the modified protein. Protein inclusion bodies that are a pathological hallmark of several neurodegenerative diseases, including Parkinson's disease, contain SUMO, and several of the aggregation-prone proteins, including alpha-synuclein, have been found to be SUMO targets. Previously, SUMO-1 has been found associated with lysosomes clustered around or embedded in cytoplasmic protein inclusion bodies in the alpha-synucleinopathy, multiple system atrophy, and a unilateral rotenone-lesion Parkinson's disease mouse model conjugated to Hsp90, suggesting a link to chaperone-mediated autophagy. Baseline SUMOylation was also shown to be increased in aged compared to young PD mice that exhibited a more severe phenotype, but a reduced SUMOylation response in the lesioned hemisphere. The current work investigated the influence of inhibiting SUMOylation on alpha-synuclein aggregation in a cell culture model of PD and compared the SUMOylation response to proteolytic stress in human olfactory neurosphere (hONS)-derived cell lines from PD and normal cases. Alpha-synuclein aggregation was induced in SH-SY5Y human neuroblastoma cells by potassium depolarization and calcium influx and cells were imaged by confocal immunofluorescence microscopy. Co-treatment with SUMOylation inhibitors (ginkgolic acid and anacardic acid) significantly reduced the frequency of SUMOylated lysosomes and alpha-synuclein inclusion bodies and increased the number of LC3-positive autophagosomes. In parallel, Western analysis revealed a significant increase in SUMO-1-conjugates (p, 0.02) after potassium depolarization that was inhibited by ginkgolic acid treatment and there was an increase in the LC3b band associated with macroautophagy in ginkgolic acid treated cells. Analysis of hONS cell lines revealed that proteasome inhibition (MG132) caused alpha-synuclein aggregates and that PD-derived cell lines (n = 5) showed significantly higher baseline lysosomal SUMOylation, but there was a more significant induction of SUMOylation, especially of Hsp90 (p, 0.05), under proteolytic stress in normal cell lines (n = 5). These data indicate that inhibiting SUMOylation could inhibit chaperone-mediated autophagy and up-regulate macroautophagy that may in turn lead to a reduction of alpha-synuclein aggregates. Although further studies are needed, PD-derived cell lines may reflect a higher baseline level of SUMOylation and reduced induction compared to normal controls, following a similar trend to that observed in the aged mouse model compared to young mice. Additional work is required to determine if SUMOylation inhibitors may have therapeutic potential for PD and other alpha-synucleinopathies.

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Exogenous guanosine to modify SUMOylation – focus on Alzheimer and Parkinson's diseases

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Post-translational modifications regulate different cellular mechanisms. Recent studies are showing important roles for protein SUMOylation (small ubiquitin-like modifier conjugation): it can interfere with mitochondrial dynamics, cellular function, activity and subcellular localization of several proteins. We are currently investigating the potential of guanosine in modulating SUMOylation, focusing on proteins related to mitochondrial fission-fusion pathways, such as dynamin-related protein 1 (Drp1). Guanosine is an endogenous molecule that acts as an intercellular signaling modulator. Although its precise mechanism of action is under investigation, several studies support that guanosine can protect cells from several insults both *in vitro* and *in vivo*. Our aim is to determine the effects of guanosine in global SUMOylation levels and cellular viability, to

later apply it in different models of Alzheimer and Parkinson's diseases. This work will reveal whether guanosine can act as a SUMO modulator, which is of great interest as the SUMOylation pathway could be a potential target for therapeutic intervention in neurodegenerative diseases.

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Symposium: Metabolic Derangements Predisposing to Neurotoxicity and Neurodegenerative Disease

Hypothalamic dysfunction in obesity

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In experimental obesity, the consumption of high-fat diets triggers an early inflammatory response in the hypothalamus. Studies have shown that saturated fats activate TLR4 signal transduction and endoplasmic reticulum stress leading to the activation of intracellular inflammatory signaling through NFkB and JNK in hypothalamic cells. Upon persistence in the consumption of large amounts of dietary fats, the hypothalamic inflammation becomes chronic leading to abnormal regulation of the neurons involved in the control of food intake and energy expenditure. POMC neurons are particularly affected by this inflammation and they may undergo apoptosis resulting in an imbalance between orexigenic and anorexigenic neuronal subpopulations. In this talk we will present the most recent advances in this field.

Hypercholesterolemia as a risk factor for neurocognitive impairments

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Epidemiological findings suggest an intriguing and complex relationship between hypercholesterolemia and the development of Alzheimer disease (AD). In contrast to traditional neuroncentric views of neuropathologies, others and we propose that neurovascular dysfunction associated to hypercholesterolemia contributes to cognitive decline, depressive behavior and neurodegeneration. In this way, we are dedicated to study and propose some molecular mechanisms which can explain this association. Employing a widely used rodent model of familial hypercholesterolemia as a pre-clinical approach, we characterized a cognitive impairment and depressive like behavior in low-density lipoprotein receptor knockout (LDLr-/-) mice. These behavior impairments were associated to blood brain disruption, neuroinflammation, cerebral mitochondrial disruption and adult neurogenesis prejudice. Finally, the understanding of the role of these vascular-related conditions in AD development, would suggest possible modifiable risk factors that may serve as targets for therapeutic strategies.

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Chronic hyperglycemia promotes hippocampal REST epigenetic gene inactivation with cognitive impairment and neurotoxicity

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Here we showed that persistent hyperglycemia, a hallmark of some chronic metabolic diseases promotes epigenetic changes in the CNS that convey in higher susceptibility to neurodegeneration, compromising learning and memory. In order to investigate of impact of chronic hyperglycemia on these neurochemical and behavioral parameters, Wistar rats received a single intraperitoneal injection of 55 mg/kg streptozotocin (STZ). Some rats also receive also insulin to control glycaemia (INS; 1.5 IU; human insulin NPH; Novolin®N twice a day). Hyperglycemia treatment compromised short- and long-term memory, as well as spatial memory, effect that was accompanied by significant CSF and plasma oxidative stress. Insulin expression was markedly reduced in the hippocampus, but with normal canonical insulin/insulin-like signaling maintained by normal expression levels of IGF-1. Oxygen consumption experiments showed uncoupled mitochondria with increased expression of complex I (NDUFA6), probably as a compensatory response to the mitochondrial stress elicited by the excess of nutrients. Furthermore, the hyperglycemic state induced a subcellular redistribution of hippocampal α-synuclein, even when the total content of the protein remained unchanged. Hippocampus was also depicted by marked reduction of IL-10 expression, increased GFAP expression, reduced content of BDNF and increased caspase-3 activation. This neurotoxic environment occurred in parallel with a specific and significant global DNA hypermethylation and hypomethylation of LINE-1 region (genome instability) in STZ rats. DNA hypermethylation affected the expression of REST, a neurocognitive transcription factor in the adult brain, which was significantly decreased in the hippocampus of STZ rats. In agreement, REST promoter was also hypermethylated. In contrast, the gene expression of the truncate toxic splice variant REST4 was significantly up-regulated. INS administration efficiently prevented all these epigenetic alterations and the cognitive impairments. Epigenetic alterations were also observed in human leucocytes obtained from obese children with also marked reduction of REST expression. It could be concluded that controlling glucose homeostasis could reduce the risk of the development of cognitive impairment and predispose to neurodegenerative diseases.

Grant sponsor: CNPq, PRONEX, CAPES.

A single dose of glucocorticoid affects synaptic plasticity markers in the human limbic system

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Joint Student Award Symposium (Abstracts with Posters)

Hypoxia-inducible factor-1α (HIF-1α) binds to HIF-response elements on the promoter region of candidate genes in the genome rat under hypoxic conditions: A bioinformatics study

Emmanuel Casanova Ortiz
University of Chile, Santiago, Chile.

Small molecules alter manganese toxicity in *Caenorhabditis elegans*

Tanara V. Peres
Albert Einstein College of Medicine, Bronx, NY, USA.

The effects of early environmental enrichment and PACAP in a rat model of Parkinson's disease

Adel Jungling
University of Pecs Medical School, Pecs, Hungary.

Symposium: Advancing Mechanistic Understanding of Neurotoxic Contributors to Autism

What we have learned about mechanisms for ASD etiology by studying risk and protective factors

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Increasing evidence points to a complex interplay between genes and environment in autism spectrum disorder. Evidence from epidemiology studies suggests a critical time for autism etiology appears to be early pregnancy when the child's methylome is being established; *in utero* environment can impact this process. We show evidence that maternal supplemental folic acid, a major contributor to methylation and DNA synthesis/repair reactions, is associated with reduced risk for ASD prevalence and recurrence in children, and could attenuate associations with ASD risk factors like pesticides. We further show evidence that environmental exposures including pesticides influence placental DNA methylation. Finally, we show that altered placental and cord DNA methylation are associated with the child's later ASD diagnosis, and these alterations are in genes relevant to synaptic and neurodevelopmental pathways. Our findings suggest that the complex nature of ASD etiology could involve interactions between risk and protective factors, early periods of susceptibility during pregnancy, and that DNA methylation could play a central role as a biomarker of both exposure and risk.

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Developmental neurotoxicity of traffic-related air pollution: studies with diesel exhaust in mice

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Escalating prevalence of autism spectrum disorders (ASD) in recent decades has triggered increasing efforts in understanding potential roles played by environmental risk factors. Several epidemiological studies show associations between developmental exposure to traffic-related air pollution and increased ASD risk. Diesel exhaust (DE) is a major contributor to traffic-related air pollution, and it has been estimated that >35% of ambient particulate matter (PM) 2.5 can be attributed to DE. PM2.5 is known for its ability to cross cellular membranes and cause oxidative damage and inflammatory responses. Elevated levels of inflammatory cytokines have been reported in fetal brain and placenta of mice exposed prenatally to DE. In the maternal immune activation model of ASD in rodents, increases in IL-6 and IL-17α levels have been reported to be sufficient to elicit ASD-like behavior in the offspring. We found that C57Bl/6J mice exposed from GD0 to PND21 to 250-300 ug/m3 DE (or filtered air as control) exhibited deficits in all three of the hallmark categories of ASD behavior, i.e. social interaction in the reciprocal interaction and social preference tests, social olfactory and vocal communication, and repetitive behavior. In brains of developmentally DE-exposed mice, levels of IL-6 were also increased. Binding of IL-6 to its receptor activates the JAK2/STAT3 pathway, allowing STAT3 to act as a transcription factor modulating expression levels of target genes. One of these target genes, DNA methyltransferase 1 (DNMT1), is expressed at high levels in both developing and adult mammalian brains and is responsible for both de novo methylation of un-methylated DNA and maintenance of existing DNA methylation patterns. Among the targets of DNMT1 is reelin, an extracellular protein secreted by Cajal-Retzius cells that plays a major role in guiding neuronal migration and dendrite formation during CNS development. Reelin-deficient mice exhibit some ASD related behavioral phenotypes as well as cortical disorganization similar to brain structural changes found in prefrontal cortex of ASD patients. In agreement with these hypotheses, we found that DE exposure increases expression of DNMT1, and decreases expression of reelin,

supporting the hypothesis that dysregulation of the reelin pathway could contribute to the ASD phenotype elicited by developmental DE exposure.

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Traffic related air pollution and autism spectrum disorder: A population based nested case-control study in Israel

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Several studies have implicated perinatal traffic-related air pollution in risk of autism spectrum disorder (ASD), although the literature from outside the United States is less consistent. However, study settings with lower exposure levels and use of autism traits in general populations as the outcome may contribute to the inconsistent findings. Therefore, we sought to explore this association in Israel—a different geographical context where we have population-based data on ASD ååcases and traffic-related exposures that are on the higher end of studies that have explored this question. We used Israeli National data to identify all children born during the years 2005-2009 in the central coastal area of Israel (the area covered by our exposure model, including ~40% of the total Israeli population, and ~45% of the births in the country in that period) who were still alive and residing in Israel at age 4 years, and who had an Israeli mother and father. ASD cases were identified from the National Insurance Institute of Israel (NII) and were based on Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria. We identified 2,098 ASD cases and selected a 20% random sample of the remaining children as controls (N=54,191). Exposure to nitrogen dioxide (NO2)—a marker of traffic pollution—was based on an optimized dispersion model, generating half-hourly 500X500m concentration maps. Average exposure concentrations were calculated for the mother's residence for different time periods around pregnancy. The mean NO_2 level during pregnancy was 16.7ppb (median: 16.8; range: 7.5 – 31.2). We used logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI). In adjusted models that included exposure windows of the 9-months before, during, and after pregnancy there was no association with exposure before pregnancy (OR=1.11; 95% CI: 0.83-1.49), exposure during pregnancy was associated with decreased odds of ASD (OR=0.60; 95% CI: 0.42-0.84), and exposure after pregnancy was associated with increased odds (OR; 1.59 (1.18-2.15). In trimester analyses, negative associations were seen in pregnancy trimesters one and two, while positive associations were seen in postnatal months 4-9. Both negative and positive associations were stronger in boys. These findings suggest that postnatal exposure to NO_2 in Israel is associated with increased odds of ASD. The lower odds with exposure during early pregnancy may relate to selection effects and could possibly contribute to differences seen between studies.

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Environmental toxins and neural-glial interactions in autism

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It is increasingly evident that diverse genes and environmental exposure(s) combine or synergize to produce a spectrum of autism phenotypes dependent upon critical developmental windows. Multiple prenatal/maternal environmental toxins and exposures have been linked to human ASDs, but the associations of single agents have been relatively weak. We now recognize that non-chemical stressors, such as limited resources or social support of the mother, can increase vulnerability of the fetus to chemical stressor exposures (e.g., pollution or toxins), which could explain why a single exposure or risk factor in isolation is a modest predictor of autism risk. We are using a novel model that employs the combined effects of an ethologically relevant maternal stressor and environmentally relevant pollutant, diesel exhaust, both of which have been implicated in

autism. Pregnant dams were exposed every third day throughout gestation to diesel exhaust particles (DEP) or vehicle, with or without maternal stress (MS; restriction of nest materials in the cage) during the last third of pregnancy. Male and female fetal brains were assessed at embryonic day (E)18 and postnatal day (P)30 for impacts on microglial colonization and development. Additional offspring were assessed for behavioral changes at distinct stages of development; including ultra-sonic vocalizations at postnatal day P5, social exploratory behavior at P15, and memory testing and anxiety at P60. Inflammatory gene expression in microglia and interactions with neurons were assessed at P30 to determine if persistent changes in function occur. Maternal DEP exposure combined with MS (but neither in isolation) produces early-life communication and social deficits, long-term cognitive deficits, and strikingly increased anxiety in male but not female offspring. DEP exposure significantly alters microglial colonization and neural interactions of the male but not female developing brain, and combined prenatal DEP/MS leads to persistent gene expression changes in the microglia of the same brain regions of males. These data demonstrate that combinations of environmental exposures converge onto the primary immune cells of the developing central nervous system, whose activation may be causally important in long-term behavioral alterations in ASDs. We hypothesize this may be due to the important functions of microglia in experience-dependent synaptic remodeling during development.

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Persisting impairment of neurobehavioral function in zebrafish caused by early developmental exposure to disrupting retinoic acid receptor and vitamin D signaling

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Autism incidence has been continually rising over the past two decades. Even after accounting for increases in diagnosis due to more refined diagnostic criteria, there is a substantial increase in Autism incidence. This suggests that there are causative factors that also have been becoming more prevalent, which contribute to the increased Autism incidence. It could be fruitful to investigate in experimental animal models chemical factors that have been found in humans to be associated with increased rates of autism. Rather than a blind screen, we chose to follow an approach using adverse outcome pathways (AOP) of signaling mechanisms for which there is evidence for connections with Autism. We chose zebrafish for a model because they have a complex brain and wide behavioral repertoire with which to characterize Autism-like behavioral phenotypes and are quite inexpensive so that a variety of compounds could be tested for autism risk. The two receptor pathways we chose to start with are retinoic acid and vitamin D receptors. Retinoic acid receptors have been long known to play key roles in early neurodevelopment. There is an increased rate among the children of women who had taken during pregnancy valproic acid, which interferes with retinoic acid receptor signaling. Valproic acid in rodent models has been shown to produce Autism-like neurobehavioral effects. We tested valproic acid in the zebrafish model and found that early developmental exposure caused significantly less social attraction to the shoaling conspecifics when the fish were adults. This was a fairly specific effect with no effect detected in a test of anxiety response. Excess vitamin A during early development also caused the same behavioral phenotype (Bailey et al., NeuroToxicology, 52:23-33, 2016). Vitamin D receptor mechanisms were tested in another study. We assessed in zebrafish the persisting behavioral effects of vitamin D deficiency during early development. A different behavioral phenotype was found. The fish with vitamin D deficiency showed an increased diving response in the novel tank diving test, which is indicative of increased anxiety-like behavior. Vitamin D deficiency also produced slower habituation to tactile startle. However, no significant effects were seen with the shoaling test. Disrupting signaling of receptors for vitamin A (retinoic receptors) and vitamin D (VDR) during early development cause persisting behavioral disruptions in behavioral function in the zebrafish model. The character of the persisting behavioral impairments are different with disrupted retinoic acid receptor signaling impairing social behavior and VDR disruption causing an anxious phenotype.

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Symposium: Long-lasting Effects of Early Development Challenge on Brain and Behavior

Developmental exposure to excess glucocorticoids alters neuronal differentiation and induces long-term behavioral changes

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Epidemiological and experimental studies have shown that alterations in the intrauterine programming occurring during critical periods of development have adverse consequences in later life. Most prenatal adverse conditions are associated to high levels of glucocorticoids (GC), and considering that GC are critical for normal development of many organs, including the nervous system, alterations in their levels may result in long-lasting detrimental consequences. In humans, conditions related to prenatal excess GC lead to intrauterine growth retardation, and low birth weight has been associated to higher risk for attention-deficit hyperactive disorder, schizophrenia and depression. In our recent studies on the long-lasting behavioral alterations induced by prenatal exposure to the synthetic GC dexamethasone (DEX) we observed that DEX-exposed mice aged 12 months, but not younger, exhibit depression-like behavior and impaired hippocampal neurogenesis, which do not respond to antidepressant treatment with fluoxetine (FLX). Unfavorable prenatal environmental factors associated to high levels of GC, such as stress or childhood abuse/neglect, have been shown to cause epigenetic changes in early life. In agreement, prenatal exposure to DEX resulted in global DNA hypomethylation in the cerebral cortex of 3-day-old mouse pups and changes in the expression of the DNA methyltransferase Dnmt3a. By using human and rodent embryonic neural stem cells in culture we were able to identify Tet-3 dependent regulation of Dkk1as major players in DEX-induced alterations in differentiation. In a more recent study on the induced pluripotent stem cells (IPSC)-derived lt-NES AF22 cell line representative of the neuroepithelial stage in central nervous system development, we showed that DEX induces heritable effects on the intracellular REDOX state that, in turn, alter the differentiation potential of lt-NES . Treatment with the antioxidant N-acetyl-cysteine (NAC) could reverse the DEX-induced adverse effects on differentiation, indicating that the increased ROS concentration plays a direct role in the long-lasting impairment of lt-NES differentiation. Altogether, our data support the hypothesis that early insults associated to high GC levels have detrimental long-term consequences on neurogenesis. Based on the positive effects exerted by NAC, it is conceivable that therapeutic strategies including antioxidants may be effective in the treatment of neuropsychiatric disorders that have been associated to impaired neurogenesis.

Long lasting neurotoxic effects of MDMA administration during adolescence

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Several results have shown that the adolescent brain may be vulnerable to long-term neurotoxic effects induced by drugs acting at the central nervous system level.
In line with these studies, previous results from our research group demonstrated that chronic exposure to 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) of adolescent mice exacerbates dopamine neurotoxicity and neuroinflammatory effects elicited by 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP) in the substantia nigra and striatum at adulthood and that combined administration of MDMA plus caffeine during adolescence may worsen the neurotoxicity and neuroinflammation elicited by MDMA. In order to study the neurotoxic effects of MDMA administered chronically at different post-natal days, the present study evaluated NET, DAT and TH -positive fibers and GAD-67 and TH-positive neurons in different motor and limbic areas. Mice received MDMA (10 mg/kg, i.p.), twice a day/twice a week from post-natal day (PND) 60 to PNDs 82, 107 or 124 and were then sacrificed at different time-points after discontinuation (PNDs 85, 110, 138, or 214). A reduction of DAT-positive fibers in the striatum and medial prefrontal cortex associated to a reduction of TH-positive substantia nigra neurons and of GAD-67-positive neurons in the striatum, medial prefrontal cortex and hippocampus were detected in mice treated from PND 60 to 107 (28 administrations). In contrast an increase in NET-positive hippocampal fibers was found in the same group. In addition to eliciting these effects, MDMA reduced TH-positive striatal fibers and substantia nigra neurons in mice treated from PND 60 to 124 (36 administrations). Finally, the effects of MDMA on nigrostriatal DA system and GABAergic transmission

persisted up to 3 months after discontinuation. Results suggested that MDMA produces long-term changes in neurotransmitter systems regulating both motor and cognitive performances.
Interruption of oxygen at delivery and permanent vulnerability to recurrent metabolic insults: in vitro and in vivo experiments

Interruption of oxygen at delivery and permanent vulnerability to recurrent metabolic insults: In vitro and in vivo experiments

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The hypothesis of enhanced vulnerability following perinatal asphyxia (PA) was investigated with a protocol combining *in vivo* and *in vitro* experiments. Asphyxia exposed (AS) and caesarean-delivered control (CS) rat pups were used at P2-3 for preparing triple organotypic cultures including tissue samples from mesencephalon (SN) and telencephalon (Str and Cx). In parallel series, AS and CS neonates were treated with a single dose of saline (100 µl, i.p.) or nicotinamide (0.8 nmol/kg, i.p.), 60 min after delivery. At DIV 18, cultures were subjected to a second challenge, consisting of different concentrations of H_2O_2, added to the culture medium for 18h. After a 48h recovery period, the cultures were either assessed for cell viability, or for neurochemical phenotype and confocal evaluation in formalin fixed cultures. Energy metabolism (ADP/ATP), oxidative stress (GSH/GSSG) and a modified ferric reducing/antioxidant power assay was applied to homogenates of parallel culture series. It was found that PA produced a long-term energetic deficit associated to regionally specific cell loss, mainly affecting mesencephalic dopamine systems. Homogenates of AS triple organotypic cultures showed a >6-fold increase in ADP/ATP ratio, assayed at DIV 21-22, compared the corresponding controls (CS). The increase in the ADP/ATP ratio reflected a permanent energetic deficit, since a single 1 mM H_20_2 insult also increased the ADP/ATP (>7-fold) in CS, further elevating the ADP/ATP ratio in AS cultures. That effect was paralleled by a decrease in the GSH/GSSG ratio, observed in AS culture homogenates, also mimicked by the H_20_2 insult. A decrease in reducing power was observed in AS homogenates compared to the CS controls, but that was not affected by H_20_2. The cell phenotype of dying/alive cells was investigated in formalin fixed cultures, co-labelling for DAPI (nuclear staining), TUNEL (apoptosis), MAP-2 (neuronal phenotype), GFAP (astroglial phenotype), and tyrosine hydroxylase (dopamine phenotype). In substantia nigra, the number of MAP-2/TH positive cells/mm^3 was decreased in AS compared to CS cultures, also by 1 mM of H_20_2, both in CS and AS cultures, prevented by nicotinamide. In agreement, the number of MAP-2/TUNEL positive cells/mm^3 was increased by 1 mM H_2O_2, both in CS (2-fold) and AS (3 fold) cultures, also prevented by nicotinamide. The number of MAP-2/TH/TUNEL positive cells/mm^3 was only increased in CS (>3 fold), not in AS (1.3 fold) cultures. No TH labelling was observed in neostriatum, but 1 mM of H_2O_2 produced a strong increase in the number of MAP-2/TUNEL positive cells/mm^3, both in CS (>2.9 fold) and AS (>5 fold) cultures, decreased by nicotinamide. In neocortex, H_2O_2 increased the number of MAP-2/TUNEL positive cells/mm^3, both in CS and AS cultures (≈3fold), decreased by nicotinamide. Thus, the present results demonstrate that PA implies a long-term energetic deficit, even when re-oxygenation is stabilized, priming cell vulnerability with both neuronal and glial phenotype. The observed effects were region dependent, being the substantia nigra particularly prone to cell death. Nicotinamide treatment *in vivo* prevented the deleterious effects observed *in vitro*.

