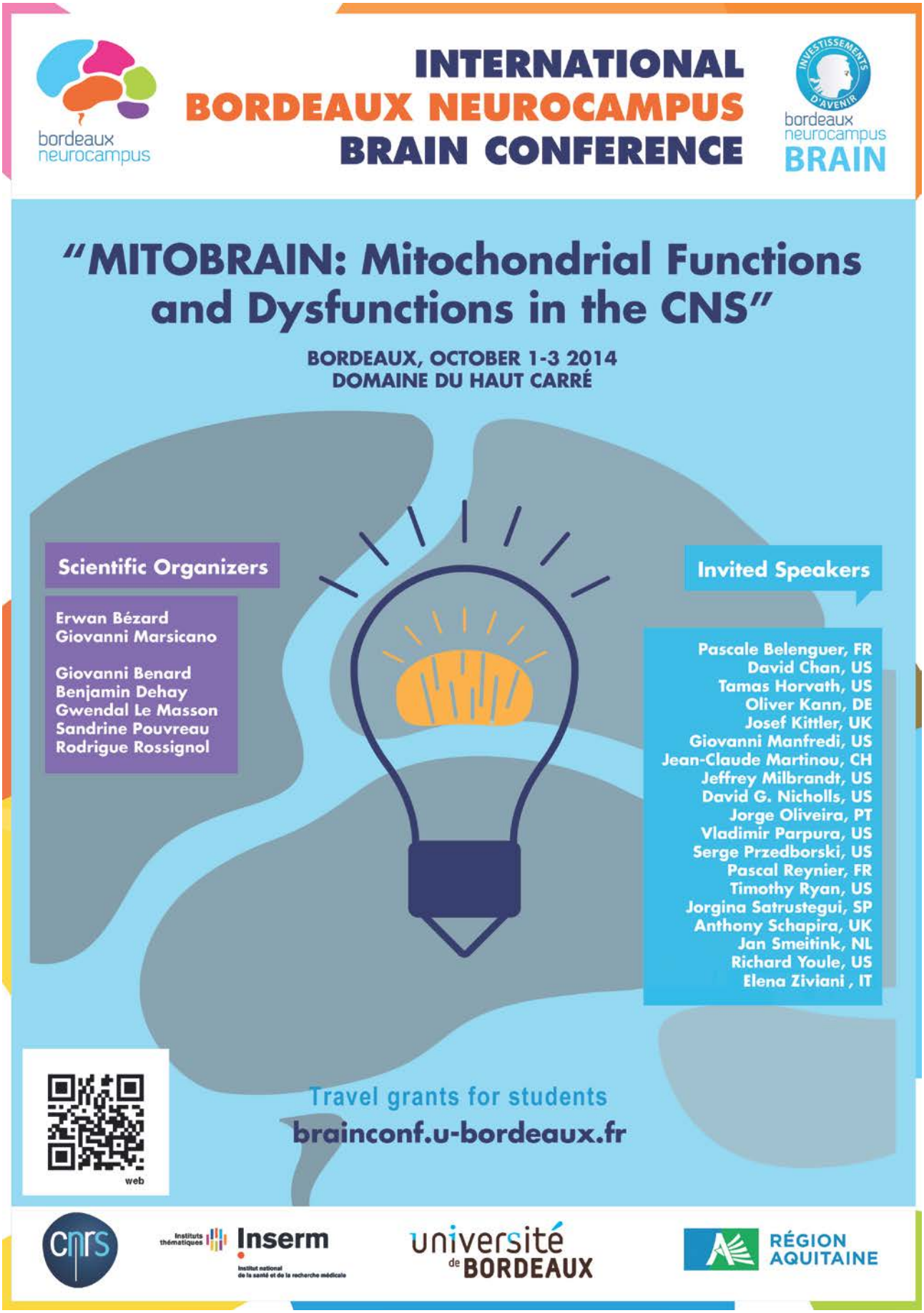
# 1st International Bordeaux Neurocampus/Brain Conference

## MitoBrain - 2014



# 1st International Bordeaux Neurocampus/Brain Conference
# MitoBrain - 2014

**The first edition of the International Bordeaux Neurocampus / Brain Conferences is scheduled in October 1 - 3, 2014. "MitoBrain" for 'Mitochondrial Functions and Dysfunctions in the Central Nervous System', will focus on the role of mitochondria in the cells of the Central Nervous System (neurons and glia), in normal and pathological states.**

This meeting aims to promote dialogue between international experts on the role of mitochondria applied to physiological and pathological processes of the CNS and to improve the therapeutic challenges.
More than 200 researchers, coming from different countries and from Neurosciences or Bioenergetics disciplines, will present and share their results.
Renowned Speakers will be there to brainstorm with scientists, in order to generate innovation, creativity and therapeutic perspectives. Some representatives of leading healthcare companies and innovative biotech start-ups will join the conference.

**The Invited Speakers** are:

| | | |
|---|---|---|
| Pascale BELENGUER | France (Paul Sabatier University, Toulouse) | PhD, Prof. |
| David CHAN | USA (California Instit. of Technology, Pasadena) | MD, PhD, Prof. |
| Tamas HORVATH | USA (Yale University, New Haven) | DVM, PhD, Prof. |
| Oliver KANN | Germany (Inst. Pathophysiology, Heidelberg) | MD, Prof. |
| Josef KITTLER | United Kingdom (University College of London) | PhD |
| Giovanni MANFREDI | USA (Weill Cornell Medical College, Ithaca) | MD, PhD |
| Jean-Claude MARTINOU | Switzerland (University of Geneva, Geneva) | PhD, Prof. |
| Jeffrey MILBRANDT | USA (Washington University, St. Louis) | MD, PhD |
| David G. NICHOLLS | USA (Buck Institute California, Novato) | PhD, Prof. |
| Jorge OLIVEIRA | Portugal (University of Porto) | PhD |
| Vladimir PARPURA | Croatie (Univ. Alabama, and Univ. of Rijeka) | MD, PhD |
| Serge PRZEDBORSKI | USA (Columbia University, New York) | MD, PhD |
| Pascal REYNIER | France (University of Angers) | MD, Prof. |
| Timothy RYAN | USA (Weill Cornell Medical College, Ithaca) | PhD, Prof. |
| Jorgina SATRUSTEGUI | Spain (CBMSO, Madrid) | PhD |
| Anthony SCHAPIRA | United Kingdom (Univ. College of London) | MD, PhD, Prof. |
| Jan SMEITINK | Netherlands (Nijmegen CMD & Khondrion) | MD, PhD, Prof. |
| Richard YOULE | USA (National Institute of Health, Bethesda) | PhD |
| Elena ZIVIANI | Italy (University of Padova) | PhD, Prof. |



## Organizing Committee:

| | |
|---|---|
| Erwan BEZARD | France - Institut des maladies neurodégénératives, IMN<br>Directeur de recherche Inserm, PhD |
| Giovanni MARSICANO | Italy - NeuroCentre Magendie, NCM<br>Directeur de recherche Inserm, PhD |
| Sandrine POUVREAU | France - Institut interdisciplinaire de neurosciences, IINS<br>Chargée de recherche CNRS, PhD |
| Giovanni BENARD | France - NeuroCentre Magendie, NCM<br>Chargé de recherche CNRS, PhD |
| Benjamin DEHAY | France - Institut des maladies neurodégénératives, IMN<br>Chargé de recherche Inserm, PhD |
| Gwendal LE MASSON | France - NeuroCentre Magendie, NCM<br>PU-PH, MD, PhD |
| Rodrigue ROSSIGNOL | France - Maladies rares : génétique et métabolisme, MRGM<br>Chargé de recherche Inserm, PhD |

## The Organizing Committee thanks the institutional and academic partners:

## as well as industrials which supported the conference:

The *Company of Biologists* gave 3 grants to help students and young scientists to attend the conference

# MITOBRAIN AT A GLANCE

## Wednesday, October 1st

**08:00 - 09:30** **REGISTRATION**

09:30-09:45 Welcome Remarks by Christophe Mulle

**09:45-17:30** - **MITOCHONDRIAL PHYSIOLOGY IN THE CNS**
*Chaired by Sandrine Pouvreau (a.m.) & Giovanni Bénard (p.m.)*

| | | | |
|---|---|---|---|
| 09:45-10:15 | Richard Youle | – | IS 001 |
| 10:15-10:45 | Giovanni Marsicano | – | S 002 |
| *10:45-11:15* | *Coffee break* | | |
| 11:15-11:30 | Alfredo Gimenez-Cassina | – | ST 1 |
| 11:30-12:00 | Oliver Kann | – | IS 003 |
| 12:00-12:30 | Josef Kittler | – | IS 004 |
| *12:30-15:00* | *Lunch break + Poster session* | | |
| 15:00-15:30 | David Nicholls | – | IS 005 |
| 15:30-15:45 | Francesc Soriano | – | ST 2 |
| 15:45-16:15 | Jorgina Satrustegui | – | IS 006 |
| *16:15-16:45* | *Coffee break* | | |
| 16:45-17:00 | Jordi Duran | – | ST 3 |
| 17:00-17:30 | Jean-Claude Martinou | – | IS 007 |

## Thursday, October 2nd

**09:00-12:30** - **MITOCHONDRIAL PHYSIOLOGY IN THE CNS**
*Chaired by Gwendal Le Masson*

| | | | |
|---|---|---|---|
| 09:00-09:30 | David Chan | – | IS 008 |
| 09:30-10:00 | Timothy Ryan | – | IS 009 |
| 10:00-10:15 | Ciro Leonardo Pierri | – | ST 4 |
| 10:15-10:45 | Pascale Belenguer | – | IS 010 |
| *10:45-11:15* | *Coffee break* | | |
| 11:15-11:30 | Marion Szelechowski | – | ST 5 |
| 11:30-12:00 | Jeffrey Milbrandt | – | IS 011 |
| 12:00-12:30 | Tamas Horvath | – | IS 012 |
| *12:30-15:00* | *Lunch break + Poster session* | | |

**15:00-17:45** - **MITOCHONDRIAL PATHOPHYSIOLOGY IN THE CNS**

*Chaired by Rodrigue Rossignol*

| | | | |
|---|---|---|---|
| 15:00-15:30 | Elena Ziviani | – | IS 013 |
| 15:30-16:00 | Gwendal Le Masson | – | S 014 |
| 16:00-16:15 | Dirk Mossmann | – | ST 6 |
| *16:15-16:45* | *Coffee break* | | |
| 16:45-17:15 | Serge Przedborski | – | IS 015 |
| 17:15-17:45 | Antony Schapira | – | IS 016 |

***19:30 -23:30*** ***Gala dinner*** *at Château Smith Haut-Lafitte*

## Friday, October 3rd

**09:00-12:00** - **MITOCHONDRIAL PATHOPHYSIOLOGY IN THE CNS**

*Chaired by Erwan Bézard*

| | | | |
|---|---|---|---|
| 09:00-09:30 | Pascal Reynier | – | IS 017 |
| 09:30-10:00 | Jorge Oliveira | – | IS 018 |
| 10:00-10:30 | Jan Smeitink | – | IS 019 |
| *10:30-11:00* | *Coffee break* | | |
| 11:00-11:30 | Giovanni Manfredi | – | IS 020 |
| 11:30-12:00 | Vladimir Parpura | – | IS 021 |

# MITOBRAIN PROGRAMME

## Wednesday, October 1st

**08:00 - 09:30** **REGISTRATION**

09:30-09:45 Welcome Remarks by Christophe Mulle

**09:45-17:30** - **MITOCHONDRIAL PHYSIOLOGY IN THE CNS**
*Chaired by Sandrine Pouvreau (a.m.) & Giovanni Bénard (p.m.)*

09:45-10:15 **Richard YOULE**, National Institute of Health, Bethesda (US)
*Damage Control - How the Pink1/Parkin pathway can regulate removal of impaired mitochondria by autophagy*

10:15-10:45 **Giovanni MARSICANO**, Université de Bordeaux (FR)
*Mitochondrial cannabinoid receptor $CB_1$ ($mtCB_1$) in the brain*

10:45-11:15 *Coffee break*

11:15-11:30 **Alfredo GIMENEZ-CASSINA**, Harvard Medical School, Boston (US)
*Programming carbon substrate metabolism for control of neuronal excitation: a role for BAD*

11:30-12:00 **Oliver KANN**, University of Heidelberg (DE)
*Bioenergetics of fast neuronal network oscillations*

12:00-12:30 **Josef KITTLER**, University College of London (GB)
*Regulation of mitochondrial transport in neurons by the Miro1 and Miro2 GTPases*

12:30-15:00 *Lunch break + Poster session*

15:00-15:30 **David NICHOLLS**, Buck Institute for Research on Aging, Novato (US)
*Assessing mitochondrial function and dysfunction in cultured neurons and isolated nerve terminals: advantages and limitations*

15:30-15:45 **Francesc SORIANO**, University of Barcelona (SP)
*Mfn2 downregulation in excitotoxicity causes mitochondrial dysfunction and delayed neuronal death*

15:45-16:15 **Jorgina SATRUSTEGUI**, CBMSO, Madrid (SP)
*Regulation by $Ca^{2+}$ of mitochondrial respiration in neurons: malate aspartate shuttle and calcium uniporter*

16:15-16:45 *Coffee break*

16:45-17:00 **Jordi DURAN**, IRB Barcelona (SP)
*Physiological and pathological roles of glycogen in brain energy metabolism*

17:00-17:30 **Jean-Claude MARTINOU**, University of Geneva (CH)
*Role of the mitochondrial pyruvate carrier in neuronal function*

## Thursday, October 2nd

### 09:00-12:30 - MITOCHONDRIAL PHYSIOLOGY IN THE CNS

*Chaired by Gwendal Le Masson*

09:00-09:30 **David CHAN**, California Institute of Technology, Pasadena (US)
*Proteolytic cleavage of Opa1 stimulates mitochondrial inner membrane fusion and couples fusion to oxidative phosphorylation*

09:30-10:00 **Timothy RYAN**, Weill Cornell Medical College, Ithaca (US)
*Energy consumption and supply at nerve terminals*

10:00-10:15 **Ciro Leonardo PIERRI**, University of Bari (IT)
*Mitochondrial carrier related neurological disorders*

10:15-10:45 **Pascale BELENGUER**, Paul Sabatier University, Toulouse (FR)
*OPA1 loss of function affects in vitro neuronal maturation and functioning through mitochondrial alterations*

10:45-11:15 *Coffee break*

11:15-11:30 **Marion SZELECHOWSKI**, CPTP Toulouse (FR)
*Bornavirus X protein localizes in neuronal mitochondria and mediates neuro- protection through stabilization of the mitochondrial chaperone Hspa9*

11:30-12:00 **Jeffrey MILBRANDT**, Washington University in Saint-Louis (US)
*Glial metabolism and axonal maintenance*