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Low-level embryonic exposure to flame retardants and related compounds causes neurobehavioral impairment in larval and adult zebrafish

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Flame retardants (FRs) are widely used in home furnishings and electronics. FRs are not chemically bound to products, and thus continuously leach out into the surrounding air and dust, resulting in ubiquitous human exposure to low levels in indoor and outdoor environments. Moreover, FR exposure is higher in toddlers and children due to crawling and hand-to-mouth contact. Organophosphate (OP) FRs have recently replaced the neurotoxic brominated FRs in many consumer products. In this study, we examined the larval (early-life) and adult (later-life) behavioral consequences of developmental exposure to low levels of OPFRs. In addition to the target OPFRs, we included in our exposures several related compounds with known neurotoxicity, namely the OP pesticide chlorpyrifos and two brominated FRs 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) and 2,2',4,4',5-pentabromodiphenyl ether (BDE-99). Zebrafish embryos were exposed to low concentrations of the test chemicals at 5-120 hours post fertilization (hpf), when they were transferred to non-dosed water. At 144 hpf the larvae were tested for locomotor activity in response to alternating light and dark conditions. To determine the long-term effects, developmentally exposed adult zebrafish were tested in a behavioral battery including assays for anxiety-related behavior, sensorimotor response and habituation, social interaction, predator avoidance, and learning. The results so far indicate that early-life exposure to the OPFRs isopropylated phenyl phosphate (IPP), t-butylphenyl diphenyl phosphate (BPDP), 2-ethylhexyl diphenyl phosphate (EHDP) and isodecyl diphenyl phosphate (IDDP), as well as the brominated FRs, cause both larval and adult behavioral impairments that are manifested in a chemical-specific manner. In some cases, at the lower doses of exposure the adult behavioral effects were not predicted by effects on larval motility, thus stressing the importance of life-long behavioral testing of multiple behavioral domains. In contrast, exposure to the OP pesticide chlorpyrifos caused a reduction in locomotor activity of the larvae, that was not evident in adults. The lack of predictability of adult behavioral impairment by larval behavioral impact can be seen in both directions. Some developmental chemical exposures cause larval effects but not adult effects and others can cause adult effects without evident larval effects.

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Lead Exposure of Children in China: Current Status and Prevention

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Children are especially susceptible to chronic lead exposure, with effects including physical, cognitive, and neurobehavioral impairments, and there is no safe concentration of blood lead below which children are not affected. The presentation provides a comprehensive review of lead exposure among children in China, the sources and new trends of emerging cases. In China, a declining tendency has become evident for children's blood lead levels in recent years. This trend of changes can be contributed by several factors such as the shutdown and relocation of severely polluting factories, the using of cleaning fuel instead of coal that containing high concentration of lead for cooking and heating, and the enhanced civil awareness of the relationship between environmental lead exposure and human health. One another major reason for declined lead poisoning in cities pertains to the banned production of the leaded gasoline in January 2000 and its sale since July 2000. However, blood lead concentrations, even those below 10 microg per deciliter, are inversely associated with children's IQ scores. While the decreasing blood lead concentration of children in Chinese cities is evident, the blood lead levels of these children are still higher than those in developed countries; the children in rural areas are now becoming the major victims of the lead pollution cases, because more and more factories have been moved from cities to rural areas. From the occupational exposure point of view, the main industrial sources of lead pollution in China are ore, and metal processing and manufacturing, as well as combustion of coal, petroleum fuel, and wastes. For daily life, the unqualified toys and materials for building and furniture, and lead-polluted food are major sources for children poisoning. Considering that childhood lead poisoning remains a public health problem in China, there is still a long way to go for lead prevention.

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<u>Symposium</u>: Neurodevelopmental Effects of Chemical Exposure

Effects of developmental alcohol exposure on neuronal plasticity and multisensory integration in the cortex

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Children with Fetal Alcohol Spectrum Disorders (FASD) often present sensory alterations such as aversion to multiple sensory stimuli presented at the same time, tactile hypersensitivity and poor visual sensory processing. There is growing evidence that disruption of Multisensory Integration (MSI) underlies these problems. MSI relies on the precise wiring of primary sensory systems and the accuracy of convergence of these systems to multisensory processing areas. This precision is acquired through neuronal plasticity processes that include sprouting and pruning of connections. Our group and others have shown that alcohol exposure during the third trimester equivalent of human gestation leads to a permanent disruption of cortical neuronal plasticity. Therefore, we hypothesized that developmental alcohol exposure disrupts the functional connectivity of multisensory cortical areas and impairs MSI. Based on this hypothesis, we used a ferret model of FASD to test the following predictions: 1-Alcohol exposed ferrets will display aberrant connectivity in the rostral posterior parietal cortex (PPr), a visual-tactile cortical area. 2-The PPr of alcohol exposed ferrets will display aberrant MSI. The degree of connectivity of PPr with its major visual (posterior parietal cortex, PPC) and tactile (third somatosensory cortex, S3) inputs was investigated by resting state functional MRI. Ferrets (n=9 per group) were exposed to 3.5 g/Kg of ethanol (BAL=~250mg/dl) or control saline between postnatal day (P) 10-30, a period that is roughly

similar to the third trimester of human gestation. Between P45-P60 animals were scanned in a 7 tesla magnet. Z-scores indicated a higher correlation of the BOLD response between PPr-S3 and PPr-PPC in alcohol animals than in controls. These findings suggest that developmental alcohol exposure leads to hyperconnectivity in ferret PPr. To test if developmental alcohol exposure affects MSI, we conducted *in vivo* electrophysiology. Single units were recorded with a 16 channel electrode after visual only, tactile only or visual+tactile stimulation. A total of 46 cells from two control animals and 103 cells from four ethanol treated ferrets were recorded. As expected, in the control group 37% of cells showed crossmodal depression, 24% crossmodal facilitation and 39% did not show MSI. This distribution is similar to what is described in the literature for normal ferrets. Strikingly, in the alcohol group, 82% of cells showed crossmodal depression, 8% crossmodal facilitation and 11% did not show MSI. Taken together, these findings suggest that developmental alcohol exposure lead to hyperconnectivity of multisensory processing areas and disrupt MSI. Further investigation of the mechanisms underlying these abnormalities will shed light on the causes of multisensory processing deficits seen in FASD.

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Enduring neurotoxic effects of perinatal exposures to pesticides in mice

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Parkinson's disease (PD) is a multifactorial neurodegenerative disorder with late-life onset. It has been hypothesized that PD could arise from events that occur early in development, but that may have delayed adverse consequences in the nigrostriatal dopaminergic system during the adult life. Although some lines of evidence indicate that developmental exposure to neurotoxicants (including pesticides) could make the nigrostriatal dopaminergic system more susceptible to subsequent challenges during the adult period, the molecular mechanisms mediating this phenomenon are unknown. Therefore, we investigated potential occurrence of late nigrostriatal dopaminergic dyshomeostasis induced by exposures to the pesticides paraquat (PQ) and maneb (MB) during the early-postnatal development. In addition, we investigated whether PQ and MB exposure during critical periods of development could enhance the vulnerability of the dopaminergic system to the toxicity induced by a subsequent re-exposure to these same pesticides in adult life. Male Swiss mice were treated with a combination of PQ and MB (PQ + MB; 0.3 + 1.0 mg/kg/day; s.c.) from post-natal (PN) day 5 to 19. Postnatal pesticide exposure neither induced mortally nor modified mouse body weight and motor function. However, significant decreases in the striatal activity of mitochondrial complex I and II were observed. Moreover, postnatal PQ + MB exposure reduced the levels of tyrosine hydroxylase (TH) and dopamine transporter (DAT) in the striatum, as well as decreased the number of TH and DAT positive neurons in the substantia nigra pars compacta (SNpc). Parallel groups of mice (3 months) developmentally exposed to PQ + MB were re-challenged to these same pesticides (PQ + MB; 10 + 30 mg/kg/day; s.c., twice a week during 6 weeks) during adulthood. Animals subjected to PQ + MB exposures during both periods (postnatal + adult exposures) presented lower motor performance compared to animals exposed to these pesticides during a single period. In addition, mice subjected to PQ + MB exposures during both periods (postnatal + adult exposures) presented significant lower numbers of TH and DAT positive neurons in the SNpc, although no significant differences among groups were observed in the striatum. Taken together, these findings indicate that exposure to PQ + MB during either the postnatal period or adulthood causes neurotoxicity in the mouse dopaminergic system and that the sum of the two exposures (postnatal period plus adulthood) causes a higher neurotoxicity when compared to exposures during a single period.

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Exposure to domoic acid during a critical period of neurodevelopment alters myelin sheath formation and leads to behavioral deficits

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Harmful algal blooms (HABs) can produce toxins that accumulate in seafood and affect human health. While regulations prevent the harvest of seafood with concentrations causing acute toxicity, emerging research shows that low□dose exposures during early development can have long□lasting, detrimental effects on nervous system function. This study seeks to identify the cellular and molecular mechanisms of developmental toxicity by characterizing cell types targeted by HAB toxins, identifying perturbations to nervous system structure and their downstream effects on behavior. We exposed zebrafish to Domoic acid (DomA; 0.18□0.14ng/embryo) by caudal vein microinjections at three different periods of neurodevelopment: i) 1 day post fertilization (dpf) when oligodendrocyte precursor cells are specified, ii) 2dpf when oligodendrocytes differentiate and begin axon wrapping, and iii) 4dpf, a later period after the early nervous system is specified. Using a transgenic zebrafish line with fluorescently□labeled myelin sheaths, tg(mbp:EGFP-°©□CAAX), we found that exposure to DomA only at 2dpf (but not 1 or 4dpf) caused myelination defects ranging from the thinning (68.8 % ± 5) to the absence of myelin sheaths (16.6 ± 6%). Time-°©□lapse imaging showed that DomA exposure at 2dpf perturbs the initial formation of myelin sheaths from 2.5□3dpf. To determine whether myelination deficits are driven by a decrease in the number of oligodendrocyte precursor cells and myelinating oligodendrocytes, we quantified cell numbers. Exposure to DomA at 2dpf reduced the number of myelinating oligodendrocytes by 23% ($p<.0001$), but did not reduce the number of oligodendrocyte precursor cells before the onset of myelination. Furthermore, whole embryo RNA sequencing showed differentially expressed genes associated with myelin sheath structures (*mbpz, mbpa*) and axon cytoskeletal proteins (*nefla, neflb, nefma, nefmb*) (FDR<0.05), suggesting that myelination defects may result from DomA disrupting pathways necessary for maintaining axonal and myelin sheath structures. We also observed an impaired startle response in DomA exposed embryos. Larvae exposed at 2dpf (but not 1 or 4dpf) had 2□3 fold reduction in bend angles and slower angular velocities ($p<.001$). These findings provide mechanistic insights into neurotoxicity of DomA exposure during critical periods of neurodevelopment. In particular, we have i) identified the vulnerable period of developmental exposure: when oligodendrocyte differentiation and myelin sheath formation occurs, ii) characterized DomA□induced structural changes in myelin sheath formation, and iii) determined the functional consequences for startle behavior. These mechanistic insights can be extrapolated to support hazard assessments for low□dose DomA exposures in humans during key periods of pregnancy and early childhood development.

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Histopathological and behavioral alterations caused by chronic intrastriatal hypoxanthine administration in striatum of young rats

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Lesch-Nyhan disease is a hereditary metabolic disorder linked to chromosome X, which affects the metabolism of purines, being characterized by the deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). This alteration results in the accumulation of oxypurines, mainly hypoxanthine. The symptomatology manifests itself early in the life of the patients and is characterized by motor and cognitive alterations, mental retardation, spasticity and self-mutilation. In the present study, we evaluated the effect of chronic administration of hypoxanthine on behavioral cognitive and motor parameters, and analyzed striatal morphology. Wistar rats of 21 days of life underwent stereotactic surgery with placement of a cannula, followed by chronic intrastriatal administration for 14 days. Animals were divided into three groups: (1) naive, (2) saline infusion (solution 0.9%), (2) hypoxanthine infusion (10 μM; 20pg/2μL). Behavioral

analyzes (open field, novel object recognition, beam walking test, cylinder test, and ladder walking test) were performed 24 hours after the last administration of hypoxanthine and the rats were perfused after the behavioral analysis. Our results show a decrease in the total distance traveled by the hypoxanthine group compared to controls in the open field test. In the novel object recognition, there was an increase in the recognition time of the new object by the control group; the same was not seen in the hypoxanthine group, suggesting a long-term memory alteration. Chronic administration of hypoxanthine also increased the number of hind paw errors presented by animals in the ladder and beam walking test. In addition, we saw in the cylinder test, a decrease in the use of the front legs in relation to the controls. Through immunohistochemical analysis, we have seen an increase in glial fibrillary acidic protein reactivity and a decrease in the NeuN and tyrosine hydroxylase labeling. According to our results, chronic intrastriatal administration of hypoxanthine alters cognitive, locomotor and histologic parameters in Wistar rats. Thus, our data corroborate with a model that mimics alterations found in patients with Lesch-Nyhan disease, becoming a viable model for a better understanding of the disease.

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Poster Presentations

1

Vinpocetine, a phosphodiesterase inhibitor, mitigates hyperactivity and memory/ learning deficits in mice exposed to nicotine and ethanol during the period equivalent to human gestation

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Epidemiological studies indicate that there is a strong association between smoking and alcohol drinking, so that co-consumption is very frequent. Co-use and co-abuse of tobacco and ethanol often occur during pregnancy. Developmental exposure to nicotine or ethanol evokes hyperactivity and memory/learning deficits, and recent reports indicate that impairments in both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) signaling cascades contribute to these phenotypes. Despite that, there is a lack of studies that investigate the effects of the combined use of nicotine and ethanol during development. Here, we used a mice model to investigate the effects of exposure to nicotine and/or ethanol in the period equivalent to human gestation on locomotor activity and memory/learning, as well as cAMP and cGMP levels in the hippocampus and cerebral cortex. Additionally, we investigated the effects of the treatment with vinpocetine (a phosphodiesterase inhibitor that increases intracellular cAMP and cGMP levels) on these parameters. Swiss male and female mice were exposed to nicotine free base (50 μg/ml) dissolved in saccharin 2% or to saccharin 2% to drink during gestation and until the 8^{th} day of postnatal (PN8) life. Ethanol (5 g/kg, i.p.) or saline were injected in the pups in alternate days from PN2 to PN8. Accordingly, four groups were analyzed: VEH, NIC, ETOH and NIC+ETOH. At PN30, animals either received vinpocetine (20 mg/kg i.p.) or vehicle before memory/learning (step-down passive avoidance test) and locomotor activity (open field) were evaluated. After the behavioral tests, animals were decapitated and the brain regions were fast-frozen and stored until cAMP and cGMP analyses (Enzyme Immunoassay - EIA). Analysis of variance followed by Fisher's Protected Least Significant Difference tests were used with $p<0.05$ as the threshold for significance. There were memory/learning deficits in NIC, ETOH and NIC+ETOH mice, and the treatment with vinpocetine mitigated ETOH and NIC+ETOH-induced deficits. Locomotor hyperactivity was identified only in the NIC+ETOH group. This effect was ameliorated with vinpocetine treatment. While cyclic nucleotides levels were reduced by NIC, ETOH and NIC+ETOH exposures, this effect was more consistent in the latter group, in which vinpocetine effectively restored cyclic nucleotides levels. These data lend support to the idea that cAMP and cGMP signaling cascades contribute to NIC+ETOH-induced hyperactivity and memory/learning deficits, and provide evidence for the potential therapeutic use of vinpocetine in individuals co-exposed to these drugs during development.

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2

Rifampicin suppresses alpha-synuclein induced microglial activation and improve neuron survival against inflammation

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Microglial cells are the resident immune cells of the brain parenchyma. Sustained activation of microglia is known to play a role in the progression of neurodegenerative diseases such as Parkinson's disease (PD). It has been suggested that the modulation of microglial activation could prevent neuronal demise and thus the progression of neurodegeneration. Based on clinical studies in the context of infectious diseases where Rifampicin seems to protect patients from neurodegeneration, we hypothesize that Rifampicin, could exert a neuroprotective effect by suppressing microglial activation induced by endogenous pro-inflammatory mediators, such as alpha-synuclein aggregates (ASa). Primary microglial cells purified from post-natal day 1 C57BL/6J mouse pup brains were pre-treated or not with Rifampicin, then challenged with ASa and incubated for 24 h. Conditioned media were collected to perform ELISA assays to measure cytokine levels (TNF-α, IL-1b, IL-6). Adherent cells were either fixed for immunostaining procedures or lysed for western blot assays. The modulatory effect of drugs on cell proliferation was also followed by thymidine incorporation. Cortical neurons purified from C57BL/6J mouse embryos were challenged to microglial induced conditioned media. The viability was measured using CCK-8 and LDH release. Rifampicin readily reduced prototypical markers of inflammation induced by ASa such as (i) Iba-1 expression, (ii) TNF-α and IL-6 production and release, (iii) morphological changes, and (iv) cell proliferation, by blocking PI3K/pAKT signaling pathway, (v) neuron survival. Globally, our results suggest that Rifampicin inhibits microglial activation induced by ASa. We thus propose that Rifampicin could be used as a novel treatment for neurodegenerative diseases such as PD.

3

Methamphetamine, 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxypyrovalerone (MDPV) induce differential cytotoxic effects in bovine brain microvessel endothelial cells

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Designer drugs such as synthetic psychostimulants are indicative of a worldwide problem of drug abuse and addiction. In addition to methamphetamine (METH), these drugs include 3,4-methylenedioxy-methamphetamine (MDMA) and commercial preparations of synthetic cathinones including 3,4-methylenedioxypyrovalerone (MDPV), typically referred to as "bath salts." These psychostimulants exert neurotoxic effects by altering monoamine systems in the brain. Additionally, METH and MDMA adversely affect the integrity of the blood-brain barrier (BBB): there are no current reports on the effects of MDPV on the BBB. The aim of this study was to compare the effects of METH, MDMA and MDPV on bovine brain microvessel endothelial cells (bBMVECs), an accepted *in vitro* model of the BBB. Confluent bBMVEC monolayers were treated with METH, MDMA and MDPV (0.5mM-2.5mM) for 24h. METH and MDMA increased lactate dehydrogenase release only at the highest concentration (2.5mM), whereas MDPV induced cytotoxicity at all concentrations. MDMA and METH decreased cellular proliferation only at 2.5mM, with similar effects observed after MDPV exposures starting at 1mM. Only MDPV increased reactive oxygen species production at all

concentrations tested whereas all 3 drugs increased nitric oxide production. Morphological analysis revealed different patterns of compound-induced cell damage. METH induced vacuole formation at 1mM and disruption of the monolayer at 2.5mM. MDMA induced disruption of the endothelial monolayer from 1mM without vacuolization. On the other hand, MDPV induced monolayer disruption at doses ≥0.5mM without vacuole formation; at 2.5mM, the few remaining cells lacked endothelial morphology. These data suggest that even though these synthetic psychostimulants alter monoaminergic systems, they each induce BBB toxicity by different mechanisms with MDPV being the most toxic.

4

The flavonoid 4,5,7–trihydroxyflavone is a neuroprotective agent against neuroinflamation and dopaminergic degeneration induced by aminochrome

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The main mechanisms responsible for neurodegeneration in Parkinson's disease is still unknown, however, some cellular and molecular disorders are involved in this process: accumulation of α-synuclein, oxidative stress, mitochondrial damage, proteosomal, autophagic dysfunction and neuroinflammation. Among these, only neuroinflammation has not yet been associated with effects induced by aminochrome. This work is mainly focused on neuroinflammatory potential of aminochrome and role of 4,5,7–trihydroxyflavone (apigenin) a well-known immunomodulator flavonoid in reversing this phenotype. Midbrain organotypic cultures from P8 wistar rats were cultivated for 3 days with DMEM/F12, incubated in 5% CO_2, at 37 ° C. Slices were treated with aminochrome (0,01 to 25 μM) and/or apigenin (10μM), and analyzed after 24 or 48 hours. Neurotoxicity was evaluated by morphological analysis and expression of tyrosine hydroxylase (TH) by Western blot. The neuroinflammatory response was assessed by immunohistochemistry for Iba1, RTq-PCR and ELISA essay for cytokines TNF and IL-1β. The modulatory effects of neurotrophic factors were evaluated by RTq-PCR. Our results demonstrate aminochrome induced tissue damage in culture associated to reduction of TH expression, which was inhibited by apigenin. Furthermore, aminochrome induced morphological changes in $Iba1^+$ cells associated to increase of TNF and IL-1β mRNA levels and increase of IL-1β and TNF levels in the supernatant of cultures treated with aminochrome, which were also inhibited by apigenin. Aminochrome reduced mRNA levels of CDNF and NGF but increased BDNF. Apigenin inhibited the effects induced by aminochrome in relation to CDNF and NGF expression. On the other hand, apigenin treatment reduced expression of BDNF and GDNF. These results demonstrated that neuroinflammation and neurotrophic factors are also involved in the neurodegeneration induced aminochrome and apigenin is a neuroprotective agent against cell damage induced by this toxin.

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5

Isolated human populations and Mercury Exposure: Blood mRNA of S100B protein as a possible biomarker of intoxication

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Mercury is a heavy metal responsible for human intoxication worldwide. The most toxic form is methylmercury due to its affinity for the central nervous system, with recognized neurotoxicity. Some regions of the Amazon, as Tapajós River basin, are well characterized by mercury exposure in humans presenting mercury levels above the WHO safety limit. However, few studies analyzed possible intoxication biomarkers in these isolated populations. One of the major factors contributing for the difficulty to carry out this kind of study is the huge limitation for the adequate conservation of samples (blood and serum), especially those destined for protein analysis. Thus, although S100B protein is a well-stablished biomarker to detect brain damage by mercury intoxication, it is not possible the accurate measure of this protein in these isolated populations of Amazon. Our propose was to use the mRNA along with a RNA stabilizing reagent, making it simple and easy to detect the desired target. The objective of this study was to determine the exposure (mercury content in the body by using mercury levels in hair samples) and access the mercury intoxication (quantifying S100B mRNA by using RT-qPCR - TaqMan gene expression assay) in Amazon riverside populations. Two hundred individuals, selected after inclusion and exclusion criteria, were studied. The median level of total mercury in hair in Tapajós was 2.63 µg/g (ranging to 0.18- 20.14) and in Tucuruí was 9.47 µg/g (ranging to 1.12- 75.80). A significant proportion of participants had mercury levels above 10 µg/g, as recommended by the WHO limit and showed a total content of mercury greater or equal to 20 µg/g). Interestingly, there was a significant difference in mRNA levels of S100B protein between individuals exposed to high and low levels of mercury, confirming this alternative measure as an effective biomarker for isolated populations. Moreover, for the first time, the S100B biomarker has been studied in Amazonian populations. This knowledge will assist the development of prevention strategies and making government decisions facing the problem of the impact of the mercury in the Amazon.