12:00-12:30 **Tamas HORVATH**, Yale University, New Haven (US)
*Brain Mitochondrial Dynamics in Integrative Physiology*

12:30-15:00 *Lunch break + Poster session*

### 15:00-17:45 - MITOCHONDRIAL PATHOPHYSIOLOGY IN THE CNS

*Chaired by Rodrigue Rossignol*

15:00-15:30 **Elena ZIVIANI**, Padova University (IT)
*Balanced ubiquitination and de-ubiquitination of Mfn2 in the regulation of ER-Mito tether: role of Parkin and its opposing DUB in the onset of Parkinson's Disease*

15:30-16:00 **Gwendal LE MASSON**, University of Bordeaux (FR)
*A theoretical framework to study bioenergetics and its relationships to pathophysiology in neuronal diseases: the example of ALS*

16:00-16:15 **Dirk MOSSMANN**, University of Freiburg (DE)
*Amyloid-β peptide induces mitochondrial dysfunction by inhibition of preprotein maturation*

16:15-16:45 *Coffee break*

16:45-17:15 **Serge PRZEDBORSKI**, Columbia University, New York (US)
*The tale of mitochondria in neurodegeneration*

17:15-17:45 **Anthony SCHAPIRA**, University College of London (GB)
*Glucocerebrosidase in Parkinson disease: a meeting of organelles*

19:30-23:30 **Gala dinner** at Château Smith Haut-Lafitte

## Friday, October 3rd

**09:00-12:00** - **MITOCHONDRIAL PATHOPHYSIOLOGY IN THE CNS**
*Chaired by Erwan Bézard*

09.00-09.30 **Pascal REYNIER**, University of Angers (FR)
*Clinical spectrum and pathophysiology of OPA1 dysfunctions*

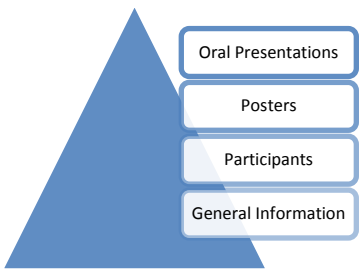
09.30-10.00 **Jorge OLIVEIRA**, University of Porto (PT)
*Modulation of neuronal mitochondrial dynamics and huntingtin aggregation by selective HDAC inhibitors*

10:00-10:30 **Jan SMEITINK**, Nijmegen Centre for Mitochondrial Disorders & Khondrion (NL)
*Mitochondrial drug development: from bench to bedside*

10.30–11.00 *Coffee break*

11.00–11.30 **Giovanni MANFREDI**, Weill Cornell Medical College, New York (US)
*Mitochondrial dysfunction in familial ALS*

11.30-12.00 **Vladimir PARPURA**, Univ. Alabama, Birmingham (US)
*The role of mitochondria in exocytotic glutamate release from astrocytes*



# INVITED SPEAKERS - IS

IS 001 Richard YOULE – *Damage control - How the Pink1/Parkin pathway can regulate removal of impaired mitochondria by autophagy*

S 002 Giovanni MARSICANO - *Mitochondrial cannabinoid receptor $CB_1$ ($mtCB_1$) in the brain*

IS 003 Oliver KANN - *Bioenergetics of fast neuronal network oscillations*

IS 004 Josef KITTLER - *Regulation of mitochondrial transport in neurons by the Miro1 and Miro2 GTPases*

IS 005 David NICHOLLS - *Assessing mitochondrial function and dysfunction in cultured neurons and isolated nerve terminals: advantages and limitations*

IS 006 Jorgina SATRUSTEGUI - *Regulation by $Ca^{2+}$ of mitochondrial respiration in neurons: malate aspartate shuttle and calcium uniporter*

IS 007 Jean-Claude MARTINOU - *Role of the mitochondrial pyruvate carrier in neuronal function*

IS 008 David CHAN - *Proteolytic cleavage of Opa1 stimulates mitochondrial inner membrane fusion and couples fusion to oxidative phosphorylation*

IS 009 Timothy RYAN – *Energy Consumption and Supply at nerve terminals*

IS 010 Pascale BELENGUER - *OPA1 loss of function affects in vitro neuronal maturation and functioning through mitochondrial alterations*

IS 011 Jeffrey MILBRANDT - *Glial metabolism and axonal maintenance*

IS 012 Tamas HORVATH - *Brain mitochondrial dynamics in integrative physiology*

IS 013 Elena ZIVIANI - *Balanced ubiquitination and de-ubiquitination of Mfn2 in the regulation of ER-Mito tether: role of Parkin and its opposing DUB in the onset of Parkinson's Disease*

S 014 Gwendal LE MASSON - *A theoretical framework to study bioenergetics and its relationships to pathophysiology in neuronal diseases: the example of ALS*

IS 015 Serge PRZEDBORSKi - *The tale of mitochondria in neurodegeneration*

IS 016 Anthony SCHAPIRA - *Glucocerebrosidase in Parkinson disease: a meeting of organelles*

IS 017 Pascal REYNIER - *Clinical spectrum and pathophysiology of OPA1 dysfunctions*

IS 018 Jorge OLIVEIRA - *Modulation of neuronal mitochondrial dynamics and huntingtin aggregation by selective HDAC inhibitors*

IS 019 Jan SMEITINK - *Mitochondrial drug development: from bench to bedside*

IS 020 Giovanni MANFREDI - *Mitochondrial dysfunction in familial ALS*

IS 021 Vladimir PARPURA - *The role of mitochondria in exocytotic glutamate release from astrocytes*

# SELECTED TALKS - ST

ST 3 Jordi DURAN - *Physiological and pathological roles of glycogen in brain energy metabolism*

ST 5 Cécile FERRE - *Bornavirus X protein localizes in neuronal mitochondria and mediates neuroprotection through stabilization of the mitochondrial chaperone Hspa9* - Talk presented by Marion SZELECHOWSKI

ST 1 Alfredo GIMENEZ-CASSINA - *Programming carbon substrate metabolism for control of neuronal excitation: a role for BAD*

ST 6 Dirk MOSSMANN - *Amyloid-β peptide induces mitochondrial dysfunction by inhibition of preprotein maturation*

ST 4 Ciro Leonardo PIERRI - *Mitochondrial carrier related neurological disorders*

ST 2 SORIANO Francesc - *Mfn2 downregulation in excitotoxicity causes mitochondrial dysfunction and delayed neuronal death*

# ABSTRACTS

## INVITED SPEAKERS - IS

### IS 010

## OPA1 loss of function affects in vitro neuronal maturation and functioning through mitochondrial alterations

Ambre M. BERTHOLET[1], Aurélie M.E. MILLET[1], Oriane GUILLERMIN[1], Marlène DALOYAU[1], Arnaud BONNAFFOUX[1], Brice RONSIN[1], Noélie DAVEZAC[1]*, Marie-Christine MIQUEL[1,2]* and **Pascale BELENGUER**[1]

[1] *Universités de Toulouse, Centre de Biologie du Développement, CNRS UMR5547/Université Paul Sabatier, Toulouse, France*

[2] *UPMC Université Pierre et Marie Curie, Sorbonne Universités, Paris, France*

**Co-seniorship*

Mitochondrial dynamics control the organelle's morphology, through a balance between a fusion process resulting in the formation of elongated tubules and a fission process leading to isolated puncta. This dynamic balance participates in the regulation of mitochondrial functions. Recent reports have shown that disruption of mitochondrial dynamics contributes to neurodegenerative diseases. Mutations of the inner membrane fusogenic GTPase OPA1 are responsible for dominant optic atrophy, by mechanisms not fully understood. We showed that in rodent cortical primary neurons, down-regulation of the OPA1 protein led to fragmented mitochondria that become less abundant along the dendrites and axons. Furthermore, this inhibition resulted in reduced expression of mitochondrial respiratory complexes as well as mitochondrial DNA, decreased mitochondrial membrane potential, and diminished respiratory levels. The onset of synaptogenesis was markedly impaired through reductions in pre- and postsynaptic structural protein expression and synapse numbers. Accordingly, preliminary data, by electrophysiological recordings, show that OPA1 depletion could be associated to a decrease in glutamatergic synaptic transmission. With longer time in culture, OPA1 extinction led to a major restriction of dendritic growth. Altogether, our findings suggest a new role for OPA1 in synaptic maturation and dendritic growth through maintenance of proper mitochondrial oxidative metabolism and distribution, highlighting the role of mitochondrial dynamics in neuronal functioning and providing insights into dominant optic atrophy pathogenesis, as OPA1 loss affecting neuronal maturation could lead to early synaptic dysfunction.

Synaptic alterations have been demonstrated to lie at the early onset of the pathological mechanisms leading to neuronal apoptosis in various neurodegenerative diseases, including Alzheimer's Disease. We have demonstrated that OPA1 down-regulation *per se* did not result in

neuronal apoptosis but however led to ROS over-production, which was buffered by increasing antioxidant defenses. Our preliminary data suggest that increasing oxidative imbalance, through application of the pro-oxidant rotenone, potentiates neuronal death rate. Thus, mitochondrial defects due to the loss of OPA1 could pre-sensitize neurons to further insults and, in conjunction with supplemental subthreshold stress, would finally induce apoptosis in late-onset diseases.

## IS 008

# Proteolytic cleavage of Opa1 stimulates mitochondrial inner membrane fusion and couples fusion to oxidative phosphorylation

MISHRA Prashant [1], CARELLI Valerio [3], MANFREDI Giovanni [4], **CHAN David C.** [1,2,4]

[1] *Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125.*

[2] *Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA 9112.*

[3] *IRCCS Istituto delle Scienze Neurologiche di Bologna and Department of Biomedical and NeuroMotor Sciences, University of Bologna, Via Altura 3, 40139 Bologna, Italy.*

[4] *Departments of Neurology and Neuroscience, Weill Medical College of Cornell University, 1300 York Avenue, A501, New York, NY 10065.*

Mitochondrial fusion is essential for maintenance of mitochondrial function. The mitofusin GTPases control mitochondrial outer membrane fusion, whereas the dynamin-related GTPase Opa1 mediates inner membrane fusion. We show that mitochondrial inner membrane fusion is tuned by the level of oxidative phosphorylation (OXPHOS), whereas outer membrane fusion is insensitive. Consequently, cells from patients with pathogenic mtDNA mutations show a selective defect in mitochondrial inner membrane fusion. In elucidating the molecular mechanism of respiration-stimulated fusion, we uncover that real-time proteolytic processing of Opa1 stimulates mitochondrial inner membrane fusion. OXPHOS-stimulated mitochondrial fusion operates through Yme1L, which cleaves Opa1 more efficiently under respiratory conditions. Artificial cleavage of Opa1 is sufficient to mediate inner membrane fusion, regardless of respiratory state. Proteolytic cleavage therefore stimulates the membrane fusion activity of Opa1, and this feature is exploited to dynamically couple mitochondrial fusion to cellular metabolism.

## IS 012

# Brain mitochondrial dynamics in integrative physiology

**HORVATH Tamas**
*Program in Integrative Cell Signaling and Neurobiology of Metabolism, Section of Comparative Medicine, Yale University School of Medicine, New Haven CT 06520*

The fundamental aspect of any living organism is its ability to convert organic molecules from the environment via chemical reactions utilizing oxygen for energy production, cellular structure development, differentiation and growth. Given the crucial role of mitochondria in metabolic pathways relating to oxygen, tight regulation of mitochondrial functions and dynamics must be critical for adequate energy balance of the organism. In complex organisms, such as mammals, it is also fundamental that the various tissues are coordinated in manner that the energetic needs of the whole body are most effectively met. The central nervous system plays a crucial organizational role in which the hypothalamus is a key brain center. Recent studies unmasked that mitochondrial dynamics within specific circuits of the hypothalamus are important for the proper adaptive responses of the whole body to the changing metabolic milieu. I will aim to provide an overview of the mechanisms by which mitochondria are involved in the central regulation of physiology and how mitochondrial dysfunction can lead to metabolic disorders.

## IS 003

# Bioenergetics of fast neuronal network oscillations

**KANN Oliver**
*Institute of Physiology and Pathophysiology, University of Heidelberg, Im Neuenheimer Feld 326 - D-69120 Heidelberg, Germany*

Fast neuronal network oscillations in the gamma frequency-band (30-100 Hz) in cortical structures have been implicated in higher cognitive functions such as sensory perception, attentional selection and memory formation. However, little is known about the bioenergetics of gamma oscillations. We addressed this issue by combining electrophysiological recordings, oxygen sensor microelectrodes and live-cell fluorescence imaging in slice preparations of the rodent hippocampus. We show that (i) gamma oscillations are associated with high energy utilization that requires rapid adaptation of mitochondrial oxidative metabolism, and (ii) gamma oscillations are exquisitely sensitive to metabolic stress as induced by lowering oxygen availability or mitochondrial poisoning. These findings might help to explain the exceptional vulnerability of higher cognitive functions during pathological processes of the brain, such as vascular disorders, mitochondrial diseases and neurodegeneration.

## IS 004

# Regulation of mitochondrial transport in neurons by the Miro1 and Miro2 GTPases

**KITTLER Josef**
*Department of Neuroscience, Physiology and Pharmacology, University College London, Gower Street, London, WC1E 6BT, UK*

Regulated trafficking of mitochondria in neurons is essential for providing ATP at the correct spatial location to power neural function and computation, and for providing $Ca^{2+}$ buffering at sites of $Ca^{2+}$ entry or release. Indeed the regulation of mitochondrial distribution, morphology and function are proposed to play an important role in neuronal and synaptic development but the regulatory mechanisms remain unclear. Miro family proteins (Miro1 and Miro2 in mammals) contain a transmembrane domain locating them to the outer mitochondrial membrane, along with two GTPase domains and two $Ca^{2+}$- sensing EF-hand domains that face into the cytosol, and play a key role in regulating mitochondrial transport. We previously showed that Miro1 mediates mitochondrial trafficking in neurons by linking mitochondria to the motor protein KIF5 for their transport in dendrites. Moreover, through its $Ca^{2+}$-sensing EF-hand domains Miro1 can stop mitochondria at individual activated synapses providing a mechanism to tune mitochondrial distribution to changes in synaptic activation and $Ca^{2+}$-signaling. Thus Miro1 mediates both constitutive transport and neural activity-dependent regulation of mitochondrial location in dendrites providing a mechanism for enhancing location-specific energy provision, $Ca^{2+}$-buffering and/or mitochondrial signaling during neuronal development and plasticity. Here I will present our recent work on the role played by Miro proteins in regulating trafficking of mitochondria. The role that Miro-mediated control of mitochondrial trafficking plays in regulating neuronal development, function and pathology *in vitro* and *in vivo* will also be explored.