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6

Atorvastatin-promoted neuroprotection against glutamate toxicity depends on GluN2B subunit of of N-methyl-D-aspartate receptors (NMDAR)

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Atorvastatin is a hypocholesterolemic drug that belongs to the class of statins and presents pleiotropic effects, as anti-inflammatory and neuroprotective. The neuroprotective effects of atorvastatin in mice may be mediated by glutamatergic receptors N-methyl-D-aspartate (NMDAR) interaction. The aim of this study was to evaluate the neuroprotective effect of atorvastatin against glutamate-induced toxicity and the involvement of NMDAR subunits GluN2A/2B in such effect. Male adult albino Swiss mice received a daily dose of atorvastatin for 7 days (1 and 10 mg/Kg p.o., sub-effective and effective doses for neuroprotection, respectively). Control animals received filtered water (p.o.) during the same period. On the 8th day the animals were sacrificed and their hippocampi rapidly removed and sliced (0.4 mm) and incubated in physiological buffer. Slices were pre-incubated for 15 minutes with ifenprodil (5-20 µM, GluN2B antagonist), D-serine (30 µM, GluN2A co-agonist) or glycine (10 µM, GluN2B co-agonist) and then subjected to the glutamate excitotoxicity (1 hour incubation of 10 mM glutamate). Cellular viability was analyzed by MTT reduction assay. Ifenprodil (20 µM, GluN2B antagonist) showed partial prevention against glutamatergic toxicity and was selected as the sub-active dose to neuroprotective effect. Hippocampal slices of mice treated with sub-active dose of atorvastatin, *in vivo,* (1 mg / kg, p.o.) and incubated with the sub-active dose of ifenprodil, *ex vivo,* had a summation effect preventing glutamate-induced toxicity. In addition, atorvastatin prevented glutamate-induced reduction of slices viability (in

the active dose, 10mg/kg). Preincubation of slices with D-serine (GluN2A co-agonist) showed a partial reduction of the neuroprotective effect of atorvastatin against glutamate. In contrast, in slices preincubated with glycine (GluN2B co-agonist) plus glutamate, the neuroprotective effect of atorvastatin was fully maintained. D-serine or glycine *per se* did not alter cell viability and did not prevent glutamate toxicity. These results suggest a role to NMDAR GluN2B subunit interaction in the neuroprotective effect of atorvastatin in mice.

7

Effects of combined exposure to low-frequency noise and CS_2 on hearing and balance

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Carbon disulfide (CS_2) is a neurotoxic solvent causing central and distal axonopathies. Long duration occupational exposure produces debilitating motor and sensory consequences in both animals and humans. However, little is known about its effects on hearing and balance, particularly when combined with noise exposure. Other solvents, including several aromatic solvents, cause hearing loss and balance disturbances, and these harmful effects are enhanced by noise. CS_2 could have a toxic effect on cochlear and vestibular hair cells. Also, CS_2 could have a neuropathic effect on the auditory and vestibular pathways, and therefore induce central hearing deficits or balance dysfunctions. The aim of the current investigations was to study the effects of exposures to either a continuous noise or to a 250-ppm CS_2 exposure alone, or to a combined exposure to noise and CS_2. Female Long-Evans rats (16 weeks old) were exposed to a) control conditions; b) continuous noise alone [0.5 to 2 kHz, 105 dB]; c) 250-ppm CS_2 exposure alone;, d) combined exposure to noise and CS_2. The animals were exposed to noise and CS_2 with the same schedule: 6 hours per day, 5 days per week, for 4 weeks. The auditory function was evaluated using distortion product otoacoustic emissions (DPOAEs) to test the cochlear performance, while balance was proved by recording the vestibulo-ocular reflex (VOR) using video-oculography. Finally, histological analyses were carried out to evaluate the induced damages of the inner ear. The functional tests were done before exposure, after exposure, and 4-week post-exposure.
The low-frequency noise alone caused an enduring auditory deficit revealed by increased DPOAE thresholds after exposure and 4-weeks post-exposure. Deficits were found in a frequency region higher (3.6 - 6 kHz) than that of the noise spectrum (0.5 - 2 kHz). The highest frequency of defective hearing, 6 kHz, corresponds to approximately one octave above the highest frequency of the band noise used in the current experiment (2.8 kHz). Therefore, low–frequency exposure can impact cochlear regions dedicated to mid-frequencies. Adding 250 ppm [10 x TLV ST (threshold limit value short term)] of CS_2 to the noise increased the window of the damaged frequencies at the end of exposure. Thus, in co-exposed conditions, a significant auditory deficit was obtained at 9.6 kHz. However, 4 weeks after recovery, the width of the window was reduced to 6 kHz, coincident with the one recorded with the noise alone. The CS_2 alone, and associated with noise, caused vestibular deficits assessed by the VOR analyses. At the end of the exposure, changes were found in all recorded VOR parameters: number of saccades, total duration, median frequency, and damping slope of saccades. However, all parameters recovered to control values after 4 weeks of recovery. Histological evaluation did not reveal any overt pathology. Hair cells, stereocilia and ganglion cells were always present with a control-like morphology. Similarly, we did not find any change in the afferent fibers connecting to hair cells in the organ of Corti and vestibule. In conclusion, the results of the present study indicate that CS_2 temporarily potentiates the noise effects, enlarging the frequency window showing an auditory deficiency. Moreover, CS_2 caused reversible VOR deficits, likely disturbing the central nervous system centers involved in balance. No obvious morphological changes were observed in the inner ear, so the recorded functional deficits cannot be explained by major pathology in the organ of Corti, the vestibular epithelia, or the ganglion neurons. As most aromatic solvents, CS_2 should be taken into consideration as a relevant factor in hearing and balancing conservation regulations, at least for its acute effects.

8

Chronic administration of L-tyrosine alters oxidative stress parameters in brain of rats supplemented with omega-3 fatty acids

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Tyrosine aminotransferase deficiency characterizes the inborn error of metabolism tyrosinemia type II, leading to increased levels of tyrosine and its by-products in plasma, resulting in eye, skin and neurological injuries. The mechanisms of brain injury are still not well known, but several studies suggest that oxidative stress in brain is involved in the pathophysiology of tyrosinemia. Docosahexaenoic acid (DHA; C22:6) and eicosapentaenoic acid (EPA; 20:5) are omega-3 fatty acids which play important roles in the development and maintenance of the central nervous system. Thus, the present study aimed to assess DHA and EPA administration effects on oxidative stress parameters, such as 2′,7′-dichlorofluorescein (DCFH) oxidation, nitrate and nitrite levels, sulfhydryl (thiol) group content and thiobarbituric acid-reactive species (TBA-RS) levels, in brain of young rats subjected to an animal model of hypertyrosinemia. Wistar rats were divided into 4 groups: control, L-tyrosine, DHA + EPA, and L-tyrosine + DHA + EPA. The animals received L-tyrosine (500 mg/kg of body weight, i.p., 12/12 hours), and DHA + EPA (0.1 g/kg body weight by gavage, once a day) from the 7th to the 28th postnatal days (control group received vehicle). Twelve hours after the last administration, the animals were killed by decapitation; hippocampus, striatum and cortex were isolated for analysis. Our results showed that L-tyrosine increased the oxidation of DCFH and TBARS levels in the cortex, and administration of DHA and EPA prevented these effects. The levels of nitrates and nitrites were increased in hippocampus and striatum of rats, but the administration of DHA and EPA did not prevent this effect. Sulfhydryl group content was decreased in striatum in the tyrosine group, and the administration of DHA and EPA partially prevented this diminution. From these results, we speculate that chronic administration of L-tyrosine may induce oxidative stress in the hippocampus, striatum and cortex and the supplementation omega-3 fatty acids can be a potent adjuvant treatment for patients with tyrosinemia type II.

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Hypoxia-inducible factor-1α (HIF-1α) binds to HIF-response elements on the promoter region of candidate genes in the genome rat under Hypoxic conditions: A bioinformatics study

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Perinatal asphyxia (PA) is characterized by interruption of oxygen bioavailability at birth. Hypoxia implies HIF-1α activation, a key sentinel protein, which, upon translocation to the nucleus, binds to response elements (HREs), promoting the transcription of several genes. Potential genes activated by HIF-1α under hypoxic conditions were identified in the rat genome by extracting promoter sequences of *Rattus Norvegicus* from the UCSC database Genome Browser, using the latest version of rat genome (Jul 2014. RGSC 6.0/rn6). A promoter sequence of 39595 transcripts was first obtained, identifying then 8762 genes with the HIF-1α binding sequence (5`-RCGTG-3`) with the "R" software. 8762 genes were introduced to the *Gen Ontology* platform for performing an enrichment analysis, selecting the following processes linked to PA: (i) Hypoxia (865 genes); (ii) Glucose Metabolism (330 genes); (iii) Neurogenesis (1243 genes); (iv) Apoptosis (814 genes); (v) Hematopoiesis (165 genes), and (vi) Regulation of Gene Expression (2076 genes). The 865 hypoxia associated genes were further selected and compared with experimental data by ChIPSeq, with 772 and 98 genes, from the human and zebrafish genomes, respectively, finding that (i) 72 genes were shared by human and rat; (ii) 10 genes were shared by zebrafish and rat; (iii) 8 genes were shared by human and zebrafish, and

(iv) 3 genes were shared by the three species (LDHA, DDIT4, CITED2). The 8762 genes were then analysed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) platform, selecting the HIF-1 pathway, identifying 47 genes. The 79 genes filtered for human, zebrafish and rat, were compared with the 47 genes obtained by the KEGG platform, yielding 12 genes. Finally, 12 genes were compared with 47 genes referred by the literature to be associated to PA, identifying 5 genes: (i) bcl2; (ii) hif-1α; (iii) ldha; (iv) pdk1, and (v) vegfa. Analysis of sequence conservation was performed for each species, using the Ensembl Genes database, obtaining an 80% of conservative sequences for all cases (5 genes). It is planned now to demonstrate that HIF-1α interacts with the epigenetic-regulating protein histone acetyltransferases p300/CBP on the promoter regions of the 5 identified genes, as a proof-of-principle for the participation of HIF-1α in the regulation of gene expression following PA.

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Mechanisms mediating paraquat and maneb-induced toxicity in neural stem cells

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Exposure to neurotoxic chemicals during perinatal period (*in utero* or early childhood) may adversely affect brain development and result in long-lasting neurological impairments later in life. In this scenario, pesticides exposures have been linked to neurodevelopmental and neurodegenerative disorders, such as autism spectrum disorders, attention deficit/hyperactivity disorder and Parkinson's disease. Exposure to these toxicants during early stages of development can lead to neuronal loss and function deficits, even at low doses that are not harmful to adult brain. It has been shown that prenatal or early postnatal exposure to the herbicide paraquat (PQ) and the fungicide maneb (MB), isolated or in combination, causes permanent toxicity in the nigrostriatal dopamine system, supporting the idea that early exposure to pesticides may contribute to the pathophysiology of Parkinson's disease (PD). However, the molecular mechanisms mediating PQ and MB neurotoxicity during development are not yet understood. Therefore, we investigated the toxic effect of low (subapoptotic) concentrations of PQ and MB, isolated or in combination, in primary cultures of rat embryonic neural stem cells (NSCs), as well as the possible molecular mechanisms involved. Rat NSCs were exposed to 1 μM PQ and/or 1 μM MB for 24 hours. In parallel experiments, cells were pre-treated with the well-established antioxidant molecule *N*-acetylcysteine (NAC, 25 μM). Exposure to PQ alone or in combination with MB (PQ + MB) led to a significant decrease in cell proliferation, while the cell death rate was not affected. Consistently, PQ + MB exposure altered the expression of major genes regulating the cell cycle, namely cyclin D1, cyclin D2, Rb1 and p19. Moreover, PQ and PQ + MB exposures induced a significant increase in the intracellular reactive species (RS) production that could be neutralized upon NAC treatment. Notably, in the presence of NAC, Rb1expression was normalized and a typical cell proliferation pattern could be restored. Taken together, these findings suggest that exposure to low doses of PQ + MB may impair neurodevelopment by mechanisms involving alteration in the redox balance.

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Poly [ADP-ribose] polymerase-1 (PARP-1) is important in adult brain neuroinflammation and neurotoxicity instigated by alcohol

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PARP-1 is a nuclear and mitochondrial DNA repair enzyme contributing >90% of total brain PARP activity that can elicit programmed necrosis of neurons (termed parthanatos) when its levels and activity (poly [ADP-ribose] or PAR production) are excessive or prolonged by cerebral ischemia or trauma. However, the roles of PARP and PAR in high alcohol (ALC; ethanol)-induced neurodamage in adult brain are basically unexplored. In an adult rat model of ALC neurotoxicity caused by repetitive binge intoxication (three gavages/d for 4 d; 9-12 g ALC/kg/d), immunoblots showed that PARP-1, as well as the phospholipase A2 (PLA2) enzymes responsible for neuroinflammatory arachidonic acid (ARA) mobilization and oxidative stress, were increased over controls selectively in brain regions incurring neurodegeneration—esp. hippocampus (HI) and entorhinal cortex (EC) (Tajuddin et al. 2014). We report here that in this severe binge model, co-treatment with veliparib, a PARP-1 inhibitor used clinically with cancer, prevented 65%-80% of ALC's neurodamage. Reinforcing these *in vivo* results, rat organotypic HI-EC slice cultures of adult brain age displayed elevated PARP-1 and PAR due to 4 d of ALC "binge" treatment (100 mM), and four PARP inhibitors including veliparib each prevented the ensuing neurodegeneration. Concurrently, PARP inhibition abrogated PLA2 elevations, for the first time linking phospholipid-derived neuroinflammation (ARA mobilization) to PARP activity. Furthermore, levels of high mobility group box-1 (HMGB1), a proinflammatory mediator and "danger signal" that activates toll-like receptor-4 (TLR4) to promote IL-1β cytokine release, were increased by binge ALC, consistent with *in vivo* reports; importantly, PARP inhibitors abolished the danger signal potentiation. The ALC-dependent time course revealed that PARP-1 elevations on d2 occurred 24 hr before PLA2 surges and 2 d before increases in HMGB1 (which could come from damaged neurons). Summarizing, the pharmacological results implicate PARP-1 augmentation by ALC in the mediation of two possibly parallel brain neuroinflammatory/pro-oxidative pathways that potentially underlie brain neurodegeneration in alcoholism—i.e., PLA2→phospholipid→ARA and HMGB1→TLR4→Ilβ.

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Antitumoral evaluation of triterpenes in glioblastoma cells

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Glioblastoma are tumors of the central nervous system, highly invasive and resistant to chemotherapy. More efforts are needed to lead to a rational development of new drugs. Triterpenes, like ursolic and betulinic acid, have demonstrated a potent cytotoxic activity against various cell lines, including gliomas. The aim of this work was to evaluate the *vitro* antitumor activity of synthetic derivatives of betulinic and ursolic acid *in* using rat glioblastoma (C6) and human glioblastoma (U-87 MG and U-138 MG) cell lines. Twenty synthetic derivatives of betulinic and ursolic acid, with modifications in C-3 and C-28 positions were tested. Glioblastoma cell lines were maintained in CO_2 incubator at 37 ºC, seeded (8.000 cells per well) in a 96-well plate and after 24 hours, incubated with samples in the range of 0,5-100 µM. After 72 hours of incubation, cells containing samples and controls (DMSO 1% and DMEM culture medium) were subjected to SRB assay. Data were submitted to ANOVA followed by Tukey test and compared to the DMSO group ($P<0.05$). Sixteen derivatives showed inhibition of cell lines viability using SRB assays (n= 4). The SRB assay, after 72 hours, showed that the most active derivatives inhibited cell viability by 50% (IC_{50}) at a range of 1.55 to 5.66 µM, about 6 times more potent that their precursors, betulinic and ursolic acid. Investigation about a possible synergic effect with TMZ, drug usually used for glioblastoma clinical treatment, are being performed. Effect in non-tumor cell line are been investigated too. The highly potent results founded are relevant for future evaluations of the signaling pathways involved in cytotoxic effect on glioblastoma cell lines treated with these derivatives.

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Involvement of GluN2B subunit containing N-Methyl-D-Aspartate (NMDA) receptors and

phosphatidylinositol-3 kinase (PI3K) pathway in the mechanism of NMDA preconditioning

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NMDA administered at subtoxic dose plays a protective role against neuronal excitotoxicity, a mechanism described as preconditioning. PI3K pathway as well as NMDA receptor subunits composition and localization have a crucial role in the achievement of a neuroprotective state following preconditioning. We aimed to evaluate the participation of PI3K signaling pathway involved in preventing seizures and cell death induced by quinolinic acid (QA), in the hippocampus of mice (*in vivo*) and the participation of GluN2A or GluN2B subunits in the neuroprotective effect of NMDA preconditioning in hippocampal slices (*in vitro*). Male adult albino Swiss mice were administered with the PI3K inhibitor (wortmannin, 1nmol; i.c.v.). Fifteen minutes later, a subconvulsive dose of NMDA (75 mg/kg, i.p.) or saline (NaCl 0.9 g%; i.p.) was administered and mice were observed for 30 minutes. Seizures were evoked by a chemical stimulus, i.e., the intracerebroventricular QA infusion (36.8 nmol; i.c.v.). Twenty-four hours after QA-induced seizures, Fluoro-Jade histochemistry was used as indicative of neuronal degeneration (N=8). In order to evaluate the participation of GluN2A or GluN2B subunits in NMDA preconditioning, slices (0.4 mm) were obtained and pre-incubated in Krebs Ringer bicarbonate buffer (KRB) for 30 min at 37°C. The preconditioning was induced for 2 hours with 50 μM NMDA/10μM glycine (GluN2B co-agonist) or 50 μM NMDA/30μM D-serine (GluN2A co-agonist) in KRB. The excitotoxic cell damage was induced by incubation of hippocampal slices for 1 hour with glutamate (10 mM) and cell viability was evaluated after 4 hours by MTT reduction assay (N=4). Results showed that inhibition of the PI3K pathway is effective in abolishing the protective effect of preconditioning on QA-induced seizures ($P < 0.05$). However, it did not interfere with the neuronal protection promoted by NMDA preconditioning ($P > 0.05$). NMDA preconditioning with GluN2B co-agonist prevented the loss of cellular viability triggered by glutamate-induced toxicity, whereas NMDA preconditioning with GluN2A co-agonist had no neuroprotective effect ($P < 0.05$). These results indicated that PI3K pathway acts by different mechanisms modulating NMDA preconditioning in prevention of seizures and hippocampal cell death induced by QA. Additionally, the mechanism of achievement of a neuroprotective state following NMDA preconditioning depends on GluN2B subunit containing NMDA receptors.

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Motor deficits, loss of nigral dopaminergic neurons and gliosis in transgenic A53T +/- mice: Role of Na^+-Ca^{2+} exchanger isoforms

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The hypothesis that elevated intracellular calcium concentrations ($[Ca^{2+}]i$) might participate to the degeneration of substantia nigra pars compacta (SNc) neurons has been recently taken in consideration in the pathogenesis of Parkinson's disease (PD), thus suggesting an involvement the Na^+-Ca^{2+} exchanger isoforms (NCXs), the major cellular Ca^{2+} extruding system.
To investigate whether alterations in the activity of NCXs in neurons and microglial cells might correlate with the motor impairment observed in mice expressing human A53T variant of α-synuclein, we evaluated dopamine neuron degeneration, astroglial and microglial activation and the expression of NCX1 and NCX3 in

dopaminergic neurons and glial cells in the SNc and caudate-putamen (CPu) of A53T and wild-type mice of 10/12 months old. Motor performance was evaluated with the beam walking test and correlated to immunohistochemical analysis of tyrosine hydroxylase (TH), glial fibrillary acidic protein (GFAP) (marker of astroglia), ionized calcium binding adaptor molecule-1 (Iba-1) (marker of microglia), and the colocalization of NCXs with TH and Iba-1 in the SNc and CPu. A53T mice showed an increase in the time to traverse the beam as compared to wild-type mice. Moreover, a significant increase of GFAP-positive cells was observed in the SNc and CPu and an increase of Iba-1-positive cells in CPu of A53T mice, as compared with wild-type mice. Furthermore, A53T mice exhibited a significant loss of TH-positive neurons in the SNc and TH-positive fibers in CPu, as compared with wild-type mice. Finally, NCX1 was expressed in Iba-1-positive cells in CPu whereas NCX3 was expressed in TH-positive neurons in SNc of A53T mice.
Results showed that changes in NCXs levels and activity might play a role in mitochondrial dysfunction and correlate to microglial activation, motor impairment and neuronal demise observed in A53T transgenic mice.

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Attenuation of sickness behavior and neuroinflammation and neuroplasticity improvement in the hippocampus of aged mice: The role of exercise training

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Exercise improves mental health and synaptic function in the brain during aging. However, the molecular mechanisms involved in this phenomenon are little understood. The modulation of the repressor element RE1-binding transcription factor (REST) and the inflammatory state by the exercise are possible mechanisms. Objectives: To evaluate the effect of voluntary exercise performed in running wheels (RW) in the sickness behavior and hippocampal neuroplasticity in adult and aged C57BL/6 mice. Material and Methods: C57BL/6 male mice of 4-6 months of age (young) and 19-21 months old (aged) (Ethics Commitee Protocol PP00760) which were divided in four groups: sedentary young (SED-young) and aged (SED-aged), and running wheel young (RW-young) and aged (RW-aged). All animals were isolated for eight weeks and the RW groups (young and aged) had free access to individual RW; while the SED groups had a locked RW. The animals daily distances races were measured by digital odometers. After 8 weeks, they were subjected to open field and tail suspension behavioral tasks, posteriorly euthanized to dissection of the hippocampus. The REST gene expression and BDNF were analyzed by RT-PCR. Results and Discussion: The aged animals exhibit a depressive-like and sickness behavior: less mobility in RW and in the open field, and great immobility in the tail suspension test. Gene expression showed a low profile neuroplasticity and high neuroinflammation in the hippocampus of aged animals. Exercise was anxiolytic and antidepressant, and improved motor behavior of aged animals. Exercise also boosted BDNF and REST expression, and decreased IL-1β and IL-10 expression in the hippocampus of aged animals. These data support a beneficial role of REST in the aged hippocampus, which can be further enhanced by regular exercise.

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Neurotoxic effect of As, Pb, and Mn – mixture developmental exposure: Impaired the learning and memory with NMDA receptor and postsynaptic signaling proteins in hippocampus of rats.

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The neurotoxic metals; arsenic (As), lead (Pb) and manganese (Mn), are a global health concern as they are considered ubiquitous contaminants causing adverse health effects in human. Exposure to these elements, particularly at chronic low dose levels, is still a major public health concern. Concurrent exposure to As, Pb or

Mn may produce additive or synergistic interactions or even new effects different from the single component exposures. The present study has been carried out to investigate the effect of heavy metals exposure from gestational day 5 to postnatal day 60 on the developing rat brain. Wistar rats were treated with three doses of each single metal, Pb (4 mg/Kg bw), As (4 mg/kg), and Mn 10 mg/Kg bw), or the same doses in a triple metal mixture. The learning and memory ability of rats was measured by assessing active and passive avoidance shuttle box, Y maze and water maze. Rats subjected to three doses of each single metal, Pb, As and Mn or the same doses in a triple metal mixture were found to impairment in the learning and memory in comparison to controls. However, no significant difference in the learning and memory was observed in Mn group as compared to controls. On PND60 the expression of NR2B and NR1subunit of NMDA receptor was found to decreased and also their level of mRNA while there is no changes was found in protein expression as well as mRNA level of NR2A subunit of NMDA receptor. Expression of NMDA receptor postsynaptic signaling protein including pCAMKII, pERK, Syngap and pCREB were also impaired due to following exposure to heavy metals as compared to controls. Intensity of these change were more marked in a metal mixture group as compared to controls. The results of the present study exhibit that functional deficits specially in learning - memory heavy metal mixture at low doses may be associated with NMDA receptor and postsynaptic signaling proteins alterations in hippocampus of rats.

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Methylmercury enhances SP1 nuclear localization concurrent with diminished Fyn gene expression in primary rat cortical astrocytes

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Methylmercury (MeHg) is an established developmental neurotoxicant, known to alter cellular growth and survival. Sp1 is a transcription factor that regulates expression of several genes involved in these processes, including the Src family kinase, Fyn. It has been demonstrated that nuclear factor erythroid 2-related factor 2 (Nrf2) can bind Sp1, and reduce its activity, as measured by Tgf-β1 gene expression. It has yet to be determined, however, whether MeHg exposure alters Sp1 activity. Therefore, we immunostained primary rat cortical astrocytes with 5 μM MeHg for 1, 6, or 24 hours (hr) in order to examine Sp1 localization. 5 μM MeHg was used because it elicits a toxic response (1 hr: 85.4 ± 14.1, $p = 0.4$; 6 hr: 76.7 ± 6.7, $p = 0.03$; 24 hr: 42.6 ± 6.0, $p = 0.0007$), and was previously established to augment Nrf2 activity. We found that Sp1 increases in the nucleus at 1 hr (1.88 ± 0.2, $p = 0.009$) and 6 hr (1.78 ± 0.3, $p = 0.05$). Sp1 also appears to increase at 24hr (1.52 ± 0.3, $p = 0.2$; $n = 2$), but additional replicates are required. Contrary to these results, we observed a significant decrease in Tgf-β1 gene expression as assessed by qRT-PCR at 1 hr (0.43 ± 0.1, $p = 0.005$) and 6 hr (0.41 ± 0.1, $p = 0.0002$), but not at 24 hr (0.54 ± 0.2, $p = 0.14$). These data are most likely attributed to Nrf2 binding Sp1, however, further experiments are required to confirm this assumption. Additionally, we determined that Fyn gene expression decreases at 6 hr (0.65 ± 0.1, $p = 0.0004$) and 24 hr (0.39 ± 0.04, $p = 0.0000008$), but not 1 hr (0.77 ± 0.1, $p = 0.09$). Fyn has been demonstrated to phosphorylate and inactivate Nrf2. Thus, by binding Sp1, Nrf2 reduces Fyn expression, and sustains its own activity. Data represent the mean ± SEM of at least three biological replicates.