## S 014

# A theoretical framework to study bioenergetics and its relationships to pathophysiology in neuronal diseases: the example of ALS

Stephanie CHEVALLIER[1], Claire LEGER[1], Virginie ROQUES[1], Rodrigues ROSSIGNOL[2] and **Gwendal LE MASSON**[1]

[1] *Team "Motor system diseases", Magendie Neurocentre INSERM U862, 146 rue Léo Saignat, 33077 Bordeaux Cedex*

[2] *Laboratoire "Maladies Rares: Génétique et Métabolisme" Université de Bordeaux. 146 Rue Léo Saignat, 33076 Bordeaux Cedex*

We developed a theoretical framework and models to link in single neurons or small networks, classical Hodgkin-Huxley formalism to describe neuronal electrical activity, together with intracellular pathways associated with the mitochondrial production of ATP and to its consumption by different processes such ATP-ases. We apply this framework to build a model of a motor neuron with impaired mitochondrial function and transport. Our model recapitulates many of the salient features of Amyotrophic Lateral Sclerosis (ALS), such as distal degeneration, hyper excitability, selectivity of some subpopulation of motor neurons to degeneration, associated with specific energy consumption profiles, and involvement of calcium, as most of the extrusion mechanisms are ATP dependants. We then underwent a detailed profiling of the bioenergetics of motor neurons from the mice model SOD1, and found many evidence for a disruption of normal ATP synthesis and delivery in both motor neuron SOD1 mice but also from fibroblasts from ALS patients. Altogether, this work, strongly suggest that mitochondria dysfunction occurs very early in the course of the disease and might be a essential step of the selective neuro-degeneration of motor neurons in ALS. Our model is now used to identify new drug target, using original and efficient optimization techniques, capable of rescuing mitochondrial functions. Some of the drug candidates found are already being tested in preclinical studies.

## IS 020

# Mitochondrial dysfunction in familial ALS

**MANFREDI Giovanni**

*Feil Family Brain and Mind Research Institute, Weill Medical College of Cornell University, New York, NY, USA*

Mitochondrial dysfunction is associated with neurodegenerative diseases, because neuronal cells are highly dependent on mitochondria. As perturbed mitochondrial function renders neurons extremely sensitive to a wide variety of insults, such as oxidative stress and bioenergetic defects, mitochondrial defects can profoundly affect neuronal fate. Several studies have linked ALS with mitochondrial dysfunction, stemming from observations of mitochondrial abnormalities in cellular and mouse models of familial forms of ALS. Mitochondrial changes have been mostly investigated in mutants of superoxide dismutase 1 (SOD1), one of the most common causes of familial ALS, for which excellent cellular and animal models are available. Through a variety of approaches, in vitro and in vivo, we showed that mitochondrial defects in SOD1 mutants involve many critical physiopathological processes, from defective bioenergetics to abnormal calcium homeostasis, to altered morphology and impaired trafficking. The majority of SOD1 is in the cytosol, but a portion is localized in organelles, including mitochondria. Using recombinant mutant SOD1 specifically targeted to the mitochondrial inter membrane space, we investigated the specific role of mitochondrial mutant SOD1 in causing neuronal dysfunction and neurodegeneration. Recently, we have started to study the involvement of mitochondrial



quality control mechanisms to understand why proteostasis and mitophagy fail to eliminate damaged mitochondria in mutant SOD1 motor neurons.

## S 002

# Mitochondrial cannabinoid receptor $CB_1$ ($mtCB_1$) in the brain

Etienne Hebert-Chatelain[1,2,3,*], Tifany Desprez[1,2,*], Edgar Soria-Gomez[1,2], Luigi Bellocchio[1,2,4], Anna Delamarre[1,2], Peggy Vincent[1,2], Arnau Busquets-Garcia[1,2], Laurie Robin[1,2], Michelangelo Colavita[1,2,5], Geoffrey Terral[1,2], Filippo Drago[5], Nagore Puente[6], Leire Reguero[6], Uzaskun Elezgarai[6], Maria-Luz Lopez-Rodriguez[7], Federico Massa[1,2], Pedro Grandes[6], Giovanni Bénard[1,2,*], **Giovanni MARSICANO**[1,2,*]

[1] *INSERM U862, NeuroCentre Magendie, Group "Endocannabinoids and Neuroadaptation", Bordeaux, France;*
[2] *Université de Bordeaux, Bordeaux, France;*
[3] *Dept. of Biology, Université de Moncton, Moncton, New-Brunswick, Canada;*
[4] *Dept. of Biochemistry and Molecular Biology I, Complutense University, Madrid, Spain;*
[5] *Department of Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, University of Catania, Catania, Italy*
[6] *Dept. of Neurosciences, Medical and Odontology Falculty, University of Bask Country, Leioa, Spain;*
[7] *Department of Organic Chemistry, Complutense University, Madrid, Spain*
**EH-C and TD share first authorship; GB and GM share senior authorship*

The G protein coupled cannabinoid type-1 ($CB_1$) receptor is a seven transmembrane G protein coupled receptor widely expressed in the brain. In the last years, we showed that $CB_1$, receptors in the brain are present both at plasma and mitochondrial membranes ($mtCB_1$). $MtCB_1$ receptors directly regulate OXPHOS activity and participate in $CB_1$-dependent modulation of synaptic plasticity and transmission. $mtCB_1$ receptors decrease brain mitochondrial respiration *via* intramitochondrial modulation of Gi and soluble adenylyl cyclase (sAC). Interestingly, *in vivo* blockade of sAC activity in the brain eliminates certain acute effects of cannabinoids (catalepsy), but not others (antinociception), suggesting the involvement of $mtCB_1$ signaling in specific pharmacological actions of cannabinoid drugs. Moreover, we generated a mutant $CB_1$ protein (DN22-$CB_1$) lacking functional localization at mitochondrial membranes, but maintaining localization and activity at plasma membranes. The viral expression of DN22-$CB_1$ *in vivo* blocked cannabinoid-induced decrease of glutamatergic neurotransmission and impairment of blockade of consolidation of object recognition memory in mice. These data show that $mtCB_1$ receptors mediate important cannabinoid-induced adverse effects, such as catalepsy and amnesia, and provide potential therapeutically-relevant approaches for discriminating between desired and undesired effects of cannabinoid drugs.

## IS 007

# Role of the mitochondrial pyruvate carrier in neuronal function

**MARTINOU Jean-Claude**, HERZIG Sébastien, BENDER Tom, VANDERPERRE Benoit, COMPAN Vincent

*University of Geneva*

During my talk, I will discuss the structure and function of the mitochondrial pyruvate carrier and its role in cell metabolism, in particular in relation to neuronal function.

## IS 011

# Glial metabolism and axonal maintenance

**MILBRANDT Jeffrey**
*Department of Genetics; Washington University School of Medicine; St Louis, MO*

Diabetic neuropathy is a rapidly increasing disorder worldwide. Our lab is studying how metabolic abnormalities in Schwann cells might contribute to axonal dysfunction in this neuropathy. We have produced mice with Schwann cell-specific deficits in mitochondrial function as well as other metabolic pathway components. A mouse model produced by mutating Tfam, a protein responsible for mitochondrial maintenance, develops peripheral neuropathy characterized by early disorganization and degeneration of Remak bundles, which contain unmyelinated fibers, followed by later demyelination of myelinated axons. These mice also demonstrated alterations in behavior and nerve histology consistent with severe neuropathy. Molecular analysis of nerves from these mice showed that aberrant mitochondrial function activates a maladaptive integrated stress response (ISR) through the actions of hemeregulated inhibitor (HRI) kinase, and causes a shift in lipid metabolism away from fatty acid synthesis toward oxidation. We also observed an accumulation in SCs of acylcarnitines (ACs), an intermediate of fatty acid ß-oxidation. We found that these ACs are released from SCs and can induce axonal degeneration. These studies along with observations from mice with disruption of other metabolic pathways, such as the Lkb1-AMPK axis, in SC will also be discussed.

IS 005

# Assessing mitochondrial function and dysfunction in cultured neurons and isolated nerve terminals: advantages and limitations

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It is important to define the terms 'function' and 'dysfunction'. The primary role of neuronal mitochondria is to be able to generate sufficient ATP to match the greatest demand that the neuron will ever be exposed to, however transient. A bioenergetic deficiency, however brief, will lead to catastrophic cell failure. Our research has employed two preparations: primary neuronal cultures and isolated nerve terminals ('synaptosomes'). Primary cultures are limited to neonatal preparations, restricting their ability to model developmental and aging-related changes, but allow a wide range of bioenergetic techniques to be applied. The development of techniques to monitor in situ respiration (1) and membrane potential (2) of mitochondria in neuronal monolayers has allowed us to assess the respective roles of bioenergetic insufficiency, calcium overload and reactive oxygen species generation in glutamate excitotoxicity (3).
We have devoted considerable effort into refining techniques to investigate synaptosomal function, including the control of glutamate exocytosis in response to induced action potential firing (4), the monitoring of respiration of microgram quantities of synaptosomes (5), and the development of optical techniques to deal with the inherent functional and transmitter heterogeneity of synaptosomes from even defined brain regions (6). These approaches have led to some surprising conclusions in the search for bioenergetic defects in dopaminergic neurons (6) and transgenic models of Alzheimer's disease (7) that indicate the need to re-examine some hypotheses of mitochondrial dysfunction.

(1) Jekabsons, M. B. and Nicholls, D. G. (2004) *J. Biol. Chem.* **279,** 32989-33000
(2) Gerencser, A. A., Chinopoulos, C., Birket, M. J., Jastroch, M., Vitelli, C., Nicholls, D. G., and Brand, M. D. (2012) *Am. J. Physiol. Cell Physiol.* **590,** 2845-2871
(3) Nicholls, D. G., Johnson-Cadwell, L. I., Vesce, S., Jekabsons, M. B., and Yadava, N. (2007) *J. Neurosci. Res.* **85,** 3206-3212
(4) Sánchez-Prieto, J., Budd, D. C., Herrero, I., Vázquez, E., and Nicholls, D. G. (1996) *Trends Neurosci.* **19,** 235-239
(5) Choi, S., Gerencser, A. A., and Nicholls, D. G. (2009) *J. Neurochem.* **109,** 1179-1191
(6) Choi, S. W., Gerencser, A. A., Lee, D., Rajagopalan, S., Nicholls, D. G., Andersen, J. K., and Brand, M. D. (2011) *J. Neurosci.* **31,** 4524-4534
(7) Choi, S. W., Gerencser, A. A., Ng, R., Flynn, J. M., Melov, S., Danielson, S. R., Gibson, B. W., Nicholls, D. G., Bredesen, D. E., and Brand, M. D. (2012) *J. Neurosci.* **32,** 16775-16784

IS 018

# Modulation of neuronal mitochondrial dynamics and huntingtin aggregation by selective HDAC inhibitors

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Huntington's disease (HD) stems from a polyglutamine expansion in the huntingtin protein that is particularly detrimental to medium spiny neurons in the striatum. Expression of mutant huntingtin in neurons has been associated with mitochondrial dysfunction and impaired proteostasis. Histone deacetylase (HDAC) inhibitors are a group of epigenetic modulatory compounds currently being investigated for their putative neuroprotective properties (1). We have previously shown that the HD vulnerable striatal neurons present a decreased mitochondrial calcium buffering capacity when compared with cortical neurons (2), and that non-selective HDAC inhibitors improve mitochondrial-dependent calcium handling in HD striatal neurons (3). Aiming to further clarify the mechanisms of HDAC inhibitors action in HD models, we tested entinostat and tubastatin A (selective HDAC1 and HDAC6 inhibitors, respectively) in primary cultures of rat cortical and striatal neurons. Drug efficacy was verified by increased histone or tubulin acetylation with entinostat or tubastatin, respectively. In young neurons, entinostat reduced neurite outgrowth while increasing mitochondrial average length and fractional occupancy, suggesting it does not impede mitochondrial biogenesis. Tubastatin increased primary axon length without affecting mitochondrial average length or distribution. When combined, tubastatin partly rescued entinostat effects on neurite outgrowth. In mature neuronal cultures, tubastatin increased the motile mitochondrial fraction in both striatal and cortical neurons, without affecting top velocity, distance, number of stops, or mitochondrial fractional occupancy. HDAC6 inhibition with tubastatin did not prevent autophagosome-lysosome fusion, actually increasing flux and number of LC3 vesicles within neuronal soma. Neither entinostat nor tubastatin altered the rate of inclusion body formation in either cortical or striatal neurons expressing mutant huntingtin. The present study contributes to the open discussion as to whether HDAC6 inhibitors to boost mitochondrial trafficking might aggravate neuropathology by impairing HDAC6-dependent autophagy (1). Our findings in primary neuronal cultures suggest that pharmacological HDAC6 inhibition, while promoting mitochondrial trafficking, neither impairs neuronal autophagy nor accelerates mutant huntingtin aggregation.

(1) Guedes-Dias P and Oliveira JMA (2013) BBA Mol Basis Dis 1832: 1345-1359;
(2) Oliveira JMA and Gonçalves J (2009) J Biol Chem 284: 5010-5020;
(3) Oliveira JMA et al. (2006) J Neurosci 26: 11174-11186

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IS 021

# The role of mitochondria in exocytotic glutamate release from astrocytes

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Astrocytes can exocytotically release the gliotransmitter glutamate. Increased cytosolic $Ca^{2+}$ concentration is necessary and sufficient in this process. Mitochondria play a role in $Ca^{2+}$-dependent glutamate release from these cells by modulating cytosolic $Ca^{2+}$ levels. These organelles affect two aspects of the cytosolic $Ca^{2+}$ kinetics in astrocytes. They play a role in immediate sequestration of $Ca^{2+}$ during the cytosolic $Ca^{2+}$ increase in stimulated astrocytes, a function carried by the $Ca^{2+}$ uniporter, which can be blocked by Ruthenium 360. As cytosolic $Ca^{2+}$ levels subside, free $Ca^{2+}$ exits the mitochondrial matrix through the $Na^{+}/Ca^{2+}$ exchanger (NCLX), and this process can be blocked by CGP37157 or by using NCLX siRNA. $Ca^{2+}$ accumulation in the mitochondria is increased by cyclosporine A (CsA) via preventing the formation of the mitochondrial permeability transition pore (MPTP), which transient openings may serve as a physiological mechanism to remove $Ca^{2+}$ rapidly from the mitochondrial matrix at high $Ca^{2+}$ loads. The above pharmacological manipulations affecting the mitochondrial $Ca^{2+}$ buffering, as seen by appropriate cytosolic $Ca^{2+}$ changes, in rat astrocytes were correlated with glutamate release from these cells, suggesting that mitochondria play a role in $Ca^{2+}$-dependent glutamate release from astrocytes.

CsA targets cyclophilin D (CypD) to inhibit opening of the MPTP. Indeed, stimulated cortical astrocytes isolated from CypD deficient mice *Ppif* $^{-/-}$ displayed reduced cytosolic $Ca^{2+}$ increases. Unexpectedly, however, these cells showed augmented exocytotic release of glutamate, when compared to responses obtained from astrocytes isolated from wild-type mice. This process is commonly referred to as down-stream of $Ca^{2+}$ modulation of secretory machinery and likely included calcineurin. The difference between rat and mouse astrocytes uncovered through contrasting the results could be explained by species difference. This difference does not detract from a general conclusion that CypD can shape cytosolic $Ca^{2+}$ responses.