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Tributyltin (TBT) chronic exposure impairs object recognition memory in female mice

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Tributyltin (TBT) is an organometallic compound, a variety of chemicals used on several areas of industry and agriculture. Recent studies have demonstrated that TBT has effects on human and rodent endocrine systems, reducing the estradiol levels in the blood. It is already known that estrogens have neuroprotective effects in

mammals, acting like antioxidant and antidegenerative factors. Considering this, exposure to TBT may possibly lead to cognitive impairments through alterations in the estradiol levels in the organism. In this study were used 18 Swiss female mice. The animals were divided into 3 groups of 6 individuals each, and kept in an acclimatized room (22°C), under a 12h light/dark cycle. Tributyltin in 0.1% ethanolic solution (Sigma, St. Louis, MO,USA) was administrated intragastricly, during 14 days, in the concentrations of 500ng/kg and 750ng/kg for the groups TBT-500 and TBT-750, respectively. A 0.1% ethanolic solution was administrated for the Control group. The estrous cycle of the animals was observed during the treatment through the analysis in the optic microscope of the vaginal mucus of the animals. For the behavioural component of the project, was utilized the Novel Object Recognition Test. The test was recorded and analyzed by the software ANY-MazeTM. 24 hours after the test, the animals were euthanized through saline intracardiac perfusion, and their brains were collected and frozen. For the statistical analysis was considered the percentage of time spent in the exploration of novel objects in each session, and was utilized the Kruskal-Wallis non-parametrical test, followed by the Dunn's test for multiple comparisons. For all the analysis was considered a significance level of $P < 0,05$. The TBT exposure during 14 days, in the concentrations of 500ng/kg and 750ng/kg caused impairment both in the short and longterm object recognition memory in female mice, in comparison with the Control group. This study indicates that chronic exposure to tributyltin in different concentrations leads to impairments in the recognition memory of mice, evaluated in the Novel Object Recognition Test. Further research is needed to identify the physiological and biochemical basis of tributyltin effects on memory.

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GluN2B-containing NMDAR antagonism reduce short-term neurodegeneration and inflammation induced by early life status epilepticus

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Status epilepticus (SE) when occurred during brain development can cause short- and long-term consequences, which are frequently associated to NMDA receptors-mediated glutamatergic excitotoxicity. NMDA receptors-mediated neurodegeneration have been associated to GluN2B-containing NMDA receptors overactivation. The aim of this study was to investigate the role of GluN2B-containing NMDA receptors on the SE-induced neurodegeneration and inflammation in young rats. Forty-eight Wistar rats (16th postnatal days) were injected with pilocarpine (60 mg/kg; i.p.) 12-18 h after LiCl (3 mEq/kg; i.p.). Fifteen minutes after pilocarpine administration, animals received i.p. injections of vehicle (0.9% NaCl – SE+SAL group), ketamine (25 mg/kg – SE+KET), CP-101,606 (10 mg/kg - SE+CP group) or CI-1041 (10 mg/kg - SE+CI group). Seven days after SE, brains were removed for Fluoro-Jade C staining and Iba1/ED1 immunolabeling. All procedures were approved by the Ethic Committee from Universidade Federal do Rio Grande do Sul (protocol number #21369). Blockage of GluN2B-containing NMDA receptors by CP-101,606 or CI-1041 was not sufficient to stop LiCl-pilocarpine-induced SE. SE+SAL group presents a massive neurodegeneration and Iba1+/ED1+ double-labeling in hippocampus (CA1 and GD) and amygdala (MePV nucleus) 7 days after the insult. Specific GluN1/GluN2B diheteromers antagonism, by CP-101,606, did not alter this pattern. However, GluN2B-containing NMDA receptors antagonism, by CI-1041, was able to reduce the damage in hippocampus and amygdala similarly to the reduction found in animals injected with the non-specific NMDA receptor antagonist, ketamine. Our results indicate that GluN2B are involved in neurodegeneration and microglial recruitment and activation induced by SE, and that stopping epileptic activity are not required to prevent brain damage.
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C elegans as in vivo model for test Quinolinic Acid neurotoxic effects

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The 2,3-Pyridinedicarboxylic acid, widely known as quinolinic acid (QA), is a metabolite of tryptophan degradation in kynurenine pathway, which acts as a NMDA receptor agonist. Within the brain, QA is produced only by microglia and activated macrophages. Furthermore, QA has been described as a potent endogenous neurotoxin, when present at high levels, related to various psychiatric disorders and neurodegenerative processes. The nematode *Caenorhabditis elegans* has a nervous system highly conserved with mammals and thus is an alternative animal model widely used in neurobiology research. However, there is no neurotoxin described that allows the study of glutamatergic system disturbance in *C. elegans*. The aim of this study was verify if QA can induce neurotoxicity in *C. elegans*, due to its action in glutamatergic system. Nematodes from N2 (wild type) and transgenic strains VM487 (nmr-1), VC2623 (nmr-2), TJ356(daf-16::GFP), CL2070 (hsp-16,2::GFP), CL2166 (gst-4::GFP) and CF1553 (sod-3::GFP) at young adult stage were treated in liquid or agar containing QA in different concentrations (5, 10, 20, 50, 100 and 200 mM) or vehicle (M9 buffer) at 20^{o}C for 1 hour. The analyses included evaluation of QA's effects on survival, behavioral parameters (pharynx pumping and locomotion), subcellular DAF-16 localization, reactive species generation and expression of superoxide dismutase 3, glutathione-S-transferase-4, and heat shock protein 16.2. When used at high concentrations (50, 100 and 200 mM) QA can induced and increase in C elegans mortality (~15%), although when used at low concentrations (10 and 20mM) QA altered some behavioral pattern of the nematodes. The QA can leave to an increase in the expression of *hsp-16.2* (~15%) as well as *gst-4* (~40%). However, *sod-3* levels were not significantly different from control group. QA also activated DAF-16/FOXO signaling pathway and increased reactive species levels compared to control group. When used specific strains of glutamatergic system, the increase in reactive species production occur in a *nmr-1*-dependent manner. Our data suggests that QA might be used for neurotoxicological studies on glutamatergic system injuries associated with oxidative stress in *C. elegans*.

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The flavonoid 4,5,7–trihydroxyflavone is a neuroprotective agent against neuroinflamation and dopaminergic degeneration induced by aminochrome

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The main mechanisms responsible for neurodegeneration in Parkinson's disease is still unknown, however, some cellular and molecular disorders are involved in this process: accumulation of α-synuclein, oxidative stress, mitochondrial damage, proteosomal, autophagic dysfunction and neuroinflammation. Among these, only neuroinflammation has not yet been associated with effects induced by aminochrome. This work is mainly focused on neuroinflammatory potential of aminochrome and role of 4,5,7–trihydroxyflavone (apigenin) a well-known immunomodulator flavonoid in reversing this phenotype. Midbrain organotypic cultures from P8 wistar rats were cultivated for 3 days with DMEM/F12, incubated in 5% CO2, at 37 ° C. Slices were treated with aminochrome (0,01 to 25 μM) and/or apigenin (10μM), and analyzed after 24 or 48 hours. Neurotoxicity was evaluated by morphological analysis and expression of tyrosine hydroxylase (TH) by Western blot. The

neuroinflammatory response was assessed by immunohistochemistry for Iba1, RTq-PCR and ELISA essay for cytokines TNF and IL-1β. The modulatory effects of neurotrophic factors were evaluated by RTq-PCR. Our results demonstrate aminochrome induced tissue damage in culture associated to reduction of TH expression, which was inhibited by apigenin. Furthermore, aminochrome induced morphological changes in Iba1+ cells associated to increase of TNF and IL-1β mRNA levels and increase of IL-1β and TNF levels in the supernatant of cultures treated with aminochrome, which were also inhibited by apigenin. Aminochrome reduced mRNA levels of CDNF and NGF but increased BDNF. Apigenin inhibited the effects induced by aminochrome in relation to CDNF and NGF expression. On the other hand, apigenin treatment reduced expression of BDNF and GDNF. These results demonstrated that neuroinflammation and neurotrophic factors are also involved in the neurodegeneration induced aminochrome and apigenin is a neuroprotective agent against cell damage induced by this toxin.

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Hospitalization rates for Parkinson's disease and pesticide use in Brazil

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Pesticide exposure has been implicated in several health problems, including Parkinson's disease (PD). Although pesticide use in Brazil has increased significantly over the last four decades, few studies have evaluated the potential effects of chronic occupational and/or environmental exposure to pesticides on the development of PD. In the current ecological study, we investigated the association between per capita pesticide expenditure and hospitalization rates for PD in 552 micro-regions in Brazil. Pesticide expenditure in 1985 and 1996, used as surrogate measures of pesticide exposure, and PD hospitalization rates (HR) from 1997-2007, stratified by age and sex, were collected for all micro-regions from the Agricultural Censuses and the Brazilian Hospital Information System, respectively. Rural micro-regions were grouped into quintiles according to the per capita pesticide expenditure, and PD hospitalization rates were compared between quintiles, using the first quintile (lowest pesticide expenditure) as a reference group. Spearman correlation tests were also performed to compare pesticide expenditure and PD hospitalizations rates for different gender, age groups, and conglomerates of urban and rural micro regions. Moderate correlations between PD hospitalization rates and pesticide expenditure, especially for individuals ³ 70 living in rural micro-regions were observed. For rural micro-regions, quintiles with higher levels of pesticide expenditure persistently displayed higher PD hospitalizations rates. In agreement with recent studies, our results suggest that pesticide exposure may increase the chance of PD hospitalization in Brazil.

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Elucidation of the molecular signature of Parkinson's disease – Altered regulons, molecular targets and innovative therapeutics interventions

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Parkinson's disease (PD) is among the most prevalent neurodegenerative diseases, with around 10 million people diagnosed globally (WHO). Currently there is no treatment available to halt, prevent or reverse PD, nor a specific differential diagnostic test that can clearly identify the disease before patient death, probably due to its complex and multifactorial characteristics. PD motor symptoms are associated with nigrostriatal degeneration followed by a rostrocaudal progression reaching cortical areas in more advanced stages. Oxidative stress, mitochondrial dysfunction and failure of the protein degradation machinery are the main mechanisms associated with the disease. Though these series of cell alterations are known to be present, the exactly combination of events that trigger the neurodegenerative mechanisms are still unclear. Analysis of microarray data by transcriptional regulatory networks is a promising approach to unravel mechanisms associated to complex diseases. This study aims to elucidate the PD molecular signature based on transcriptional regulatory units and identify transcription factors that can act as master regulators in phenotype disease determination. Transcriptional regulatory units were inferred for the *substantia nigra* and the frontal cortex using normal post-mortem tissue microarray data available in the public repository Gene Expression Ominibus (GEO). Disease molecular signatures centered in altered transcriptional regulatory units were evaluated by analyses of PD case-control studies for the same regions. Three transcription units regulated by ATF2, SLC30A9 and ZFP69B master regulators were found altered in both regions, being these regions affected in different stages of the disease. All these transcription units were shown repressed in disease compared to control. Afterwards, drugs with potential to reverse the molecular signature of PD based on the altered transcriptional units in both regions were searched by connectivity maps approach. Four drugs with therapeutic potential were identified. Proteomic validation of *in silico* results and evaluation of the drugs neuroprotective effect on the PD *in vitro* model established in our lab are being conducted.

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6-Hydroxydopamine decreases brain aldehyde dehydrogenase 2 (ALDH2) expression: Implications for neurotoxicity in a Parkinson´s disease model

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Parkinson's disease (PD) is the second most common neurodegenerative disorder and is characterized pathologically by the loss of dopaminergic neurons in the substantia nigra (SN). Although motor symptoms are the main clinical features of PD, increasing evidence has shown that PD patients also have non-motor symptoms, where cognitive dysfunction is one of the most common and devastating in this neuropathology. Among the different hypothesis related to PD etiology, an abnormal ALDH2 functionality in neurotransmitter degradation that leads to the accumulation of neurotoxic metabolites such as DOPAL and DOPEGAL has been described. These molecules have been associated with neuronal cell death and neurodegeneration. OBJECTIVES: In this study we aimed to evaluate ALDH2 expression and cognitive function in a 6-hydroxydopamine (6-OHDA) animal model of PD. METHODS: Male Wistar rats were bilaterally injected in dorsal striatum (CPu) with either the neurotoxicant (6-OHDA rats) or vehicle (SHAM rats). Twenty days after the lesion the animals were tested for short-term spatial memory with a modified version of Y-maze test. At the end of the study the rats were perfused, their brains fixed and immunohistochemistry performed for TH and ALDH2 in CPu, SN, dorsal hippocampus (CA1) and prefrontal cortex (PFC). All data were compared by Student´s t-test and 2-way ANOVA ($p<0.05$ considered as statistically significant). RESULTS: At the behavioral level we first observed that both groups of rats made a similar ($p>0.05$) number of visits to the two available arms during the training phase indicating no baseline differences between them. During the test session, the results revealed that only the control rats spent significantly more time in the novel arm in comparison to chance level (33% of time) ($p<0.05$), whereas the 6-OHDA-treated rats spent similar time exploring the three arms. The observed differences between groups were unrelated to alterations of locomotion since both groups made a similar ($p>0.05$) total number of entries in all the arms during the test session. At the cellular level, and

as expected, 6-OHDA treatment induced a reduction in TH positive dopaminergic neurons in the brain areas involved in nigrostratial pathway (CPu and SN) ($p<0.05$). Interestingly, we also observed a reduction in ALDH2 expression in 6-OHDA rats in CPu, SN, CA1 and CPF compared to the SHAM rats ($p<0.05$). CONCLUSION: Our results suggest that a reduction in TH immunostaining is related to the cognitive dysfunction that we observed in this experimental animal model of PD. Moreover, the decreased ALDH2 expression may be associated to the neurotoxicity and neurodegeneration characteristic of this model.

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Prenatal hypoxia-ischemia causes loss of retinal ganglion cells followed by pupillary light reflex alteration in rats

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Prenatal hypoxia-ischemia (HI) is one of the main causes of neurodevelopmental impairment in the newborn and is associated with cerebral palsy, attention problems, hyperactivity, epilepsy, and sensory alterations, including visual processing problems. The retina is widely recognized as a neural circuit model used in the study of the development and plasticity of neuronal circuits. Thus, the investigation of the effects of prenatal HI on retinal development offers great potential to elucidate mechanisms related to the effects of HI during pregnancy. Here we studied the effects of prenatal HI on retinal morphology and function. Specifically, we evaluated the number of retinal ganglion cells (RGCs) and the pupillary light reflex (PLR) after prenatal HI of Wistar rats. Prenatal HI was induced by occlusion of uterine arteries for 45 minutes on the eighteenth gestational day (HI group, n = 4 rats). Control animals were obtained from pregnant females submitted to the same surgical procedures except for the occlusion of the uterine arteries (SH group, n = 4 rats). Immunohistochemical labeling of RGCs with Brn3-α was done at postnatal (P) day 2, 9, 23, and 30. PLR was evaluated in a separate group of animals at P30 after two hours of dark adaptation. For all studied ages, there was a significant reduction in the number of RGCs in the HI group compared to control, with exception of P9. While at the onset of the light stimulus both HI and SH animals showed equal pupil constriction times and sizes, HI animal were not able to sustain pupillary constriction under continuous illumination. Sustainability of the PLR is attributed to the activation of intrinsically photosensitive RGCs (ipRGCs). Therefore, our results suggest that prenatal HI could be preferentially eliminating ipRGCs.

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Methylmercury induces oxidative stress and rock-1 activation in primary astroglial cells

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Methylmercury (MeHg) is a highly toxic environmental contaminant that produces neurological and developmental impairments in animals and humans. Although its neurotoxic properties have been widely reported, the molecular mechanisms by which MeHg exerts its toxic effects are not completely understood. The Rho-associated protein kinase 1 (ROCK-1), is a key regulator of actin cytoskeleton and a direct substrate of caspase-3. The activation of ROCK-1 is necessary for membrane blebbing during apoptosis. The present study investigated the hypothesis that the oxidative stress induced by MeHg in astrocytes triggers the activation of the caspase-dependent apoptotic pathway, leading to the cleavage and activation of ROCK-1 and its downstream targets. Primary astrocytes were isolated from cortical tissue of postnatal day-1 C57BL/6 mice. After 2 weeks in culture, the astrocytes were treated with 10 μM MeHg for 6 h or 24 h and two assays for evaluating cell viability (ATP and lactate dehydrogenase (LDH)) were performed. Two fluorescent dyes were used to measure the intracellular and mitochondrial reactive oxygen species formation (CM-H2 DCFDA and CM-H2 XRos, respectively). The intracellular NADH and NAD^+ concentrations were measured with a sensitive cycling assay. To test the hypothesis that MeHg induces the activation of ROCK-1 signaling pathway, we carried out western blotting analysis of the active forms of caspase-9 and caspase-3, ROCK-1, LIMK1 and MYPT1. Our results establish that MeHg induced a significant cellular toxicity only in astrocytes treated with 10 μM MeHg for 24 h. An increase in intracellular and mitochondrial reactive oxygen species levels, as well as a higher NADH/NAD^+ ratio were observed 6 h in response to MeHg treatment. Furthermore, western blotting

analysis revealed activation of both caspase-9 and caspase-3 and a significant increase in ROCK-1 cleavage and LIMK1 and MYPT1 phosphorylation, 6 h after MeHg treatment. Taken together, these findings suggest that the activation of ROCK-1 signaling pathway in astrocytes may play an essential role in MeHg-induced apoptosis.

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Agmatine improves behavioral impairments observed in an animal model of the attention deficit hyperactivity disorder (ADHD)

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Attention-deficit/hyperactivity disorder (ADHD) is a persistent neurodevelopmental disorder that affects 5% of children and adolescents and 2.5% of adults worldwide. This disorder is characterized by inattention and/or hyperactivity–impulsivity symptoms. The association of genetic and environmental factors is related to increased susceptibility to ADHD. The current pharmacological treatments for ADHD are palliative focused in the improvement of behavioral impairments, with many side effects. The current study was conducted to evaluated the effects of agmatine (an endogenous neuromodulator with neuroprotective properties) as therapeutic strategy to improve behavioral and neurochemical impairments observed in an animal model of the ADHD, the spontaneously hypertensive rats (SHR). Adult male inbred SHR (4 months old) were randomly divided in two groups: Control that received saline (NaCl 0,9%, i.p.) and agmatine treatment (30 mg/kg/day, i.p.) during 21 consecutive days (n=9-10/treatment). A battery of behavioral tests was conducted and after that dopamine transporter (DAT) and tyrosine hydroxylase (TH) levels were addressed in the striatum by Western Blot analysis. The agmatine treatment improved the behavioral impairments of SHR displayed in the olfactory discrimination, object recognition and social recognition memory tasks. Agmatine administration did not alter the locomotor activity of SHR in the open field. Regarding the anhedonic-like behavior, agmatine treatment did not alter the grooming time in comparison to respective controls in the splash test. No changes were observed in systolic blood pressure and heart rate. However, agmatine did not alter striatal DAT and TH densities in SHR. This study provides the first evidence of the beneficial effects of agmatine on behavioral impairments observed in an animal model of the ADHD. All procedures of the present study were approved by the Ethics Committee of the Federal University of Santa Catarina (PP0830).

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Evaluation of neurochemical, inflammatory and behavioral parameters in hyperphenylananic female rats

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Phenylketonuria (PKU) is an autosomal recessive inborn error of amino acid L-phenylalanine (Phe) metabolism caused by deficiency of phenylalanine hydroxylase (PAH) activity. Biochemically, it is characterized by hyperphenylalaninemia (HPA). Clinically, patients present psychomotor impairment and severe intellectual disability. The pathogenesis of brain alterations related to PKU is based on the neurotoxicity exerted by HPA, which is not completely uderrstood. In this context, the aim of this study was to evaluate neurochemical, inflammatory and behavioral parameters in rats submitted to an experimental HPA model. For this, five-day-old

female Wistar rats received 2 daily subcutaneous administrations of Phe (5,2 µmol/g; 12 hours interval between administrations) and daily subcutaneous administration of p-chlorophenylalanine (0,9 µmol/g), a PAH inhibitor, from the 5th to the 30th day of life. Control goup received saline solution under the same conditions. Twenty-four hours after the last administration, behavioral tasks (open field and radial maze) were evaluated. Immediately after, animals were euthanized and cerebral cortex, hippocampus and striatum were dissected and used for the determination of brain derived neurotrofic fator (BDNF), interleukin (IL)-1beta (IL-1β), IL-6, IL-10 and tumoral necrosis fator alpha (TNFα) levels and synaptophysin immunocontent. It was observed that HPA caused cognitive déficit in animals in the open field test, without any alteration the radial maze. It was also found a decrease in immunocontent of synaptophysin, a marker of synaptic integrity, and increased levels of the IL-6 (proinflammatory interleukin) and IL-10 (anti-inflammatory interleukin) only in cerebral cortex of hyperphenylalaninemic animals. On the other hand, IL-1β, TNFα and BDNF levels were not altered. The present study demonstrated that chronic HPA caused cognitive damage, which could be explained by synaptic changes and neuroinflammation. These results may contribute to the understanding of the pathological mechanisms of cognitive damage observed in phenylketonuric patients.

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Prenatal infection affects inhibitory synaptic function and plasticity: Role of endocannabinoids

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Schizophrenia is one of the neurodevelopmental disorders which is sensitive to early insults to the central nervous system. Epidemiological studies have shown a clear association between maternal infection and schizophrenia. Parallel studies in rodents that employed prenatal infections have revealed maternal immune activation (mIA) to be a profound risk factor for neurochemical and behavioral abnormalities in the offspring. In this study, we used offspring from rat dams administered bacterial lipopolysaccharide (LPS) during pregnancy (gestational days 15 and 16) as an animal model that expressing schizophrenia-related behavioral phenotypes, and we investigated the effect of prenatal infection on metabotropic glutamate receptors (mGluR) dependent synaptic function in dorsal and ventral hippocampal inhibitory synapses from adolescent male offspring and the role of endocannabinoid. We firstly examined the effect of maternal infection on the endocannabinoid system. Western blot analysis results showed that there was no difference in expression of CB1 receptor, MAGL and FAAH in dorsal and ventral hippocampi between prenatal saline and LPS offspring. However, DAGL expression was significantly higher in prenatal LPS group than in saline group. Autoradiography results revealed no difference in CB1 receptor activity in dorsal hippocampus between prenatal saline and LPS offspring. In contrast, CB1 receptor activity was significantly enhanced in ventral hippocampus from prenatal LPS offspring compared with prenatal saline offspring. MGAL activity was higher in LPS group than that in saline group in both dorsal and ventral hippocampus. Electrophysiological results showed that bath application of mGluR agonist DHPG can induce long term depression in both saline and LPS offspring. However, CB1R-selective antagonist AM251 with the concentration of 2 µM completely blocked DHPG-induced LTD in prenatal saline group but failed to block LTD in prenatal LPS group. When applied 5 µM AM251, DHPG-induced LTD was blocked in prenatal LPS group. In contrast, DHPG did not induce LTD in slices of the ventral hippocampus in prenatal saline group, while it can depress synaptic transmission in prenatal LPS group. We confirmed that the DHPG-induced LTD was mediated by CB1 receptor. We also compared DSI in slices of dorsal and ventral hippocampus from both groups. Our results showed that there is no difference in the maximum level of DSI between prenatal saline and LPS group. The amplitude of IPSCs 60s after depolarization was significantly lower in dorsal hippocampus from LPS group compared with saline group. In prenatal LPS offspring the depressed IPSC took longer time to recover to baseline compared with prenatal saline animal. However, we found no differences in these parameters in slices from ventral hippocampus from prenatal saline and LPS animals. We inferred that 2-AG release might be higher in prenatal LPS group, which result in the impaired synaptic transmission in dorsal and ventral hippocampus. These results showed that prenatal infection can

affect inhibitory synaptic function in dorsal and ventral hippocampus and this might be associated with endocannabinoid system.