In addition to cytosolic $Ca^{2+}$, cytoplasmic glutamate levels can regulate exocytotic release from astrocytes. This was evident in a Huntington's disease (HD) animal model, BACHD mice, where full-length human mutant huntingtin expression perturbs astrocyte gliotransmitter release. BACHD astrocytes show augmented exocytotic glutamate release with unaltered $Ca^{2+}$ dynamics. These astrocytes had a biochemical footprint that would lead to increased availability of cytosolic glutamate, i.e. augmented *de novo* glutamate synthesis due to an increase in the level of the astrocyte specific mitochondrial enzyme pyruvate carboxylase. This work identifies a new mechanism in astrocytes that could lead to increased levels of extracellular glutamate in HD and thus may contribute to excitotoxicity in this devastating disease.

## IS 015

# The tale of mitochondria in neurodegeneration

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Adult-onset neurodegenerative diseases are a group of heterogeneous disorders of the central nervous system in which specific subsets of neurons are dying for unknown reasons and by unknown mechanisms. For decades, Parkinson's disease (PD) has been among the most common of such disorders and the "poster-child" for mitochondrial defects in neurodegeneration. However, for almost the past three decades, the notion that a mitochondrial defect could be linked to PD pathogenesis has had a rather bumpy ride, given the succession of periods of high enthusiasm followed by high disappointment for this view. Even though the neurotoxins MPTP and rotenone, both mitochondrial poisons, have been reported to reproduce some of the hallmarks of PD, evidence for mitochondrial respiration as a primary cause of PD is still dubious. Furthermore, excluding a few exceptions where a clinical picture of parkinsonism is associated with a mitochondrial DNA mutation, preclinical and clinical studies have failed to identify any genetic mutations in the genes encoding for the electron transport chain complexes in PD patients. More recently, it has been discovered that mutations in the genes encoding for Parkin, PINK1 and DJ-1 are associated with the familial forms of PD and with mitochondrial alterations, including morphological abnormalities. These results have led many researchers to revisit the question of mitochondrial biology as a primary mechanism in PD pathogenesis but, this time from the angle of a perturbation in, for example, mitochondrial dynamics and not from the angle of a deficit in respiration. Currently, if the consensus is that a defect in bioenergetics is unlikely a primary pathogenic factor in neurodegeneration, it can still be a key determinant in the differential neuronal vulnerability so characteristic of neurodegenerative disorders.

## IS 017

# Clinical spectrum and pathophysiology of OPA1 dysfunctions

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Since the first description in 2000 of OPA1 mutations in isolated Dominant Optic Atrophy, the clinical spectrum of OPA1-related disorders has been considerably extended to adult and pediatric syndromes. Neurons are particularly vulnerable to OPA1 mutations since visual impairment, neurosensory deafness, peripheral neuropathies and encephalomyopathies are the predominant associated defects. The natural history of OPA1 disorders remains difficult to establish. Indeed, pathophysiological studies on patients, as well as on cell and mouse models, have revealed that in addition to impaired mitochondrial dynamics, several other dysfunctions contribute to the phenotype, such as increased susceptibility to apoptosis, heterogeneous OXPHOS defects and instability of mitochondrial DNA. Premature axonal and myelin degeneration, increased mitophagy, and instability of the respiratory chain supercomplex precede cell degeneration in the mouse model carrying the most recurrent OPA1$^{delTTAG}$ mutation. Taken together, these results strongly support the idea that OPA1 protects against age-related neuronal degeneration.

## IS 009

# Energy consumption and supply at nerve terminals

**RYAN Timothy**
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Synapses are the points of all fast neurotransmission in the brain and require numerous molecular processes to carry out their function. These processes present large ATP demands. We sought to determine how fuel availability and electrical activity impact synaptic ATP levels and how ATP availability controls synaptic function. We recently developed a quantitative genetically encoded optical reporter of presynaptic ATP, Syn-ATP, and found that electrical activity imposes large metabolic demands that are met via activity-driven control of both glycolysis and mitochondrial function. Using this approach we are carrying out experiments to determine which steps in presynaptic function represent the greatest energetic burden, which are the most vulnerable to compromises and how activity drives synthesis of ATP.

## IS 006

# Regulation by $Ca^{2+}$ of mitochondrial respiration in neurons: malate aspartate shuttle and calcium uniporter

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Calcium is thought to regulate respiration but it is unclear whether this is dependent on the increase in ATP demand caused by any $Ca^{2+}$ signal or to $Ca^{2+}$ itself. $[Na^+]_i$, $[Ca^{2+}]_i$ and $[ATP]_i$ dynamics in intact neurons using glucose and exposed to different workloads in the absence and presence of $Ca^{2+}$ clearly showed that $Ca^{2+}$-stimulation of coupled respiration is required to maintain $[ATP]_i$ levels. $Ca^{2+}$ may regulate respiration by activating metabolite transport in mitochondria from outer face of the inner mitochondrial membrane, or after $Ca^{2+}$ entry in mitochondria through the calcium uniporter (MCU). The Ca2+-regulated mitochondrial aspartate-glutamate exchanger Aralar/AGC1/Slc25a12, a component of the malate-aspartate shuttle, is activated by $Ca^{2+}$ with $S_{0.5}$ of 300 nM. The lack of ARALAR reduced basal OCR (by 46%) and the $Ca^{2+}$-dependent responses to all workloads, with a 65-70% reduction in the response to the high workload imposed by veratridine, and complete suppression of the OCR responses to moderate ($K^+$-depolarization) and small (carbachol) workloads, effects reverted by pyruvate supply. For $K^+$-depolarization, this occurs in spite of the presence of large $[Ca^{2+}]_{mit}$ signals and increased reduction of mitochondrial NAD(P)H. These results show that ARALAR-MAS is a major contributor of $Ca^{2+}$-stimulated respiration in neurons by providing increased pyruvate supply to mitochondria. In its absence and under moderate workloads, matrix $Ca^{2+}$ appears unable to stimulate pyruvate metabolism in mitochondria suggesting a limited role of MCU in these conditions.

## IS 016

# Glucocerebrosidase in Parkinson disease: a meeting of organelles

**SCHAPIRA Anthony**
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Mutations of glucocerebrosidase (GBA) represent the most important risk factor for Parkinson disease (PD). The downstream effects of these influence endoplasmic reticular, lysosomal and mitochondrial function. The ramifications of GBA mutations on the pathogenesis of PD will be discussed.

## IS 019

# Mitochondrial Drug Development: from bench to bedside

**SMEITINK Jan**[1,2], KOOPMAN Werner[1,2], De HAAS Ria[1], RUSSEL Frans[1], KOENE Saskia[1], De GROOT Imelda[1], WILLEMS Peter[1,2], BEYRATH Julien[2]
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The development of new drugs finally leading to clinically relevant improvement for mitochondrial disease is a challenging, time-consuming, sometimes frustrating and expensive process involving many different stakeholders. The foundation for developing such drugs lies in a detailed understanding of the biological processes of normal and hampered energy metabolism at the different levels of complexity. In executing such a mitochondrial drug development program we came across several, first to be solved issues, including the development and validation of relevant clinical outcome measures for the mitochondrial disease group,. In this lecture I will present the state of the art of our public-private drug development program, with a focus on different classes of new chemical entities, and discuss the various unsolved issues enabling so far a proper judgement of the clinical relevance of mitochondrial disease intervention strategies in general the field is facing.