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Cognitive impairment in an experimental model of Parkinson´s Disease

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BACKGROUND: Parkinson´s disease is a neurodegenerative disorder that results from a progressive dopaminergic neuronal loss. It is considered a multifactorial condition due to the multiplicity of symptoms experienced by patients. While this condition is known for its characteristic motor deficits, patients also have wide variety non-motor symptoms among them, impaired learning and memory deficits which severely affect their quality of life. These non-motor symptoms result from the dysfunction of interconnected systems, including the striatum, the neocortex and the hippocampus. OBJECTIVES: To determine, in an experimental model of the neuropathology, the progression of working memory deficits and its correlation with the loss of dopaminergic neurons. METHODS: Adult male Wistar rats were stereotaxically injected with the neurotoxin 6OHDA in dorsal lateral striatum (DLS) or with the control solution of ascorbic saline. Independent groups of animals (12-15 rats per group) were tested only once in the behavioral tasks after 7, 14, 20 and 28 days. A group of animals were tested in the working memory task Y-maze and motor function was characterized using locomotor activity with amphetamine administration, stick and hot plate tests. After that, the rats were perfused and their brain processed for immunohistochemical of tyrosine hydroxylase (TH) in the substantia nigra (SN), DLS, dorsal hippocampus (CA1) and prefrontal cortex (PFC). RESULTS: Working memory deficits were observed in 6OHDA rats compared to Vehicle rats after 20 and 28 days of neurodegeneration ($p<0.05$). By the other hand, motor deficits were increased at 28 days after lesion in 6OHDA ($p<0.05$) and we discarded these rats for cognitive performance consideration. At 7 and 15 days post lesion there were no significant changes in any behavioral task ($p>0.05$). In parallel, we observed that this early memory impairment occurring at a premotor stage of PD is associated with a partial lesion of the nigrostriatal dopaminergic system. CONCLUSION: 1) bilateral intra-DLS injection of 6-OHDA was sufficient to cause working memory impairments without locomotor alterations 2) Knowledge of this neurodegenerative progression could result in potential new therapeutic strategies, which motivates us to further studies under this experimental model.

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Repeated 5-day administration of L-BMAA, microcystin-LR, or as mixture, in adult C57BL/6 mice - lack of adverse cognitive effects

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The cyanobacterial toxins β-methylamino-$_L$-alanine (L-BMAA) and microcystin-LR (MC-LR) are suspected to cause human developmental and degenerative neurological diseases. MC-LR is in addition a potent liver toxin. Here, male adult C57BL/6JOlaHsd mice (ex-breeders) aged approximately 11 months were subcutaneously injected for five consecutive days with L-BMAA and microcystin-LR alone, or in combination. A dose-range study determined a tolerable daily dose of ~31 µg MC-LR/day/kg BW. The L-BMAA (not acute toxic) dose and latency time prior to behavioral testing was based upon published results from others. Thus, the mice were given 30 µg MC-LR/kg BW and/or 30 mg L-BMAA/kg BW, either alone or in mixture for five consecutive days (cumulative doses were 150 µg MC-LR/kg BW, 150 mg L-BMAA/kg BW, or 150 µg MC-LR +150 mg L-BMAA/kg BW). Initially, LC-MR exposed mice rapidly lost up to ~5% body weight, but regained weight from day 8 and onwards. After 4 weeks, spatial learning and memory performance of exposed mice was compared to controls using a Barnes maze with video tracking during three days. After 8 weeks, anxiety, general

locomotor activity, willingness to explore, hippocampal and peri-postrhinal cortex dependent memory was investigated using Open Field combined with Novel Location/Novel Object Recognition tests. The mice were also re-tested for long-term memory effects 10 weeks after exposure using the Barnes maze. Several parameters were evaluated and will be presented.

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The effects of early environmental enrichment and PACAP in aging rat model of Parkinson's disease

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The causative therapy of Parkinson's disease (PD) is still under investigation. One of the well-studied effects of enriched environment and pituitary adenylate cyclase-activating polypeptide (PACAP) is the strong neuroprotective effect. We have previously described the neuroprotective effects of PACAP and postnatal enriched environment in Parkinson's disease in young animals. The aim of our present study is to investigate the protective effects of these factors in aging (12-18-months-old) rats after unilateral 6-OHDA-induced lesion of the substantia nigra (s.n.). Wistar rats were used in our experiment (n=35). Animals were divided into standard (n=17) and enriched groups (n=18).Animals of the standard group were placed under regular conditions. For environmental enrichment, during the first five postnatal weeks we placed pups in larger cages supplemented with toys, objects, running tunnels and rotating rods of different shape, size and material.In aging animals PD was induced by unilateral injections of 6-OHDA (2 µl,5 µg/µl) into the left substantia nigra, control animals received 2 µl physiological saline Following the 6-OHDA injections half of the animals received 2 µl (1µg/µl) PACAP treatment into the s.n.. On the 7th postoperative day brain of the animals were removed and samples of the substantia nigra were collected. Dopamine levels and DJ-1 protein content of the substantia nigra were measured by HPLC-Q Exactive orbitrap MS system and ELISA method, respectively. The substantia nigra of the 6-OHDA-treated standard and enriched animals showed significantly lower DA levels compared to the saline-treated animals of the same groups. Consistent with our previous studies in young animals the PACAP treatment could also increase the DA levels after 6-OHDA-induced lesion in aging rats. Also, the DJ-1 protein content of the substantia nigra was significantly higher in groups receiving PACAP treatment following the lesion. However, early environmental enrichment did not have any protective effects in this experiment. Although the protective effect of early postnatal environmental enrichment is described in young animals, we could not prove it in aging animals. However, similarly to younger animals PACAP could restore the decrease of DA and DJ-1 protein levels, which could play a role in its neuroprotective effect in Parkinson's disease.

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Effects of postnatal enriched environment in a model of Parkinson's disease in adult rats

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Environmental enrichment is a widespread neuroprotective strategy during development and also in the mature nervous system. Several research groups have described that enriched environment in adult rats has an

impact on the progression of Parkinson's disease (PD). The aim of our present study was to examine the effects of early, postnatal environmental enrichment after 6-OHDA-induced lesion of the substantia nigra in adulthood. Newborn Wistar rats (n=29) were divided into control (n=16) and enriched (n=13) groups according to their environmental conditions. For environmental enrichment, during the first five postnatal weeks animals were placed in larger cages and exposed to intensive complex stimuli such as toys, objects, running tunnels and rotating rods of different shape, size and material. In 3-month-old rats dopaminergic cell loss, postoperative hypokinetic and asymmetrical motor signs were evaluated after inducing PD with unilateral injections of 6-OHDA (2 µl 6-OHDA,5 µg/µl) into the left substantia nigra. Treatment with 6-OHDA led to a significant cell loss in the substantia nigra of control animals, however, postnatal enriched circumstances could rescue the dopaminergic cells. Although there was no significant difference in the percentage of surviving cells between 6-OHDA-treated control and enriched groups, the slightly less dopaminergic cell loss in the enriched group resulted in less severe hypokinesia. In case of the number of free rearings we found reduced rearing activity after 6-OHDA injections in both groups. However, enriched animals showed a recovery on the tenth postoperative day after the acute decrease on the first day. Regarding the distance taken, enriched animals performed better, as there was no significant impairment in their movement. In summary our present results are the first to provide evidence for the neuroprotective effect of early, postnatal enriched environment in Parkinson's disease in adult rats.

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Protein SUMOylation in models of Parkinson's disease

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Protein SUMOylation (SUMO: small ubiquitin-like modifier) is a post-translational modification that regulates the function and subcellular localization of several proteins. Recent studies suggest that protein SUMOylation can interfere with mitochondrial dynamics. Impaired mitochondrial function has emerged as a central feature in the pathogenesis of Parkinson's disease and a number of key proteins associated with PD are modified by SUMO. We are currently investigating the SUMOylation profile in *in vitro* and *in vivo* models of PD with a focus on proteins related to mitochondrial fission-fusion pathways, such as dynamin-related protein 1 (Drp1). The results of this work will provide valuable information about whether drugs that interfere with disease-modified SUMO targets could be beneficial for PD patients.

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NOVEL non-estrogenic endpoints of phenolic metabolites toxicity in fish: Using zebrafish as a model for study

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Evidence of the severe effects of phenolic metabolites on different fish species exists in a number of axes including endocrine and non-endocrine as well. Regardless the classical issues of such metabolites as

endocrine disrupting chemicals, novel non-estrogenic points are poorly studied. This article demonstrates the non-estrogenic attribution of certain phenolic metabolites in fish species. Confirming this non-estrogenic action of such compounds, zebrafish (*Danio rerio*) embryos were subjected to different doses of Polybrominated diphenyl ethers (BDEs) to study the effect of such class of chemicals on the fish embryogenesis during the first 120 hours of fertilization.

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Assessing the role of the intermediate filament (nanofilament) protein nestin in poststroke plasticity

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Stroke is a devastating disease that affects millions of people every year, being one of the leading causes of death worldwide and permanent disabilities. The mechanisms by which stroke leads to neurological deterioration are not fully understood; however, key roles have been ascribed to glutamate excitotoxicity, brain energy failure and inflammation, which ultimately result in neural cell death. The only available treatment for ischemic stroke is thrombolysis, for which a small part of patients is eligible. Novel treatment strategies for stroke aim at promoting neuronal survival and enhancing brain plasticity and for that reason modulation of astrocytes emerge as a new potential strategy. It has been demonstrated that the astrocyte intermediate filament (IF, nanofilament) system provides neuroprotective role in different neurologic diseases, such as neurotrauma or stroke. The IF nestin is unregulated in a subpopulation of astrocytes during reactive gliosis, one of the hallmarks of the stroke lesions. Therefore, the aim of this study was to investigate the role of nestin in post- stroke plasticity. Nestin deficient (Nes-/-) and their wild-type controls (Nes+/+) underwent photothrombotic stroke lesions induced by Rose Bengal technique. The target brain area was the motor cortex at -1.5 ML relative to Bregma, inducing impairment in the right fore paw. Mice were monitored during 3 to 7 weeks after stroke and underwent grid walk and cylinder behavior tests aiming at evaluating motor recovery. Brains were collected for analysis of infarct volume and markers of brain plasticity and glia responses after stroke. No difference in infarct volume was observed between Nes-/- and Nes+/+ mice either at 3 or 7 weeks after stroke. In addition, both groups of animals had complete motor recovery when evaluated by the grid walk test at 7 weeks after stroke. Nes+/+ and Nes-/- exhibited comparable responses in the expression of GAP43 and synaptophysin in the ipsilesional hemisphere, astrocyte activation (GFAP immunostaining), and microglia density at the site of injury (Iba-1 immunostaining). In conclusion, our results suggest that nestin deficient mice when exposed to ischemic stroke exhibit no differences in motor recovery or post-stroke brain plasticity.

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Correlation between oxide nitric synthase and catalase in neonatal brain exposed to perinatal asphyxia

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Catalase is an antioxidant enzyme that matures in parallel with brain development, showing lower levels in brain than in peripheral tissues. Hypoxia-ischemia can produce H_2O_2 accumulation, implying the action of catalase. The aim of the present study was to determine the effect of perinatal asphyxia (PA) on protein and catalase activity (luminescence), comparing the expression of inducible (iNOS) and neuronal Nitric Oxide synthase (nNOS) in different brain regions of control and asphyxia-exposed animals assayed by Western blots. Perinatal asphyxia was induced in rat according to Herrera-Marschitz *et al*. (2014), sampling brain at 1, 3, 7 and 14 postnatal (P) days. Data were analysed with multiple ANOVA and

Spearman's correlation, defining the $P<0.05$ as the level for statistically significant differences. Western blots experiments showed a lack of effect of PA on protein catalase levels, but a decrease of catalase activity in mesencephalon and hippocampus, evaluated at P1-14. No changes were observed in telencephalon, but the expression of iNOS and nNOS was progressively increased by PA in all regions along the evaluated postnatal days. In conclusion, the present study shows that PA induces a decrease of catalase activity and an increase of iNOS and nNOS levels, suggesting a sustained oxidative stress afftecting brain development.

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Persisting effects of separate and combined nicotine and benzo-a-pyrene exposure during gestation on motor, emotional and cognitive functions in rats

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Tobacco smoke is a complex mixture containing thousands of different chemicals. Maternal tobacco smoking has been found to be associated with impaired neurobehavioral development in their children. Tobacco smoke extract has been shown in our earlier studies to cause persisting hyperactivity and cognitive impairment in rats. The primary psychoactive compound in tobacco smoke is nicotine. Nicotine has been shown in rats to cause cognitive impairment as well. There are a variety bioactive compounds in tobacco in addition to nicotine. Prominent among these are polyaromatic hydrocarbons (PAHs). The prototypic PAH is benzo-a-pyrene (BaP). Developmental BaP exposure has been shown to cause persisting neurobehavioral impairment in rats. The current study was conducted to characterize the individual and combined neurotoxicity of nicotine and BaP. Female Sprague-Dawley rats were implanted with osmotic minipumps delivering nicotine (2 mg/kg/day), BaP (0.03 mg/kg/day), both or neither. The male and female offspring were assessed in a behavioral test battery including tests of locomotor activity, as well as emotional and cognitive function. BaP exposure caused significant locomotor hyperactivity in male, but not female offspring when they were adolescents. Gestational BaP exposure eliminated the normal sex difference in locomotor activity. Gestational BaP exposure also eliminated the normal sex difference in fear response on the novel environment suppression of feeding. Gestational exposure of rats to low-doses of BaP as well as nicotine, two constituents of tobacco smoke, cause lasting neurobehavioral effects particularly in male offspring, which diminish normal differences between males and females.

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Impairment of spatial working memory and biochemical changes induced by direct crack-cocaine inhalation and cocaine pyrolysis product, anhydroecgonine methyl ester (AEME)

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Crack-cocaine dependence has become a public health problem in Brazil. During inhalation of crack, cocaine heating generates anhydroecgonine methyl ester (AEME). The effects of AEME and crack-cocaine on cognitive function and oxidative stress was not studied yet. The objective of this study was to investigate the effects of direct crack-cocaine inhalation or AEME on spatial working memory and parameters of oxidative stress. Crack-cocaine experiment: 25 male Wistar rats well trained in 8-arm radial maze (8-RM) were

submitted to direct crack-cocaine inhalation (3 g) for five minutes, once a day, along five consecutive days, or simulated inhalation (sham group). 24-h after the last exposure to crack-cocaine or sham, animals were submitted to 1-h delayed tasks in 8-RM. At the end of experiment animals were submitted to biochemical assays. AEME experiment: 18 male Wistar rats, previously trained in 8-RM, received acute intracerebroventricular (icv) administrations of AEME at doses of 10, 32 or 100 µg, 5 min before 1-h delayed tasks in 8-RM. Saline (SAL) was used as a control solution. For biochemical assays 20 animals received an acute intracerebroventricular (icv) administrations of AEME at doses of 10, 32, 100 µg or saline. Prefrontal cortex (PFC), striatum (STT) and hippocampus (HPC) were removed for biochemical assays: advanced oxidation protein products (AOPP), thiobarbituric acid-reactive species (TBA-RS) and activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Animals submitted to crack-cocaine inhalation made more errors ($p<0.01$) on spatial working memory when compared to sham group. Animals submitted to crack-cocaine inhalation showed increase in levels of AOPP ($p<0.001$) and GPx ($p < 0.05$) in STT and decrease in levels of TBA-RS in HPC ($p<0.05$). Animals that received AEME at doses of 32 µg ($p<0.05$) and 100 µg ($p< 0.05$) made more errors of spatial working memory in 8-RM when compared to control group (SAL). Acute AEME administration at a dose of 100 µg showed increases in levels of AOPP in STT when compared with SAL ($p < 0.01$) and 10 µg ($p<0.05$). Direct crack-cocaine inhalation and AEME at doses of 32 µg and 100 µg impairs long termed spatial working memory of rats in 8-RM and induced oxidative stress in STT of rats by analysis of AOPP. As result of a compensatory mechanism, there was a decrease in levels of TBA-RS in HPC of animals submitted to direct crack-cocaine inhalation.

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Molecular mechanisms of neurotoxicity induced by chlorpyrifos and chlorpyrifos-oxon in HT22 cells

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Pesticides are compounds widely used in agriculture and responsible for innumerable cases of intoxication in humans. Exposure to these compounds occurs during their application, but also by contact with crops treated with these compounds, ingestion of contaminated food or water, as well as intentional exposures to suicide attempts. It is noted that the main cases of intoxication occur due to exposure to organophosphorus pesticides (OFs). Among them, we can highlight chlorpyrifos (CPF), one of the most used in Brazil, mainly in the agricultural environment. CPF is a lipophilic compound that, when metabolized, generates a toxic and more potent active metabolite, chlorpyrifos-oxon (CPF-O). The main mechanism of toxicity of these OFs compounds is related to the inhibition of the enzyme acetylcholinesterase (AChE), triggering a variety of signs and symptoms that characterize the "cholinergic syndrome". On the other hand, some evidence indicate that acute or chronic intoxication with OFs may lead to toxic effects that are not only attributed to inhibition of the AChE enzyme (so-called non-cholinergic effects). However, little is known about the molecular mechanisms related to non-cholinergic neurotoxicity induced by OFs. In this context, the aim of this study was to identify possible non-cholinergic events involved in the neurotoxicity of the CPF pesticide and oxon metabolite in cell line of hippocampal neurons of mice (HT22). Exposures to the CPF pesticide and metabolite CPF-O were able to decrease the cell viability of HT22 cells in a concentration-dependent manner. This cytotoxicity was not protected by atropine (muscarinic antagonist) and pralidoxime (AChE reactivator), indicating that non-cholinergic effects could be involved in the mechanism of toxicity of these compounds. In addition, exposure of HT22 cells to CPF and CPF-O induced reduction of glutathione (GSH) levels and GSK3β inactivation, but only CPF was able to activate MAPKs, ERK1/2 and $p38^{MAPK}$. The use of ERK1/2 and $p38^{MAPK}$ inhibitors did not protect HT22 cells from CPF and CPF-O induced cytotoxicity. On the other hand, the pre-treatment with N-acetylcysteine showed protective effect against this pesticide-induced cytotoxicity. Taken together, our results suggest the involvement of oxidative stress in the mechanism of toxicity of these compounds, as well as the MAPKs activation and GSK3β inhibition as alternative events to cholinergic overstimulation.

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Influence of early life NMDAR antagonism and early life GluN2B-containing NMDAR antagonism on adult behavior: Interaction with rearing environments

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Although early development NMDA receptor (NMDAR) signaling disruption can cause long-term behavioral abnormalities with relevance to schizophrenia, the NMDAR subunits involved in this neuronal disarangement it is still unkown. Brain and cognitive reserve (BCR) stimulation positively modulates brain disorders susceptibility and age-dependent dysfunctions, via neuroprotective and/or compensatory mechanisms. Here, we studied if neonatal GluN2B-containing NMDAR blockade or neonatal full NMDAR population blockade causes similar long-lasting behavioral abnormalities and if these behavioral changes are prevented by BCR stimulation during prenatal and early postnatal periods. Pregnant female Wistar rats were kept in standard (SH; N=9) or enriched housing (EH; N=11). From PND5-10 pups received two daily i.p. injections of saline 0.9% (SAL), ketamine (25 mg/kg - KET) or CI-1041 (10 mg/kg – CI). At PND30, all pups were kept in standard housing. Elevated plus maze (EPM), open field (OF) and inhibitory avoidance tasks were conducted from PND60-66. Data were analyzed by Principal Component Analysis with Varimax Rotation and Kaizer Normalization. Principal Component scores, which were extracted by regression method, were then analyzed by Two-way ANOVA followed by Sidak's multiple comparisons post hoc test. Although full NMDAR population blockade did not induce any behavioral alteration when compared to SAL groups, rats from SH+CI group presented better habituation performance than SH+SAL and SH+KET groups. Maintaining animals in EH reversed this effect. Also, EH animals presented reduced locomotion in the EPM related to decreased exposure to potentially unsafe places as the environment becomes familiar and reduced neophobic response to alternate between two distinct environments. Our results indicate that different neonatal NMDAR populations blockade lead to distinct expression of behavior during adulthood. Our data gives further support to the concept of BCR stimulation promoting neuroplastic changes in the brain and reinforce the need of using tools that allow the identification of subtle alterations in behavioral patterns of rodents.
Keywords: schizophrenia, ketamine, CI-1041, environmental enrichment, development, behavior.

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Triton WR-1339 exerts cellular impairment and depressive-like behavior

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Triton WR-1339 is a nonionic detergent that prevents catabolism of triacylglycerol-rich lipoproteins by Liprotein Lipase and is commonly used for the induction of dyslipidemia in rodents. Recent studies have pointed to the correlation between dyslipidemia and Central Nervous System disorders such as depression. Depression is a mood disorder characterized by emotional as well as pathological changes in regions such as hippocampus and frontal cortex. In this context, the current study was conducted to determine if the dyslipidemia induced by Triton WR-1339 is able to alter hippocampus cell viability as well as animal behavior. Male adult Swiss mice (N=10/treatment) were treated with Triton WR-1339 (200 – 400 mg/kg) and after 24h they were submitted to Tail Suspension Test (TST) and Open Field Test (OFT) in order to achieve depressive-like behavior. After behavioral task, animals were killed by decapitation, hippocampus was dissected and maintained in Krebs Ringer Bicarbonate (KRB in composition:122 mM NaCl, 3 mM KCl, 1.2 mM MgSO4, 1.3 mM CaCl2, 0.4 mM KH2PO4, 25 mM, NaHCO3, and 10 mM D-glucose) buffered medium. The buffer was bubbled with 95% O2– 5% CO2 up to pH 7.4. Slices (0.4 mm thick) were rapidly prepared using a McIlwain Tissue Chopper, separated in KRB at 4 °C, and allowed to recover for 30 min in KRB at 37 °C to afford stabilization. After the stabilization period, cellular viability was determined by MTT assay (0.5 mg/ml, in KRB for 30 min at 37 °C). The Lipid peroxidation was assessed by Thiobarbituric acid reactive substances (TBARs) assay. Comparisons among groups were performed by one-way analysis of variance (ANOVA) followed by Tukey post hoc test. P <

0.05 was considered as statistically significant. Triton WR-1339 at the 200 mg/kg dose significantly impaired hippocampus cell viability and increased lipid peroxidation. At the same dose and treatment schedule, Triton WR-1339 increased immobility time of animals in the TST, without any locomotor alteration. These results point for the cellular impairment as well as depressive-like behavior promoted by Triton WR-1339.

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Guanosine prevents olfactory impairment and mitochondrial disruption induced by intranasal MPTP infusion

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Parkinson's disease (PD) is a neurodegenerative disorder that affects approximately 1% of the population older than 50 years. Some evidence suggest that areas in the central nervous system (CNS) processing olfactory information are affected at the early stages of PD, even before the development of classical motor symptoms. The intranasal MPTP infusion induces impairment in olfactory, emotional, cognitive and motor functions. This protocol of MPTP administration is associated with a time-dependent disruption of dopaminergic neurotransmission in different brain structures analogous to those observed in different stages of PD. In this study, we investigated the effects of the neuroprotective nucleoside guanosine (GUO) treatment (7.5 mg/kg i.p., daily for 20 days) in a temporal evaluation of behavioral tasks (n=8-10) and biochemical assays (n=5) after a single intranasal administration of MPTP (1 mg/nostril). MPTP infusion induced olfactory discrimination impairment (3 days) and mitochondrial membrane potential disruption in olfactory bulb (21 days). However, no alteration were observed in social recognition test (7 days), splash test (9 days), Morris water maze in a procedure memory version (12-15 days), forced swimming test (17-18 days), and motor impairment in open field test (20 days). Additionally, biochemical analyses were performed in slices of olfactory bulb, cortex, striatum and hippocampus of rats 21-days after MPTP. GUO prevented olfactory discrimination impairment and mitochondrial membrane potential disruption at 3 and 21 days after MPTP infusion, respectively. In summary, these results provide the first evidence of the neuroprotective effects of GUO on MPTP-induced impairment in a rodent model of non-motor symptoms associated with PD.

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Intranasal administration of sodium dimethyldithiocarbamate induces persistent motor deficits related to Parkinson's disease in mice that are prevented by melatonin treatment

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The fungicide zinc dimethyldithiocarbamate (ziram) exposure has been recently related to the development of Parkinson's disease (PD). The aim of the present study was to evaluate the of behavioral and biochemical alterations in mice submitted to repeated intranasal (i.n.) administration of sodium dimethyldithiocarbamate (NaDMDC), a more soluble salt of dimethyldithiocarbamate and the putative neuroprotective effects of melatonin treatment. Male Swiss mice (4 months-old) were anaesthetized with isoflurane (0.96%), and subject to i.n. administration of NaDMDC (1 mg/nostril/day) or vehicle (saline, 10 µL/nostril/day) for 4 consecutive days. Mice were evaluated up to 35 days after NaDMDC administration using body weight and motor deficit measurements. Animals were submitted to behavioral tests including Neurological Score of Severity (NSS) (at 2, 6, 13, 20, 27 and 34 days), open field (OF) (at 4 and 33 days), and rotarod (at 7, 14, 21, 28 and 35 days). In another set of experiments, animals were euthanized at 2 or 24 h after the last NaDMDC administration, and samples of striatum and olfactory bulb (OB) were sliced and incubated with propidium iodide and 2',7'-dichlorodihydrofluorescein diacetate for cell viability and reactive oxygen species (ROS) measurements,

respectively. We also evaluated the effects of pretreatment with melatonin (30 mg/kg, intraperitoneally) 1 h before each NaDMDC administration on behavioral parameters. NaDMDC administration was able to induced body weight loss and locomotor deficits, as indicated by a significant ($p<0.05$) increase in the NSS, decreased total distance traveled in OF, and decreased latency to fall in the rotarod, at all times evaluated. ROS levels were significantly increased in the OB at 2 h, and in the striatum at 2 and 24 h after the last NaDMDC administration. In addition, cell viability was reduced in striatum at 2 h after the last NaDMDC administration. In addition, the pretreatment with melatonin, a potent antioxidant molecule, significantly prevented the behavioral alterations and weight loss induced by NaDMDC. These findings indicate for the first time the existence of behavioral and neurochemical impairments in mice infused intranasally with NaDMDC and the protective effect of melatonin.

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Short-time forebrain glutamate uptake impairment related to reduction of GFAP expressing cells after kainic acid induced epileptic seizures in adult zebrafish

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Astrocytes are fundamental for brain excitatory control. Glutamate is involved in *Status Epilepticus* (*SE*) initiation and termination, as well as postictal brain injury. Across time changes of animal behavior, GFAP positive cells, S100B and glutamate uptake activity are studied within seven days after epileptic seizure. To increase the translational impact of zebrafish kainic acid epileptic seizure protocol, behavior and neurochemical analysis after *SE* must be explored. The aim of this study was to identify a potential alterations from behavioral and biochemical astrocytic aspects after epileptic seizure induction. The time for the animals return to general aspects of locomotion similar to control fish was evaluated until 7 days after *SE*. Brain GFAP and intracellular S100B were analyzed 3 h, 12 h and 72 h after *SE*. At last, glutamate uptake was evaluated in different structures until 72 h after kainic acid administration. The results indicate lethargy of zebrafish swimming until 72 h after *SE*, reduced GFAP positive cells 12 h after *SE*, reduced intracellular S100B 12 h and 72 h after *SE*, and reduced glutamate uptake in forebrain 3h and 12 h after *SE*. Forebrain region of adult zebrafish after *status epilepticus* induced by kainic acid recapitulates neurochemical alterations of hippocampus in rodent model of *status epilepticus* regarding main aspects of glutamatergic system. There is a clear and critical time window to explore this model in neurochemical changes until 72 h after *SE* similar to rodent and mammalian condition, as well as a potential window to behavioral analysis 96 h after *SE*.

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Brain metabolic preference shifts under prolonged epileptic seizure episodes in adult zebrafish pentylenetetrazole model

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Altered brain metabolism is a key point of epilepsy. Even though prolonged epileptic seizure is a critical moment of energy demand, there are evidences which suggest glucose brain concentration reduction. Therefore, others energy substrates may be used for energy supply this event. The aim of this study was to

identify the brain energy contribution of L-glutamate, L-glutamine, β-hydroxybutyrate and L-lactate under prolonged epileptic seizure. Adult zebrafish brain was submitted to high-resolution respirometry technic (Oroboros®) after 2.5, 5, and 20 min of 10 mM pentylenetetrazole fish immersion, as well as 1 and 3 h after exposure of the animals. Optimal oligomycin, decoupler (FCCP) and cyanide concentrations were prior characterized to obtain ATP synthesis, maximal respiration, and extramitochondrial O2 consumption, respectively. L-Glutamate (100 µM and 1mM), L-glutamine (100 and 500 µM), β-hydroxybutyrate (0.1 and 0.7 mM), and L-lactate (1 and 10 mM) were individually added to the Oroboros® chamber in presence of glucose 5.5 mM in control and treated animals (20 min of pentylenetetrazole exposure). Optimal oligomycin, FCCP and cyanide chamber concentrations were 4 µg/ml, 0.25 µM and 1mM, respectively. Brain samples of zebrafish immersed by 20 min in pentylenetetrazole 10 mM presented glucose utilization reduction coupled to O2 consumption to ATP synthesis (8.283 ± 0.8457 to 2.298 ± 1.461) ($p \leq 0.05$). Brain samples of animals induced to prolonged seizure exposed to glutamate 100 µM and 1mM, or glutamine 500 µM in the presence of glucose 5.5 mM presented no difference with control O2 consumption to ATP production. Our data indicate a shift from brain glucose metabolism preference to other downstream metabolic sources, which might be used to supply prolonged epileptic seizure episodes.

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Neurotoxicity in rats exposed to exhaust emissions from biodiesel fuels – The FuelHealth project

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Road traffic is the most important source of local air pollution and accounts for about 20% of greenhouse gas emissions in Norway. Introducing carbon-neutral alternatives to conventional fossil fuels may be a way of reducing greenhouse gas emissions. Recent epidemiological studies show associations between air pollution and acceleration of cognitive decline in the elderly and neurodevelopmental effects in children. However, strong evidence for traffic-related air pollution exposure and neurotoxicity is still lacking. The aim of the project is therefore to elucidate gene expression in rat brain frontal cortex and hippocampus after exposure to diesel exhaust emissions (DEE) from fuels containing varying percentages of 1st and 2nd generation biodiesel, both in the presence and absence of a diesel particle filter (DPF). *Methods:* DEE were generated from a Euro 5 engine (Fiat Panda 2014) running on diesel fuel with varying contents of biodiesel; B7 and B20 with 7% and 20% 1st generation biodiesel, respectively, and SHB20 containing 7% 1st generation biodiesel and 13% 2nd generation biodiesel. One group of animals was exposed in whole-body chambers to particulate matter (PM) concentrations of 1977 µg/m^3 without DPF and 182 µg/m^3 with DPF for 7 days (6h/day), and another group for 28 days (6h/day, 5 days/week). Unexposed rats were included as controls. Levels of gene expression of 27 genes tied to cognition, oxidative stress and inflammation were studies in rat frontal cortex and hippocampus. Statistical analysis were performed with Microsoft Excel 2010 and JMP Pro 11 software. *Results:* The following genes were differently expressed in rat frontal cortex or hippocampus compared to controls after 7 day and 28 days DEE exposure from B7, B20 and SHB20 fuels with and without DPF technology: 7 days with DPF, B7: Cat, Tnf B20: Tnf SHB: Bdnf, Gsr, 7 days without DPF, B7: Il18, Hmox1, Cat, Gpx1 SHB: Vamp2, Cat, Gsr, 28 days with DPF, B7: Bdnf, Tnf, Hmox1, Ccl3 SHB: Vamp2, Sod2, Sod1, 28 days without DPF, B7: Il1b, Ccl3 B20: Gpx2, SHB: Sod2, Sod1, Both 7- and 28 day- exposure to emission from B7, B20 and SHB20 fuel resulted in differently expressed genes associated with cognitive processes, oxidative stress and inflammation compared to controls in both rat frontal cortex and hippocampus. Surprisingly, DPF technology did not reduce

the number of differently expressed genes, and B20 with highest level of 1st generation biofuel seems be less affected compared to B7 and SHB20.

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Effects of flavonoid agathisflavone on differentiation of rat bone marrow mesenchymal stem cells

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Mesenchymal stem cells (MSC) are characterized by the ability to self-renew and differentiate into various cell types contributing to the functional reconstitution of certain tissues. Because of mesodermal origin these cells can differentiate into osteocytes, chondrocytes, adipocytes, muscle cells and epithelial cells. Some studies also have been evidenced that MSC can differentiate into neurons. The biflavonoid agathisflavone is a phytoestrogen which has the ability to induce neurogenesis in neurons/glia co-cultures. In this study, we investigated the effects of agathisflavone (0.1 – 1 μM) and conditioned medium of glial cells treated with agathisflavone (ACM) on viability and differentiation of MSC. Differentiation protocol to characterize mesenchymal stem cells was performed for osteocytes and adipocytes. As revealed by MTT test to FAB at 0.1 - 1μM and 17β estradiol 100nM was not toxic to MSC cells after 72h treatment. However, the cell viability decreased in about 50% after 72h exposure to FAB 10 μM. Rosenfeld staining showed different types of morphology MSC cells. In the treatment, FAB 0.1 and 0.5 μM cells presented pavement morphology similar as control, in the treatment with FAB 1.0μM the cells presented morphology with extensions in form of Y. Immunocytochemistry analyses revealed nonspecific labeling for GFAP and β-tubulin, which corroborate the literature, that these cells have the ability to express these markers in a nonspecific manner suggesting that a differentiation in nerve cells is possible.

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New insights in neuronal protection against methylmercury-induced cytotoxicity and proposed new targets for upcoming research

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Methylmercury is an environmental toxicant with detrimental effects on the developing brain and adult nervous system. The main mechanisms identified include oxidative stress, changes in intracellular calcium, mitochondrial changes, inhibition of glutamate uptake, of protein synthesis and disruption of microtubules. However, little is known about mechanisms of protection against MeHg neurotoxicity. In this work, we aimed at finding additional targets that may be related to MeHg mode of action in cell toxicity with special emphasis in cell protection. In addition to the already published benefits of probucol, in the present study we found that resveratrol (10μM) and ascorbic acid (200μM) are also able to protect MeHg-induced cell death in primary cultures of cortical neurons. In relation to new targets we wonder whether neurotransmitters may affect the MeHg effects on neuronal death. Our findings show that neurons exposed to low MeHg concentrations exhibit less mortality if co-exposed to 10μM dopamine (DA). However, DA metabolites, HVA (homovanillic acid) and DOPAC (3,4-dihydroxyphenylacetic acid) are not responsible for such protection. Furthermore, we studied DA D_1 and D_2 receptors by agonistic effects through SKF38393 (10μM) and apomorfine (10μM), respectively. They both showed a protective effect against MeHg toxicity. It is striking though that D_1 SKF83566 (10μM), and D_2 reclopride (20μM) and haloperidol (10μM), antagonists did not inhibit DA protection against MeHg. In this sense, it seems whatever receptor is available for DA union is sufficient for protection even if both receptors have an antagonic effect on adenylate cyclase. In addition, the protective effect of 10μM DA against MeHg-induced toxicity was not affected by additional organochlorine pollutants exposure. Our results also demonstrate that cells exposed to MeHg in presence of 100μM acetylcholine (ACh), show an increase in cell mortality at the “threshold value” of 100 nM MeHg. The striking point of this result is that as we know ACh act on muscarinic and ionotropic receptors and both of them are involved in regulation of several physiological

activities such as proliferation, differentiation and apoptosis which are especially important during development. Our final results also show that norepinephrine (10μM) and serotonin (20μM) have an effect on cell protection. Altogether, we propose to further investigate the additional mechanisms that may be playing an important role in MeHg-induced cytotoxicity.

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Monocrotaline pyrrole from *Crotalaria retusa* induces glial response and behavioral changes in adult Wistar rats

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Crotalaria plants are considered toxic and can cause damage to livestock and human health problems. Previously we demonstrated that both MCT from *C. retusa* and dehydromonocrotaline, its main active metabolite, induce changes in the levels and patterns of expression of the main protein from astrocyte cytoskeleton, glial fibrillary acidic protein (GFAP) and that glial cells metabolism are involved in MCT induced neurotoxicity. Twenty male adult Wistar rats (60 days old, weighing 250–300g) were randomly divided into 2 experimental groups (n = 8 animals in saline group and 12 animals in MCT group). The animals were administered by oral gastric gavage. Seventy-two hours after the MCT (109mg/Kg) treatment, the behavioral was assessed by Open field and Elevated Pluz Maze (EPM) tasks, and histopathological analysis and astrogliosis were assessed after perfusion and fixation of the brain with 4% paraformaldehyde and subsequent hematoxylin/eosin staining or immunocytochemistry for GFAP. Our results demonstrated that MCT promotes a decreased in normal motor activity without any change in novelty habituation profile in rats submitted to open field task and anxiolytic-like effects in rats submitted to Elevated Pluz Maze (EPM). Moreover, histopathological analysis revealed hyperemic vascular structures in the hippocampus, parahippocampal cortex and neocortex, discreet perivascular edema in the neocortex, haemorrhagic focal area in the brain stem, and telangiectasia, with haemorrhage and edema in the thalamus.Furthermore immunohistochemical analysis revealed morphological changes on GFAP positive cells. We conclude that MCT ingestion induce neurotoxic effects on rats, characterized by behavioral changes, CNS vascular alterations and astrocytes response. MCT has been used as model of pulmonary hypertension, however elucidating its effects on CNS may bring this alkaloid as a model of CNS damages.

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Towards new solutions against mercury intoxication in Amazon: pre-clinical results of Euterpe oleracea (açaí)

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Mercury intoxication is a public health problem in Amazon and worldwide. Its levels found in Amazonian riverside populations exceed the limits recommended by the World Health Organization. Methylmercury, the most toxic form of mercury, interferes in both cellular and subcellular structural functions, generating free radicals and leading to damage, especially in the brain. Thus, substances with antioxidant effects show the potential of decrease the deleterious effects of mercury. One of the most consumed fruits in the Amazon, açaí, is from the common palm Euterpe oleracea, available in both isolated areas of the Amazon and international market. Açaí previously revealed potent antioxidant properties (higher than those of vitamin C) and antiinflamatory effects. This work investigated the neuroprotective activity of açaí against the acute methylmercury exposure in a murine model, analyzing possible behavioral alterations and oxidative stress (lipid peroxidation and nitrite levels) in brain. Açaí (10 µl/g/day, v.o.) or saline was administrated for 4 days; then, animals were treated with 2,5 mg/kg/day of methylmercury i.p. and/or açaí v.o. for additional 4 days. No differences were observed in open field (ANOVA: $p>0,05$) and rotarod (Kruskal-Wallis: $p>0,05$) tests, however, results of pole test revealed an behavior improve caused by açaí (Kruskal-Wallis: $p<0,05$). Treatment with this fruit also prevent the lipid peroxidation (MeHg: 68 ± 8,64 ηmol/mg and MeHg+Açaí: 44,7 ± 7,15 ηmol/mg) and the increase of nitrite levels (MeHg: 68,7 ± 21,36 µM and MeHg+Açaí: 5,65 ± 4,6 µM) in the brains of animals intoxicated with mercury (ANOVA: $p<0,05$). This work demonstrates for the first time that açaí is able to in vivo protect against behavioral alterations and oxidative stress caused by acute exposure to methylmercury. Our results already support the regular consumption of açaí juice to protect against damage caused by this substance.

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Involvement of the endocannabinoid system on neuroinflammation in the prefrontal cortex and hippocampus of adolescent rats submitted to chronic binge alcohol

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Heavy episodic consumption (binge) of alcohol has been quite common, especially among young people, generating inflammatory processes in regions such as the prefrontal cortex (CPF) and hippocampus, involved in complex cognitive processes and the development and maintenance of drug dependence. The endocannabinoid system has been proposed as a neuroprotective mechanism. The objective of this study was to investigate the possible neuroprotective effect of the endocannabinoid system on neuroinflammation processes in the CPF and hippocampus of adolescent rats submitted to chronic binge alcohol. 36 adolescents male Wistar rats (28-30 days) were submitted to a chronic model of binge drinking divided into groups: vehicle (VEH) + distilled water (AD), URB 597 0.3 mg/kg + AD, VEH + alcohol dose 3 g/kg (ALC 3), VEH + alcohol 6g/kg (ALC 6) URB 597 + ALC 3 and URB 597 + ALC 6. URB597 was administered intraperitoneally (IP) in dose of 0.3mg/kg and alcohol was administered intragastrically (IG). The VEH or URB597 was administered 40 minutes before administration of AD or alcohol. The administrations were conducted for three consecutive days along four weeks. After treatment animals were euthanized and their hippocampus and CPF removed for cytokine analysis and neurotrophin BDNF by ELISA. Treatment with ALC 3 and ALC 6 preceded by VEH produced significant increases in IFN-γ concentrations ($P<0.001$), TNF-α ($p<0.001$), IL-4 ($p<0.01$), IL-10 ($p<0.01$) and BDNF ($p<0.001$) at CPF when compared to VEH IP + AD IG. The previous administration of URB597 IP significantly decreased the concentrations of cytokines and BDNF. In the hippocampus, the treatment with ALC 3 IG and ALC 6 IG preceded by VEH IP produced significant increases in IFN-γ

concentrations ($p < 0.01$), TNF-α ($p < 0.001$), and BDNF ($p < 0.01$). Pretreatment with URB597 IP significantly decreased the concentrations of cytokines and BDNF. The URB597 had no effect *per se*. Chronic binge alcohol increased cytokines and BDNF concentrations in CPF and hippocampus of adolescent rats. The previous treatment with URB597, the inhibitor of the enzyme that degrades the endocannabinoid anandamide (fatty acid amide hydrolase – FAAH) decreased the concentration of cytokines and BDNF. Therefore, the endocannabinoid system seems to be involved in the neuroinflammatory processes related to alcohol consumption in chronic binge by adolescent rats.

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Small molecules alter manganese toxicity in *Caenorhabditis elegans*

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Manganese (Mn) is essential for several species and daily requirements are commonly met by an adequate diet. Mn overload may cause motor and psychiatric disturbances and may arise from an impaired or not fully developed excretion system, transporter malfunction and/or exposure to excessive levels of Mn by air, water, food or total parenteral nutrition (TPN). Therefore, deciphering processes regulating neuronal Mn homeostasis is essential to understand the mechanisms of Mn neurotoxicity. Our groups recently performed a high throughput screen of 40,167 small molecules for modifiers of cellular Mn content in a mouse striatal neuron cell line and demonstrated cell-level regulation of Mn content across neuronal differentiation *in vitro*. In the present study we selected two of those molecules to test their effects on Mn toxicity parameters *in vivo* using *Caenorhabditis elegans*. At both toxicological and physiological Mn exposures, VU0063088 (‘088) is an Mn level decreasing molecule and VU0026921 (‘921) is Mn level increasing. In *C. elegans,* excess Mn has been shown to induce dopaminergic degeneration and oxidative stress. We pre-exposed worms to ‘921 and ‘088 for 30 min followed by co-exposure for 1 h with Mn and evaluated worm survival and dopaminergic degeneration. Control worms were exposed to vehicle (DMSO) and NaCl only. In BY200 (pdat-1::GFP) worms, that express GFP in dopaminergic neurons, we observed a decrease of Mn-induced dopaminergic degeneration in the presence of both small molecules. This effect was also observed in MAB300 worms, an *smf-2* knockout strain. SMF-2 is a regulator of Mn transport in the worms. We did not observe improved survival in the presence of small molecules. Our results suggest that ‘921 and ‘088 may modulate Mn levels in the worms through a mechanism independent of SMF-2. The role of other Mn transporters in small molecule exposed worms remains to be determined.

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The effects of guanosine in neural proliferation, neurogenesis and antidepressant-like behavior in adult mice

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Advanced research on stem cells in adults has shown promising discoveries that may contribute to the maintenance, functioning and improvement of regenerative responses throughout life. In the adult brain, stem cells are capable of differentiating generating new neurons in a process of several steps called neuro-genesis. This process is regulated by neurotransmitters, hormones, neurotrophic factors, pharmacological agents and environmental factors. Guanosine is a purine nucleoside with important functions in cell metabolism and we have previously shown its protective role on neurodegenerative diseases or injury. The past decade has seen

major advances in identifying the modulatory role of extracellular action of guanosine in the central nervous system (CNS) including increasing levels of trophic factors. Here we investigated the effects of guanosine treatment (100 μM, for 4 days) on neural stem cells (NSC) of the subventricular zone (SVZ) from young adult mice. Guanosine treatment promotes significative ($P<0.05$) proliferation of NSC forming neurospheres in suspension cultures, without having a significant effect in neurospheres diameter. To test proliferative effect of guanosine in vivo, young adult mice (C57BL/6) were treated with guanosine (8 mg/kg, i.p, for 26 days) and 5-bromo-2'-deoxyuridine (BrdU 50 mg/kg, i.p) was administered during 5 days. Guanosine treatment significantly ($P<0.05$) increases the number of BrdU+ cells in both SVZ and in the subgranular layer (SGL) of the dentate gyrus (DG). In the hippocampus, guanosine treatment increases survival of mature neurons with significant number ($P<0.05$) of BrdU+, NeuN+ cells colocalization. Guanosine treatment also exerts a significant ($P<0.05$) anti-depressant-like behavior in the tail suspension test. Although the mechanisms involved require further study, together these data suggest that guanosine plays an important role in regulating neural stem/pro-genitor cells *in vivo* and *in vitro* and produces anti-depressant-like effects that might be related to the promotion of hippocampal neurogenesis.

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Interruption of oxygen at delivery and permanent vulnerability to recurrent metabolic insults: in vitro and in vivo experiments

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The hypothesis of enhanced vulnerability following perinatal asphyxia (PA) was investigated with a protocol combining *in vivo* and *in vitro* experiments. Asphyxia exposed (AS) and caesarean-delivered control (CS) rat pups were used at P2-3 for preparing triple organotypic cultures including tissue samples from mesencephalon (SN) and telencephalon (Str and Cx). In parallel series, AS and CS neonates were treated with a single dose of saline (100 μl, i.p.) or nicotinamide (0.8 nmol/kg, i.p.), 60 min after delivery. At DIV 18, cultures were subjected to a second challenge, consisting of different concentrations of H_2O_2, added to the culture medium for 18h. After a 48h recovery period, the cultures were either assessed for cell viability, or for neurochemical phenotype and confocal evaluation in formalin fixed cultures. Energy metabolism (ADP/ATP), oxidative stress (GSH/GSSG) and a modified ferric reducing/antioxidant power assay was applied to homogenates of parallel culture series. It was found that PA produced a long-term energetic deficit associated to regionally specific cell loss, mainly affecting mesencephalic dopamine systems. Homogenates of AS triple organotypic cultures showed a >6-fold increase in ADP/ATP ratio, assayed at DIV 21-22, compared the corresponding controls (CS). The increase in the ADP/ATP ratio reflected a permanent energetic deficit, since a single 1 mM H_20_2 insult also increased the ADP/ATP (>7-fold) in CS, further elevating the ADP/ATP ratio in AS cultures. That effect was paralleled by a decrease in the GSH/GSSG ratio, observed in AS culture homogenates, also mimicked by the H_20_2 insult. A decrease in reducing power was observed in AS homogenates compared to the CS controls, but that was not affected by H_20_2. The cell phenotype of dying/alive cells was investigated in formalin fixed cultures, co-labelling for DAPI (nuclear staining), TUNEL (apoptosis), MAP-2 (neuronal phenotype), GFAP (astroglial phenotype), and tyrosine hydroxylase (dopamine phenotype). In substantia nigra, the number of MAP-2/TH positive cells/mm^3 was decreased in AS compared to CS cultures, also by 1 mM of H_20_2, both in CS and AS cultures, prevented by nicotinamide. In agreement, the number of MAP-2/TUNEL positive cells/mm^3 was increased by 1 mM H_2O_2, both in CS (2-fold) and AS (3 fold) cultures, also prevented by nicotinamide. The number of MAP-2/TH/TUNEL positive cells/mm^3 was only increased in CS (>3 fold), not in AS (1.3 fold) cultures. No TH labelling was observed in neostriatum, but 1 mM of H_2O_2 produced a strong increase in the number of MAP-2/TUNEL positive cells/mm^3, both in CS (>2.9 fold) and AS (>5 fold) cultures, decreased by nicotinamide. In neocortex, H_2O_2 increased the number of MAP-2/TUNEL positive cells/mm^3, both in CS and AS cultures (≈3fold), decreased by nicotinamide. Thus, the present results demonstrate that PA implies a long-term energetic deficit, even when re-oxygenation is stabilized, priming cell vulnerability with both neuronal and glial phenotype. The observed effects were region dependent, being the substantia nigra particularly prone to cell death. Nicotinamide treatment *in vivo* prevented the deleterious effects observed *in vitro*.

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Vulnerability of basal ganglia to metabolic insults following perinatal asphyxia: metabolic impairment and nicotinamide protection

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Enhanced vulnerability following perinatal asphyxia (PA) was investigated using asphyxia exposed (AS) and caesarean-delivered control (CS) rat pups for preparing triple organotypic cultures including Substantia Nigra (SN), Striatum and Cortex. AS and CS neonates were treated with nicotinamide (0.8 nmol/kg, i.p.), 60 min after delivery. Cultures were subjected to different concentrations of H2O2, for 18h. Cell viability, neurochemical phenotype, energy metabolism (ADP/ATP), oxidative stress (GSH/GSSG) and reducing power were measured. PA produced a long-term energetic deficit with cell loss, affecting dopamine systems. AS cultures showed a > 6fold increase in ADP/ATP ratio, compared to CS. The increase in the ADP/ATP ratio reflected a permanent energetic deficit, since a single 1mM H202 insult also increased the ADP/ATP (>7fold) in CS, further elevating the ADP/ATP ratio in AS, along with a decrease in the GSH/GSSG ratio, observed in AS homogenates, and also mimicked by H202. A decrease in reducing power was observed in AS compared to the CS, but that was not affected by H202. Cell phenotype of dying/alive cells was investigated co-labelling for TUNEL, MAP-2, GFAP and tyrosine hydroxylase. In SN, the number of MAP-2/TH positive cells/mm3 was decreased in AS compared to CS cultures, also by 1 mM of H202, both in CS and AS cultures, prevented by nicotinamide. The number of MAP-2/TUNEL positive cells/mm3 was increased by 1 mM H2O2, both in CS (2fold) and AS (3fold) cultures, also prevented by nicotinamide. The number of MAP-2/TH/TUNEL positive cells/mm3 was only increased in CS (>3 fold), not in AS (1.3 fold) cultures. No TH labelling was observed in neostriatum, but 1 mM of H2O2 produced a strong increase in the number of MAP-2/TUNEL positive cells/mm3, both in CS (>2.9 fold) and AS (>5 fold) cultures, decreased by nicotinamide. In neocortex, H2O2 increased the number of MAP-2/TUNEL positive cells/mm3, both in CS and AS cultures (≈3-fold), decreased by nicotinamide. The present results demonstrate that PA implies long-term deficits, priming cell vulnerability of neurons and glia. The observed effects were region dependent, being SN particularly vulnerable. Nicotinamide treatment *in vivo* prevented the effects observed *in vitro*.

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Alteration of the PAC1 receptor expression in the basal ganglia of MPTP-induced parkinsonian macaque monkeys

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Pituitary adenylate cyclase activating polypeptide (PACAP) is a well-known neuropeptide with strong neurotrophic and neuroprotective effects. PACAP exerts its protective actions via three G protein-coupled receptors: the specific Pac1 receptor (Pac1R) and the Vpac1/Vpac2 receptors, the neuroprotective effects being mainly mediated by the Pac1R. The protective role of PACAP in models of Parkinson's disease and

other neurodegenerative diseases is now well-established in both in vitro and in vivo studies. PACAP and its receptors occur in the mammalian brain, including regions associated with Parkinson's disease. PACAP receptor up- or downregulation has been reported in several injury models or human diseases, but no data are available on alterations of receptor expression in Parkinson's disease. The model closest to the human disease is the MPTP-induced macaque model. Therefore, our present aim was to evaluate changes in Pac1R expression in basal ganglia related to Parkinson's disease in a macaque model. Monkeys were rendered parkinsonian with MPTP, and striatum, pallidum and cortex were evaluated for Pac1 receptor immunostaining. We found that Pac1R was markedly downregulated in the caudate nucleus, putamen, as well as in the internal and external parts of the globus pallidus, while expression remained unchanged in the cortex of MPTP-treated parkinsonian monkey brains. This decrease was attenuated in some brain areas in monkeys treated with L-DOPA. The strong, specific downregulation of the PACAP receptor in the basal ganglia of parkinsonian macaque monkey brains suggests that the PACAP/Pac1R system may play an important role in the development/progression of the disease.

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Effects of sulfasalazine administration on nociceptive responses in rats following intranasal MPTP administration, an animal model of Parkinson´s disease

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Pain is a frequent non-motor symptom of Parkinson's disease (PD). However, the mechanisms responsible for this symptom are not well understood. Tetrahydrobiopterin (BH4) is an essential cofactor for synthesis of serotonin, epinephrine, norepinephrine, dopamine and nitric oxide. Previous studies demonstrated increased BH4 levels in the brain of PD patients and that BH4 overproduction in sensory neurons increases pain sensitivity in humans and animal models. Therefore, the aim of this study was evaluated the inhibition of BH4 synthesis by sulfasalazine (SSZ) on nociceptive responses of rats infused intranasally (i.n.) with MPTP, an animal model of early stages of PD. A total of 32 male Wistar rats (90 days-old) were administered with a single i.n. infusion of MPTP (1 mg/nostril) or saline and 14 days later their nociceptive responses were evaluated in the von Frey and hot plate tests. After that, the animals were treated with sulfasalazine (50 mg/kg) or vehicle by gavage twice a day during 3 days. Von Frey and hot plate tests were performed at 0, 1, 2 and 3 h, and open field (OF) test 1.5 h after the last SSZ administration. Our results indicated that MPTP induced mechanical and hot hypersensitivity at 14 days after i.n. administration. The SSZ treatment reduced MPTP-induced hot and mechanical hyperalgesia at 1 and 2 h after the last drug administration. None treatment altered the locomotor activity of animals in the OF. This study provides the first evidence that inhibition of BH4 synthesis is able to reduce MPTP-induced hyperalgesia in rats. These findings indicate the potential role of BH4 pathway on mechanisms involved in the pain symptoms in PD.

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Noradrenaline replacement in prefrontal cortex restores both short- and long-term recognition memory impairments induced by *locus coeruleus* lesion in rats

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Locus coeruleus (LC) degeneration, the main source of cerebral noradrenaline (NA), has been related with neurodegenerative disorders such as Alzheimer's (AD) and Parkinson's diseases (PD). LC neurodegeneration frequently is more extensive than the neuronal lost found at the substantia *nigra* and hippocampus of PD and AD carriers, respectively. Additionally, growing evidence support earlier NA deficiency in several brain areas resulting from selective degeneration of LC neurons. For instance, LC projections to the prefrontal cortex (PFC) have a critical role on the cognitive functions. Therefore, here we evaluated the learning and memory of rats after the selective noradrenergic lesion by 6-hydroxydopamine (6-OHDA) in LC and the involvement of the likely resulting NA deficit in the PFC. For this, adult male Wistar rats received stereotaxic bilateral injections of 6-OHDA at a dose of 5 µg/side into the LC (AP -9.9 mm; ML ±1.4 mm, DV -7.0 mm) and two stainless-steel guide cannulas were implanted aimed at the PFC (AP +3.3 mm; ML ±0.75 mm, DV -1.7 mm). Previous data from our lab indicated that this 6-OHDA dose did not cause any motor alterations. SHAM group received just vehicle (0.2% ascorbic acid in saline). In order to induce a selective noradrenergic lesion, animals received nomifensine (10 mg/kg/ml, i.p.), a dopamine transporter blocker, one hour before surgery. The behavioral tests were carried out at 7, 21 and 42 days after the 6-OHDA administration. 6-OHDA into the LC induced short-term (STM) recognition memory impairment addressed on object recognition test (ORT) in all periods evaluated. Regarding aversive memory evaluated by the step-down passive avoidance task (SPA), 6-OHDA-lesioned rats decreased the step-through latency 1.5 h after the training session at 7 and 21 days after injection. Long-term memory (LTM) was disrupted by LC lesion 7 days after the surgery in both behavioral tests. In order to analyze the PFC involvement, bilateral NA infusion (1 µg/side) into this region was performed immediately before of the training sessions of both cognitive tests. The SPA and ORT were carried out at 7 and 14 days after the 6-OHDA injection, respectively. The NA administration into the PFC restored both 6-OHDA-induced STM and LTM recognition memory deficits observed in the ORT, but not the aversive memory impairment found in the SPA. These results indicate that the LC lesion, and the likely consequent NA reduction in the PFC, could be involved in the recognition memory impairments observed in both AD and PD patients.

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Aminochrome induces glial activation and neuronal degeneration in primary culture from rat CNS

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Neuroinflammation is one of the mechanisms involved in the loss of dopaminergic neurons in Parkinson´s Disease (PD). Aminochrome has been suggested as a more physiological preclinical model capable of inducing five of the six mechanisms of PD. However, there is no evidence that aminochrome induces glial activation related to neuroinflammation, an important mechanism involved in the loss of dopaminergic neurons. In this study, potential role of aminochrome on glial activation was studied in primary mesencephalic neuron-glia cultures from E14 Wistar rats and microglial primary culture from P01 Wistar rats. We demonstrated that aminochrome induced reduction in the number of viable cells on cultures exposed to concentration between 10 to 100µM, for 48h. Moreover, aminochrome induces neuronal death determined by Fluoro-jade B. Furthermore, we demonstrated that aminochrome (10µM, for 24h) induced reduction in the number of TH-immunoreactive neurons and reactive gliosis, featured by morphological changes in $GFAP^+$ and $IBA1^+$ cells, more increase in the number of $OX\text{-}42^+$ cells. These results demonstrate aminochrome neuroinflammatory

ability and support the hypothesis that it may be a better PD preclinical model to find new pharmacological treatment that stop the development of this disease.

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Agmatine reverses emotional impairments in the intranasal MPTP model of Parkinson's disease – The role of neurotrophic factors and neuroinflammation

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Depression is the most common non-motor symptom of Parkinson's disease (PD) and previous studies suggest that neuroinflammation and neurotrophic factors are involved in its pathophysiology. In addition, many studies have demonstrated that agmatine, an endogenous neuromodulator, has neuroprotective and antidepressant activities. Therefore, in this study we investigated the effects of repeated treatment with agmatine on the emotional deficits observed in an experimental model of PD. C57BL/6 female mice (3 months old) were divided in CONTROL and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-groups. The animals received a single intranasal (i.n.) administration of saline (NaCl 0,9%) or MPTP (1 mg/nostril), respectively. Animals were treated with vehicle or agmatine (0.1, 1 or 10 mg/kg, p.o.) during 15 days, and a battery of behavioral tests was conducted during 15 days after i.n. MPTP treatment. After that, GFAP and the ratio of Bax/Bcl-2, BDNF, GDNF and VEGF levels were addressed in the prefrontal cortex and hippocampus by Western blot analysis. MPTP administration did not alter the locomotor activity of the animals evaluated in the open field test. The anhedonic and depressive-like behaviors were addressed in the splash, tail suspension and forced swimming tests. The animals of the MPTP-vehicle group showed a reduced grooming time after a sprayed of sucrose 10% solution and an increased on immobility time when compared to control-vehicle, indicative of anhedonic and depressive-like behaviors. All tested doses of agmatine reversed the anhedonic and depressive-like behavior induced by i.n. MPTP. Furthermore, MPTP induced a more pronounced depletion of neurotrophins in the prefrontal cortex and hippocampus of these animals while the treatment with agmatine was able to prevent these neurochemical alterations. In addition, MPTP induced neuroinflammation indicated by the increase in the GFAP and the ratio of Bax/Bcl-2 levels in prefrontal cortex. The present study provides new evidence of emotional impairments in the i.n MPTP model of PD and that the treatment with agmatine prevented the development of these emotional deficits. Also this study provides the first evidence of the role neurotrophic and neuroinflammatory mechanisms in the beneficial effects of agmatine in PD. Protocol: CEUA-UFSC PP380

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Levodopa improves fatigue tolerance in reserpine-treated mice – An animal model of Parkinson's disease

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Fatigue has been recognized as a troublesome symptom with increased prevalence (32-50%) in Parkinson disease (PD) patients. It is a complex picture worsened by the addition of other two fatigue-associated symptoms, poor sleep and depression, which deeply compromised the quality of life of these individuals. It is important to distinguish symptoms of peripheral neuromuscular fatigue from the symptoms of central fatigue

characteristic of basal ganglia disorders, in order to understand the mechanisms of fatigue. Here, we investigate fatigue-like behavior in a reserpine-induced experimental PD model. Striatal DA was depleted in mice (Swiss mice, 10-12 weeks age, 35-45 g) by systemic administration of reserpine (2 x 1 mg/kg, i.p.). This treatment reduced striatal DA levels and increased the 5-HT/DA ratio. Reserpine administration also induced early fatigue (or exercise intolerance) in the voluntary running wheel and in an incremental treadmill test. The systemic administration of levodopa (100 mg/kg, i.p) plus benserazide (50 mg/kg, i.p) partially reversed DA depletion, and parkinsonism-like and fatigue-like symptoms. The impaired exercise performance was not influenced by emotional or anhedonic behaviors, and the mechanical nociceptive thresholds were not altered by reserpine treatment. In addition, the electrocardiogram profile, recording rate or heart rate 2h or 20h after reserpine administration, or the quadriceps mitochondrial content were not modified by reserpine, ruling out secondary central and peripheral effects on the exercise performance. In addition, reserpine *per se* did not change cell viability neither mitochondrial anatomy or cellular respiration in L6 muscle cells. Altogether, these results suggest that fatigue from in PD patients might be caused by impaired dopaminergic neurotransmission, pointing out to a central origin, rather by compromised muscle metabolism, which does not discard other non-motor components, such as motivation or attention.

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SUMOylation profiles in an animal model of epilepsy

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Epilepsy is a chronic neurological disorder with a high prevalence worldwide. This disease affects people from all age range. The most common type of epilepsy in adults is the temporal lobe epilepsy (TLE) and among TLE patients there is the mesial subtype related to hippocampal sclerosis (MTLE-HS). These patients show drug resistance, psychiatric impairment and neurochemical changes. SUMOylation is a reversible posttranslational modification in which small ubiquitin-like modifier (SUMO) is covalently attached to lysine residues in target proteins regulating several cellular pathways. A recent study demonstrated that SUMO plays a role in the onset of spontaneous crises and sudden death in epilepsy. Our goal is to determine the SUMOylation profiles in an animal model of MTLE-HS. The protocol was approved by UFSC Ethics Committee on the Use of Animals (PP00772). Adult male Wistar rats (280–350 g) received a single intraperitoneal (i.p.) dose of pilocarpine (300 mg/kg dissolved in 0.9% saline) for the induction of status epilepticus (SE). Two months after SE induction, corresponding to the chronic phase, the dorsal and ventral hippocampi were dissected and analyzed by Western blotting. Antibodies against SUMO-1 and SUMO-2/3 (main SUMO isoforms) and UBC9 (sole SUMO conjugating enzyme) were used to determinate the global levels of SUMOylation. Even though no significant changes in the SUMOylation profile were observed in the animal model, probably due to the analysis carried out during the chronic period, we cannot discard that SUMO alterations could occur at earlier stages, i.e. in the acute period or during the epileptic crises. The results of this work will contribute to a better understanding of the cellular mechanisms underlying epilepsy.

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Neural cell death induced by piperidine alkaloids from *Prosopis juliflora* (Mesquite) leaves involves programmed cell death via caspase-9 activation and autophagy

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Mesquite is a shrub that has been used to feed animals and humans. However, piperidine alkaloids have been suggested to be responsible for neurotoxic damage observed in animals. We investigated the involvement of programmed cell death (PCD) and autophagy on the mechanism of cell death induced by a total extract (TAE) of alkaloids and fraction (F32) from *P. juliflora* leaves composed majoritary of juliprosopine in a model of neuron/glial cell co-culture. We saw that TAE (30 µg/mL) and F32 (7.5 µg/mL) induced caspase-9 activation, nuclear condensation and neuronal death at 16 h exposure. After 4h, they induced autophagy characterized by decreases of P62 protein level, increase of LC3II expression and increase in number of GFP-LC3 cells. Interestingly, we demonstrated that inhibition of autophagy by bafilomycin and vinblastine increased the cell death induced by TAE and autophagy induced by serum deprivation and rapamycin reduced cell death induced by F32 at 24 h. These results indicate that the mechanism neural cell death induced by these alkaloids involves PCD via caspase-9 activation and autophagy which seems to be an important protective mechanism.

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Physical exercise attenuates cortical neuroinflammation induced by lipopolysaccharide in mice

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Neuroinflammation is an essential process for host defense which evokes metabolic and motivated behavioral adaptations known as sickness behavior; however, it can become harmful when sustained. In this regarding, physical exercise has been used in modulating the immune response, and possibly in neuroinflammation. OBJECTIVES. We investigated the effects of physical exercise on neuroinflammation and sickness behavior induced by lipopolysaccharide (LPS) in Swiss mice. MATERIAL AND METHODS. Mice (Swiss strain, male, 12-15 weeks age, 47,1±0,7 g BW) were divide into sedentary (SED) and exercise group (voluntary running wheels, RW). After six weeks of exercise, the animals were treated with LPS (0,33mg/kg, i.p.) or vehicle (0.9% saline solution, SAL), and experiment reached four groups: SED-SAL (N=23), SED-LPS (N=22), RW-SAL (N=16) and RW-LPS (N=15). We evaluated the following physiological parameters during all experiment: RW distance, body mass, food and water intake. After 4-h of LPS administration, the animals were evaluated in the open field, splash and tail suspension test. RESULTS AND DISCUSSION**.** LPS-treated mice expressed a depressive-like behavior and impaired locomotion on RW and open field, food and water intake. LPS also increased serum IL-1β levels, and IL-1β and IL-6 levels in the prefrontal cortex of mice; and depleted dopamine (DA) and its metabolite 3,4-dihydroxyphenylacetic (DOPAC) in the prefrontal cortex of mice. Exercise partially attenuated this neuroinflammatory profile and sickness behavior. CONCLUSIONS. These data suggest that physical exercise partially prevent neuroinflammation and sickness behavior in an animal model of neuroinflammation.

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Acute and long-term effects of intracerebroventricular administration of α-ketoisocaproic acid on oxidative stress parameters

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Patients affected by Maple Syrup Urine Disease (MSUD) are biochemically characterized by elevated levels of leucine, isoleucine and valine, as well as their corresponding transaminated branched-chain α-keto acids in tissue and biological fluids. Neurological symptoms and cerebral abnormalities, whose pathophysiology is still unknown, are typical of this neurometabolic disorder. In the present study, we evaluated the early effects (1 hour after injection) and long-term effects (15 days after injection) of a single intracerebroventricular administration of α-ketoisocaproic acid (KIC) on oxidative stress parameters. Wistar rats at 30 days old were divided into two groups: KIC and control (which received vehicle: **artificial cerebrospinal fluid**; ACSF). KIC was administered by stereotaxic into the lateral ventricle of the animal at a concentration of 0.8 µmol of KIC dissolved in 2µL ACSF; in the control group, ACSF was injected in the same way. One hour or 15 days after the administration the animals were killed by decapitation and cortex, hippocampus and striatum were isolated for analysis. Our results showed that KIC induced early and long-term effects; we found an increase in TBARS levels, protein carbonyl content in the hippocampus, striatum and cerebral cortex both one hour and 15 days after KIC administration. Moreover, a remarkable increase in SOD activity was found in the hippocampus and striatum one hour after injection, whereas after 15 days SOD activity was increased only in the striatum. On the other hand, KIC significantly decreased CAT activity in the striatum one hour after injection, but 15 days after KIC administration, we found a decrease in CAT activity in the hippocampus and striatum. From these results biochemical, we speculate that KIC provokes short- and long-term oxidative stress in brain; this fact may play an important role in the pathophysiology of the neurological damages present in patients with MSUD.

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Perinatal asphyxia induces changes in oligodendrocytes phenotype in telencephalon of rat brain

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Perinatal asphyxia (PA) implies interruption of oxygen supply at birth, leading to a cascade of pathophysiological events, resulting in short and long-term neuropsychiatric disorders. PA can affect development, by affecting connectivity, neurotransmission and survival of neuronal and glial cells including oligodendrocytes (OLs). In the brain demyelination is the consequence of damaged OLs, vulnerable to metabolic insults during delivery, due to their high metabolism rate for the synthesis of poly unsaturated phospholipids, the constitutive product of myelin. Demyelination can disrupt brain development leading to motor, sensory and cognitive deficits. We investigated the effect of PA on the density of mature state of OLs in external capsule and corpus callosum at the level of telencephalon at post-natal day (P) 7, the time when myelination starts. Severe global PA was induced by immersing foetuses-containing uterine horns into a water bath at 37°C for 21 minutes (AS; n=5). Caesarean delivered pups were used as controls (CS; n=5). Coronal sections of the telencephalon (20 µm thick) were treated for immunocytochemistry, using antibodies for Myelin Basic Protein (MBP, mature OLs marker) and DAPI (nuclear marker), evaluated with confocal microscopy. It was found that the number of MBP/DAPI positive cells/mm^3 was decreased in the external capsule and corpus callosum of AS, as compared to that of CS animals. PA induced reactivity in OLs, characterized by smaller soma and fewer processes compared to that in control animals. The present results support the idea that PA affects the oligodendroglial phenotype, resulting in long-term demyelination affecting the telencephalic connectivity of rat brain.

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Metabotropic glutamate receptors as prognostic markers in Glioblastomas

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Glioblastoma (GBM) is the most common primary malignant tumor in adults. Despite the surgical resection followed by radiotherapy and chemotherapy, GBM patient's average survival is around 15 months. Thus, there is a growing need to develop new therapies targeting molecules that specifically regulate GBM proliferation. Several studies demonstrated the involvement of metabotropic glutamate receptors (mGluR) in progression, aggressiveness and recurrence of GBM. The aim of this study was to assess the potential prognostic and predictive value of the eight mGluR subtypes, proposing a gene signature as potential biomarker to predict the patient's outcome. Tissue microarray data was used to evaluate mGluR gene expression (GRM) association with survival in a GBM cohort through hierarchical clustering and survival analyses. The results obtained showed that the expression of three receptors was the main responsible to cluster the patients into two groups and this cohort subdivision had prognostic value. The potential value of this receptors signature was tested pharmacologically in vitro. Western Blot analyses performed to assess protein immunocontent of these three mGluRs in U87-MG GBM cell line showed that only two of them are presented in these cells. U87-MG cells were treated with mGluR ligands and chemotherapeutic agents (TMZ and BCNU). For this, cells were incubated with the ligands at double concentration for 30 min in DMEM to assess the involvement of these receptors on proliferation. After, DMEM supplemented with FBS was added to achieve a final concentration of 10% FBS and incubated for 72h. Pharmacological blockade and activation treatments, attempting to simulate the profile observed in the gene signature, did not show any significant change in the percentage of U87-MG cells. A synergic effect between ligands was not seen and they also did not enhance the chemotherapeutic agents' antiproliferative action. Although in vitro treatment with mGluR ligands showed no significant results, more experiments in a different proliferative condition are required. Nevertheless, gene expression profile suggests that mGluR gene expression levels were able to predict GBM patient outcome.

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Effects of hearing isolation on the development of ultrasonic vocalization in rat pups

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Perinatal hypothyroidism causes serious damage to auditory functions that are essential for vocalization development. In rat pups, perinatal hypothyroidism potentially affects the development of ultrasonic vocalization (USV) as a result of hearing deficits. This study examined the effect of hearing isolation on the development of USVs in rat pups and compared with the results of perinatal hypothyroidism. Four pregnant rats were purchased from Japan SLC Inc. (Hamamatsu, Japan) and divided into two groups: the hearing isolation group and the control group. On postnatal days (PNDs) 6, 9, 12, and 15, the pups from the hearing isolation group were covered their ears with plastic film and the quick-drying glue, Aron Alpha A "Sankyo" (TOAGOSEI, Takaoka, Japan). This glue is exclusive for surgical operation. The pups from the control group were covered their head with plastic film and the glue on the same PNDs. USVs were recorded from the pups on PNDs 4 (before hearing isolation), 7, 10, 13, and 16 (after hearing isolation). A pup was individually separated from the dam and littermates, put into a translucent cup and left alone in a sound-insulated box for a 5-min period of habituation, followed by 5 min of USV recording. USVs were recorded and analyzed using an ultrasonic microphone and the Sonotrack system version 2. 1. 5 (Metris, Hoofddorp, the Netherlands). The

preliminary analyses of USVs indicated significant alterations of USV duration. Comparing with the control group, the hearing isolation group produced longer and shorter durations of USVs on PND 7 and 13, respectively. However, the number and frequency of USVs did not exhibit significant changes between the isolation and control groups. The body weight gains were not affected by hearing isolation. Perinatal hypothyroidism causes acoustic alterations of USVs in rat pups (Wada, 2016). The number of USVs for the hypothyroid group was increased on PNDs 10 and 15 compared with that for the control group. The duration was longer on PND 15 than that for the control group. However, the present study indicated significantly longer durations on PND7 and shorter durations on PND 13. These results were slightly different from those of the previous study. Perinatal hypothyroidism may affect the development of USVs due to different factors other than hearing deficits.

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Effect of acute and chronic administration of L-tyrosine on working memory in young rats

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Tyrosinemia type II is an inherited autosomal recessive disorder of L-tyrosine metabolism caused by a deficiency in the hepatic enzyme tyrosine aminotransferase (TAT); due to this deficiency tyrosine accumulates in blood and other fluids. Previous studies reported that acute and chronic administration of L-tyrosine affect both some neurotrophins as well as the cholinergic system. Thus, the objective of this study was to evaluate the effects of acute and chronic administration of L-tyrosine on working memory in young rats. In the acute protocol, L-tyrosine (500 mk/kg body weight) was administered once and 1 hour after administration the animals were subjected to the object recognition task; control group received vehicle. Chronic administration of L-tyrosine (500 mk/kg body weight) was performed 12/12 hours from the 7th to the 28th day of life and 12 hours after the last administration the animals were submitted to the object recognition task; control group received vehicle. We verified that short- and long-term memory was impaired by acute administration of L-tyrosine. On the other hand, chronic administration of L-tyrosine did not affect this behavioral task. Based on the present findings, we speculate that the administration of high doses of L-tyrosine may impair cognition in animals, corroborating with other studies that show neurological alterations in patients with tyrosinemia.

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Antidyskinetic effect of acute guanosine administration in reserpinized mice.

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Dyskinesia is characterized as involuntary movements that affect several body parts. Several neurological disorders can exhibit this symptom, such as Parkinson's disease. Dyskinesia can be induced by the alkaloid reserpine that acts as an inhibitor of vesicular monoamine transporter (VMAT-2). The consequent monoamine neurotransmitters depletion induces hypolocomotion, muscle rigidity and involuntary movements. Guanosine (GUO), an endogenous nucleoside, has been evidenced as a neuroprotective agent, although the exact mechanism of GUO action is not fully characterized. This study evaluated the therapeutic potential of GUO as

an antidyskinetic agent in mice treated with reserpine (1 mg/kg, subcutaneously, every other day). GUO (7.5 mg/kg p.o.) was administered 24 h after the last reserpine injection and 20 min before behavioral test. GUO prevented the increase of orofacial dyskinesia induced by reserpine. Additionally, the antidyskinetic effect of GUO was abolished by prior administration of the A_1 adenosine receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, 0.75 mg/kg). Reserpinized mice also showed a cataleptic state when evaluated in the bar test. Likewise, this behavior was prevented by GUO. Interestingly, DPCPX also abolished the anti-cataleptic effect of GUO, besides presenting an anti-cataleptic effect *per se*. Reserpine increased cells damage and reactive oxygen species (ROS) levels in the striatum of treated mice. GUO was effective in reducing the increase of ROS levels, but it did not alter cells damage induced by reserpine. This study shows for the first time an antidyskinetic effect of GUO and its effect of modulating motor and neurochemical impairments induced by reserpine.

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Alterations on the gene expression on cerebellar thyroid hormone homeostasis in perinatally rats exposed by glyphosate-based herbicide

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Glyphosate-Based Herbicides (GBHs) are widely used in modern agriculture. Recently several animal and epidemiological studies have been conducted to understand the effects of these chemicals as an endocrine disruptor. The aim of the present study was to determine whether GBHs could also disrupt the cerebellum thyroid hormone homeostasis. Female pregnant Wistar rats were exposed to a solution containing GBHs Roundup Transorb (Monsanto). The animals were divided into three groups (control, 5 mg/kg/day and 50 mg/kg/day) and exposed from gestation day 18 (GD18) to post-natal day 5 (PND5). Male offspring were euthanized at PND 90, and tissues samples from cerebellum were collected for gene expression evaluation (Thyroid receptors; Transporter proteins and activation enzymes) by qPCR. The gene expression of all thyroid hormone receptors (TRα1; TRβ1; TRβ2) of animals exposed were altered in comparison to controls as well as the proteins of thyroid hormone transport. The gene expression of enzyme conversion of active thyroid hormone, iodothyronine deiodinase 2 and 3 (DIO2/DIO3) were altered in exposed animals in comparison to controls likewise. The exposure to GBHs disturbs the gene expression involved in thyroid hormone homeostasis in cerebellum of adulthood animals that were exposed to GBHs by pregnant mothers.

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Brain bioenergetics in rats with acute hyperphenylalaninemia

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Phenylketonuria (PKU) is a disorder of phenylalanine (Phe) metabolism caused by impaired phenylalanine hydroxylase activity, resulting in increased levels of Phe and its metabolites in fluids and tissues of patients. PKU patients present neurological signals and symptoms, such as hypomyelination and intellectual deficit. It has been shown that oxidative stress, calcium homeostasis and neurotransmitters metabolism are affected in

PKU. The aim of this study was to analyze brain bioenergetics in the pathophysiology of hyperphenylalaninemia (HPA). For this study, 30-day old Wistar rats were randomized in two groups (n=6 per group). HPA group received a single subcutaneous administration of Phe (5.2 μmol/g) plus p-Cl-Phe (PAH inhibitor) (0.9 μmol/g). Control group received a single injection of NaCl 0.9%. One hour after injections animals were euthanatized and cerebral cortex, hippocampus and striatum were collected. The activities of lactate dehydrogenase (LDH), creatine kinase (CK), Krebs cycle enzymes and respiratory chain complexes were measured. It was also assayed concentrations of glycogen, free and total phosphate levels, as well as mRNA expression for PGC-1α, TFAM, NFR-1, SIRT3, SIRT5, MFN1, Polγ and DRP-1. In cerebral cortex, HPA group showed decreased CK activity, glycogen levels, complex I-III and IV activities and CS activity, as well as increased LDH (pyruvate-lactate), α-KGDH and IDH activities, and the levels of free and total Pi. In striatum, HPA animals presented increased LDH (pyruvatelactate) and IDH activities, and decreased α-KGDH and complex IV activities. In hippocampus, HPA rats had increased α-KGDH and IDH activities, decreased complex I and IV activities, and increased mRNA expression for MFN1. Our data demonstrated impaired bioenergetics in cerebral cortex, striatum and hippocampus of HPA rats. In conclusion, it may be speculated that disruption of brain bioenergetics is involved in neuropathology seen in PKU patients.

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Carnosine alters redox homeostasis in cultured primary cortical astrocytes

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Carnosine is an imidazole dipeptide with alleged neuroprotective activity. However the exact role of carnosine in different cell types remains controversial. The objective of the present study was to investigate the mechanisms involved in the biological effects of carnosine on bioenergetics and redox homeostasis, as well as cell morphology and viability in cultured primary cortical astrocytes. Cerebral cortices from one- to two-day-old Male Wistar rats were used. Primary astrocytic cultures were prepared carnosine was added to the incubation medium in increasing concentrations: 0.1, 1 and 5 mM. Morphological, biochemical and cell viability tests were performed after 72 hours of pre-incubation. More than 92% of the cultured cells were GFAP-positive. It is observed that the morphology of the astrocytes is modified with increasing carnosine concentrations and it is possible to observe several reactive astrocytes at the highest tested concentration. Carnosine also caused an increase in DCF-DA oxidation, apparently in a dose-dependent manner. The formation of mitochondrial superoxide anion was enhanced with increasing concentrations of carnosine. Our next step was to evaluate lipid peroxidation of astrocytes exposed carnosine and found an increase of this parameter at 5 mM carnosine. Finally, the mitochondrial content using Mitotracker probe in cultured primary cortical astrocytes was evaluated and it was identified a decrease in the mitochondrial content with increased carnosine concentrations. Taken together, the results of the present study demonstrated that high concentrations of carnosine may impair mitochondrial homeostasis in cultured primary cortical astrocytes.

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Colonic inflammation is accompanied by glial alterations in 6-OHDA mouse model of Parkinson's disease

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Parkinson's disease (PD) is classically known as a neurodegenerative disease that affects the central nervous system. However, currently, researchers already classifies as a gastrointestinal (GI) illness that affects the enteric nervous system (ENS). The ENS is recognized as second brain due to its independent action e complex signalling. The ENS is composed of a neuroglial network that extends throughout the GI tract. During the last decades several aspects of glial cells phisiology were discovered including its role in the modulation of the enteric neurotransmission (impacting directly on enteric functions, such as GI motility) and its interaction with immune system that is abundant in the intestines. Thus, the objective of this work is to study the state of colonic SNE through the animal model of Parkinson's disease induced by 6-hydroxydopamine (6-OHDA). In particular, the aim is access inflammatory and glial changes in different the layers of the colon. Methods: Adult C57Bl6 mice were subjected to unilateral administration of 6-hydroxydopamine (6-OHDA) (a neurotoxin, analogue of dopamine) in the left striatum region by stereotactic procedures for induction of a Parkinson's disease model. Another group of animals operated uninjured was used as control. The groups had survival times of 1, 2 and 4 weeks. The large intestine of the animals from both groups were removed, divided into oral and anal portion and processed to be used in different techniques such as histochemistry (Hematoxylin & Eosin staining), immunofluorescence for localization of IBA1 protein (a macrophagic biomarker) and GFAP protein (glial cells biomarker) and western blotting for detection of GFAP protein in the neuromuscular layer. Morphological analysis showed an inflammatory response in both oral and anal colonic tissue of animals subjected to 6-OHDA model of Parkinson's disease as indicated by the presence of inflammatory infiltrate, and an intense tissue disruption – with edema formation and loss of cytoarchitecture. The immunofluorescence for IBA1 showed an increased expression of this macrophage protein in mucosal layer in all analyzed survival times and in both portions of the colon when compared to control animals. Concomitantly, the immunofluorescence for GFAP in 1 and 2w post lesion times showed an increased expression in mucosal layer. One week after 6-OHDA administration, animals showed an increase in GFAP expression in the neuromuscular layer of both oral and anal portions of the colon. Within two weeks, significant GFAP changes were not observed between groups in the neuromuscular layer. In the last time window we observed an increase in GFAP levels in the anal muscle layer of the animals treated with 6-OHDA compared to the control group. Conclusion: The animal model of Parkinson's disease induced by 6-OHDA develop an inflammatory response in the gastrointestinal tract, specifically the colon. The colonic tissue from animals treated with 6-OHDA showed an expressive alteration of the mucosal layer. Macrophage population in this region increased as revealed by augmented expression of IBA1 protein. In addition, the glial protein GFAP were altered over the period studied in mucosal and neuromuscular intestinal layers. Increase in GFAP levels after lesion is associated with glial reactivity and/or glial proliferation. It is possible that the reactive glia interactions stablished with imune cells contribute to inflammatory responses involved in the pathologic scenarium observed in this Parkinson's disease animal model.

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Doxycycline can efficiently switch alpha-synuclein early aggregation oligomers into non-toxic species: repurposing an old drug as neuroprotector

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Synucleinophaties are progressive disorders with no cure to date. An attractive strategy to tackle this problem is discovering new uses for approved drugs to provide the quickest possible transition from bench to bedside.We used a combination biophysical techniques like fluorescence and infrared spectroscopy, electronic microscopy, small angle X Ray scattering and NMR together with cellular biology approaches to assess the impact of doxycycline on α-synuclein aggregation. We demonstrate the ability of doxycycline to interfere with the pathologic cycle involved in synucleinopathies at the aggregation level. We proved that doxycycline interacts with alpha-synuclein early aggregation intermediates leading to the formation of off-pathway species, with parallel beta-sheet content, that do not evolve into fibril formation. These aggregates are neither cytotoxic to dopaminergic cell lines, nor capable of disrupting the integrity of liposomes membrane. Furthermore, doxycycline is also able to block the seeding capacity of alpha-synuclein preformed aggregates. This novel mechanism of action proposed for doxycycline might act in a synergistic way with its anti-inflammatory and antioxidant properties to exert neuroprotection on animal models in vivo. Moreover, administration of doxycycline at subantibiotic doses would deliver a concentration high enough in the brain to interfere with the production of α-synuclein toxic species. These results place doxycycline as a pleiotropic drug becoming an attractive therapeutic strategy against synucleinopathies.

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Effect of nanocapsules with naringin and naringenin against brain damage induced by streptozotocin in mice

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Oxidative stress contributes to the pathophysiology of neurological diseases. An innovative alternative to induce protection for brain is the use of antioxidant bioactive compounds, such as citric fruit flavonoids, naringin (NA) and naringenin (NAR). However, these substances have low bioavailability when administered orally. Na alternative to overcome this limitation is the use of nanocarriers, such as nanocapsules (NC), which can increase bioavailability and improve effect of the active substance. The objective of the study is evaluating neuroprotective effect of naringin and naringenin in a model of brain damage induced by streptozotocin (STZ) in mice. The NA and NAR loaded-NC at the concentration of 2 mg/mL for each were produced by the technique of interfacial deposition of preformed polymer. Mice were pretreatment for 15 days orally (v.o.), once daily with NC. The animals received 10 mg/kg of suspension of free-active NC (FN), NA and NAR loaded-NC (NNAR), or suspension of NA and NAR in free form (FNAR). At 16th day, stereotactic surgery was performed for STZ infusion. Subsequently, the animals were left for 15 days without any manipulations. At 31st day, aversive memory of mice was evaluated by inhibitory avoidance task. Just after cognitive test, animals were killed, brain removed for oxidative stress assays: reduced glutathione (GSH), catalase (CAT) activity, determination of lipid peroxidation by acid reactive substances (TBARS), protein oxidation by carbonyls measure. Mice treated with STZ had memory impairment assessed in inhibitory avoidance task, and pre-treatment with NNAR prevented this cognitive deficit. Regarding the levels antioxidants in brain, there were no changes in the levels of GSH and CAT. However, STZ induced increased TBARS and protein carbonyl levels and NNAR prevented. The present study demonstrates that pre-treatment with NNAR prevented memory deficits, and lipid and protein oxidation caused by STZ in mice. The results showed for the first time the protective effect of NC within bioactive compounds associated at animal model of damage to brain.

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NAC decreases both dopaminergic cell death and inflammatory changes in old-Parkinsonian mice

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Experimental and clinical evidence suggests that inflammatory factors, controlled by glial cells may play an important role in the development of neurodegenerative processes associated with Parkinson's disease. Even though PD is an aging-related disorder, only few studies have been performed in old animals. Otherwise, it has been demonstrated that c-Jun N-terminal kinase (JNK), an important kinase member of the MAPK family, is implicated in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxic mechanisms. Therefore, the inhibition of JNK could prevent or delay the dopaminergic injury of MPTP intoxication and the secondary inflammatory response. In this study, we analyzed two different agents which mechanisms of action are related to JNK pathway: i) the N-acetyl-L-cysteine (NAC), a glutathione precursor, and ii) HA-1077, a ROCKinase inhibitor and microglia polarizer. Additionally, we evaluated the combination of both treatments (NAC+HA-1077). 75 twenty-weeks-old C57BL/6 mice were used in this study; 33 of them were control groups and the other 42 animals were acutely intoxicated with MPTP and divided into 4 groups: i) MPTP; ii) MPTP+NAC; iii) MPTP+HA1077; iv) MPTP+NAC+HA1077. Postmortem quantitative analysis showed a significant decrease of TH expression in the SNpc and in the striatum as well as significant increased Iba-1 and GFAP levels in all MPTP-animals comparing with control groups. However, old-Parkinsonian mice treated with NAC had TH+ cells and
fibers, GFAP and Iba-1 expression similar to control animals. Surprisingly, microglial and astroglial cells were significantly increased in MPTP-intoxicated animals treated with both drugs (NAC+HA1077) compared with all the other MPTP groups. Unfortunately, these unexpected results discard the use of the combined treatment (HA1077+NAC) in old mice. However, NAC treatment does have significant neuroprotective effects in old Parkinsonian mice probably due to its anti-oxidant properties (associated to JNK inhibition).

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Opposing roles for ascorbic acid and glutathione in the chick embryonic retina

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Neuroglia interactions are essential, they ensure signaling and metabolism homeostasis in central or peripheral nervous systems. The retinal environment is a well conserved neuron-glia structure, of which several features are common between animal species, ranging from avian to mammals. In this specialized and active tissue, there is large demand for effective antioxidant agents. Glutathione (GSH) and ascorbic acid (AA) are the main agents protecting the retina from stress, and now data point towards a communicating role for these antioxidants. Previous works suggest that GSH, in addition to a redox role, could act signaling calcium shifts at the millimolar range, particularly in Müller glia, and could regulate the release of GABA, with additional protective effects on retinal neuroglial circuit. Similarly, AA is capable of highly selective modulation of calcium signaling in Müller glia at the milimolar range, but is incapable of inducing GABA release from both neurons and glial cells. Also, toxicity data suggest that GSH is a highly potent reducing agent in the retina, preventing cell death, production of reactive oxygen species (ROS) and H2O2-induced [Ca2+]i stress, while AA may even extenuate previously existent stress conditions by promoting further synthesis of ROS and delaying the decrease of [Ca2+]i levels, as measured by live single cell calcium imaging.

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Running for REST: the effects of exercise in the hippocampus of aged mice

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Exercise improves mental health and synaptic function in the brain during aging. However, the molecular mechanisms involved in this phenomenon are little understood. The modulation of the repressor element RE1-binding transcription factor (REST) and the inflammatory state by the exercise are possible mechanisms. To evaluate the effect of voluntary exercise performed in running wheels (RW) in the sickness behavior and hippocampal neuroplasticity in adult and aged C57BL/6 mice. Material and Methods: C57BL/6 male mice of 4-6 months of age (young) and 19-21 months old (aged) (Ethics Commitee Protocol PP00760) which were divided in four groups: sedentary young (SED-young) and aged (SED-aged), and running wheel young (RW-young) and aged (RW-aged). All animals were isolated for eight weeks and the RW groups (young and aged) had free access to individual RW; while the SED groups had a locked RW. The animals daily distances races were measured by digital odometers. After 8 weeks, they were subjected to open field and tail suspension behavioral tasks, posteriorly euthanized to dissection of the hippocampus. The REST gene expression and BDNF were analyzed by RT-PCR. The aged animals exhibit a depressive-like and sickness behavior: less mobility in RW and in the open field, and great immobility in the tail suspension test. Gene expression showed a low profile neuroplasticity and high neuroinflammation in the hippocampus of aged animals. Exercise was anxiolytic and antidepressant, and improved motor behavior of aged animals. Exercise also boosted BDNF and REST expression, and decreased IL-1β and IL-10 expression in the hippocampus of aged animals. Conclusions: These data support a beneficial role of REST in the aged hippocampus, which can be further enhanced by regular exercise.

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Tnf-alpha plays a role in neuroplasticity mediated by microglia activation after monocular enucleation

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Lesions in the central nervous system often induce structural reorganization within intact circuits of the brain. However, cellular and molecular mechanisms involved in lesion-induced plasticity remain unknown. Calcineurin (CaN), a phosphatase associated with synaptic pruning and immune function also mediates microglia activation and TNF-a release. Here we evaluated the role of microglia activation and TNF-a in the sprouting of intact retinotectal axons following monocular enucleation (ME). Lister Hooded rats were submitted to ME at P10. Animals received systemic injections of cyclosporin A (50mg/kg, sc) or minocycline (125 mg/kg, sc) 3h following ME. A third experimental group received local delivery (ELVAX) of a TNF-a neutralizing antibody 3 days before ME. Neuroanatomical tracers mapped structural plasticity while immunofluorescence and western blot were used to study microglia morphology, TNF-a and CaN content. A progressive increase of activated microglia in the contralateral superior colliculus (SC) 24h after ME, peaking at 72h presented a temporal correlation with an increase in CaN immunoreactivity. Inhibition of microglia or TNF-a reduced sprouting of intact uncrossed retinotectal axons, amoeboid microglia and TNF-a expression. The treatment effect was reverted after longer survival time, showing the absence of neurotoxicity. Taken together our data support the hypothesis that TNF-a released by microglia may regulate neuroplasticity induced by lesions during early brain development. This study was approved by the local animal care committee (CEUA/UFF - protocol 0015109).

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Toxicity evaluation of nanoparticles with simvastatin in hippocampal slices from rats and C. elegans

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Statins are cholesterol-lowering agents due to the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Studies in human and rodents have demonstrated preventive effect of statins against neurological diseases (PARLE et al., 2007; WU et al., 2008). The nanoparticles (NPS) have become an important focus of therapeutic research on brain because they are an especially effective form of drug delivery thought BBB. However, there is a need to evaluate the toxicity of these nanoparticles. The objective of the work is to produce two NPS with SIN by different methods, a nanoemulsion (NES) and a nanocapsule (NS), both containing SIN, and to evaluate the toxicity in vitro and in vivo. The NS at the concentration of 2 mg/mL produced by the technique of interfacial deposition of preformed polymer described by Fessi et al. (1989) and adapted by Venturini et al (2011). NES containing 1 mg/mL were prepared by according to described by Santos-Magalhães et al (2000) by the spontaneous emulsification method with some adaptations. The nanoformulations produced evaluated for the physico-chemical characteristics through the distribution of particle size, polydispersity index, Zeta potential determination and pH. The toxicity of NES was evaluated in vitro at concentrations of 0.1 µg/mL; 1 µg/mL and 10 µg/mL in hippocampal slices from rats. The in vivo toxicity test was performed with C. elegans. The nematodes were exposed to 300 µL, 600 µL and 800 µL of the NES, NS, or free-drug nanoformulations (FN) for 1 h. After 24 h at 20 °C, the animals were quantified as live or dead. The NS showed particle size to 226 nm. The polydispersity index was below 0.2 for all formulations prepared. The Zeta potential was close to -8.4 mV and the formulations pH was close to neutrality (+/- 6.6). The NES or NB showed particle size close to 204 nm and 139 nm respectively. The polydispersity index was below 0.3 for all formulations. The Zeta potential was close to -3 mV for FN and -5 mV for the NES. The pH approximately 6.7. There was no toxicity indication after NES treatment in the hippocampal slices viability measured by MTT assay. The survival rate in the in vivo test with C. elegans did not change with treatment with all nanoformulations compared to the control group. The results indicate security of nanoformulations for biological assays. Nevertheless, further studies need to be performed to evaluate whether simvastatin nanoformulations can be used as therapeutic agent at acute or chronic treatment.

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