## IS 001

# Damage control - How the Pink1/Parkin pathway can regulate removal of impaired mitochondria by autophagy

**YOULE Richard**
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The products of two genes mutated in autosomal recessive forms of Parkinson's disease, Pink1 and Parkin, have been identified in Drosophila to work in the same pathway to maintain healthy flight muscles and dopaminergic neurons. PINK1 is a kinase located on mitochondria whereas Parkin is an E3 ubiquitin ligase located in the cytosol. Normally, the protein level of PINK1 is maintained at low to undetectable levels because PINK1 is constitutively imported to the inner membrane of healthy mitochondria where it is cleaved by the rhomboid protease PARL and then degraded in the cytosol by the proteosome through the N-end rule. When mitochondrial import is impaired PINK1 accumulates on the outer mitochondrial membrane where it can recruit Parkin from the cytosol and activate Parkin's E3 ubiquitin ligase activity. Parkin in turn ubiquitinates mitochondrial proteins to trigger autophagic elimination of the

damaged mitochondrion. Thus PINK1 represents a sensing mechanism to allow detection of individual damaged mitochondria and Parkin mediates their removal through mitophagy. Recent results reveal how mitochondria outer membrane proteins guide autophagosomal membrane encapsulation following Parkin ubiquitination. How PINK1 recruits Parkin to mitochondria and the kinase substrate of PINK1 involved in Parkin activation will be presented. These results indicate that the maintenance of mitochondrial quality control may be disrupted in patients with PINK1 and Parkin mutations and lead to parkinsonism.

## IS 013

# Balanced ubiquitination and de-ubiquitination of Mfn2 in the regulation of ER-Mito tether: role of Parkin and its opposing DUB in the onset of Parkinson's Disease

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Parkin, an E3-Ubiquitin ligase and a PD- related protein, is selectively recruited to impaired mitochondria and promotes their elimination via autophagy, a process called mitophagy. In several model systems, Parkin selectively ubiquitinates mitochondrial pro fusion proteins Mitofusins (MFNs). In coordination with pro-fusion member dynamin related GTPases optic atrophy 1 (OPA1) and pro-fission members dynamin like protein 1 (DRP1) and Fis1, MFNs regulate mitochondria size, shape and ultrastructure, which changes are tightly controlled under stress. While MFN1 is primarily responsible for mitochondrial fusion in cooperation with the inner membrane pro fusion protein OPA1, MFN2 is also required to bridge ER to mitochondria, a structural feature essential for phospholipid synthesis and exchange, $Ca^{2+}$ transfer between the organelles and $Ca^{2+}$ dependent cell death. ER-mitochondria juxtaposition is emerging as one of the most important interface in cell biology, yet we do not know how the tethering is regulated. Is ER-mitochondria physical connection decreased upon stimuli that promote $Ca^{2+}$ release from the ER and how this is achieved? Does ER-mitochondria phospholipid exchange require a closer ER-mitochondria connection? Ubiquitination has recently emerged as a powerful tool to modulate proteins activity, via regulation of their subcellular localization and ability to interact with other proteins to form signaling complexes. Similar to other post-translational modifications, such as phosphorylation and acetylation, ubiquitination is also a reversible modification, mediated by a large family of deubiquitinating enzymes (DUBs). Balanced ubiquitination/deubiquitination events might provide a potential molecular switch to modulate MFN2 ability to interact *in trans* on opposing organelles and form



the molecular bridges that affects ER-mitochondria tethering. We found that Parkin, by ubiquitinating MFN2, controls ER-mitochondria tethering. We also identified deubiquitination enzyme USP8 that is controlling Parkin in the ubiquitination of MFN2 so that balanced ubiquitination/deubiquitination of MFN2 provides a reversible mechanism to regulate ER-mitochondria tether upon specific stimuli.

## SELECTED TALKS - ST

### ST 3

# Physiological and pathological roles of glycogen in brain energy metabolism

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The role of brain glycogen has been traditionally associated with the preservation of neuronal function during energetically challenging states such as hypoxia, hypoglycaemia, ischemia, and seizures. Nevertheless, glycogenolysis also occurs in euglycaemia during an increase in neuronal activity, thus indicating that brain glycogen also supports neuronal function in non-pathological conditions. In order to address the role of glycogen in the brain, we generated a brain-specific glycogen synthase (GS) knockout (KO) mouse. Our results show a significant deficit in learning capacity and in the concomitant activity-dependent changes in synaptic strength in these animals.
In some diseases glycogen is abnormally accumulated in neurons. The most striking example is Lafora disease (LD), a fatal neurodegenerative condition which is caused by mutations affecting two enzymes, namely malin or laforin. Both enzymes interact functionally to promote the degradation of GS and its activator Protein Targeting to Glycogen (PTG). The causal role of glycogen accumulation in neurodegeneration in LD remains controversial, since the malin-laforin complex may have additional functions to that of the regulation of glycogen synthesis, such as the control of autophagy. In fact, KOs of malin and laforin present autophagy impairment. We have generated several mouse models with altered capacity to accumulate glycogen. Our findings reveal that glycogen accumulation is indeed responsible for the neurodegeneration of the LD models, as well as for the impaired autophagy observed. These results identify the regulation of glycogen synthesis as a key target for the treatment of LD.

ST 5

# Bornavirus X protein localizes in neuronal mitochondria and mediates neuroprotection through stabilization of the mitochondrial chaperone Hspa9

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Bornavirus, a non-cytolytic RNA virus, persists lifelong in the central nervous system of infected animals. Viral persistence is mainly driven by the expression of the viral X protein, which is addressed to the nucleus and mitochondria, where it interferes with both antiviral responses and apoptosis. Moreover, our team has recently reported that the ectopic expression of X protein was able to protect neurons against toxins of the mitochondrial respiratory chain in vivo and in a mouse model of Parkinson's disease. Here, our goal was to gain further insight on the molecular mechanisms underlying X-mediated neuroprotection. We first assessed the intra-mitochondrial localization of the X protein by various biochemical approaches using mitochondrial fractions prepared from X-expressing neurons. We next determined the minimal mitochondrial targeting sequence (MTS) of the protein by constructing chimeras fused to an eGFP reporter gene, and identified mutations in the X sequence that were crucial in controlling mitochondrial accumulation. In parallel, we searched for X cellular partners within mitochondria. In the nucleus, X has been shown to interact with the nuclear chaperone Hsc70 (or Hspa8), which has a mitochondrial homolog called Hspa9 (or mtHsp70, Mortalin), which was recently shown to be involved in many neurodegenerative diseases, such as Parkinson's or Azheimer's diseases. We confirmed a direct and specific interaction between the X protein and Hspa9 in mitochondria. Furthermore, Hspa9 down-regulation resulting from exposure to mitochondrial toxins was reduced in X-expressing neurons, suggesting that X may stabilize Hspa9 in mitochondria. Using an innovative oriented neuronal culture based on microfluidics, we demonstrated that similarly to the X protein, overexpression of Hspa9 enhanced mitochondrial filamentation and protected neurons from mitochondrial dysfunctions. We are currently generating X mutants that have lost the capacity to interact with Hspa9. The resulting impact of these mutations on the neuroprotective potential conferred by the X protein will be presented.

ST 1

# Programming carbon substrate metabolism for control of neuronal excitation: a role for BAD

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Neuronal excitation can be substantially modulated by alterations in metabolism, including changes in glucose utilization. Manipulating carbon substrate metabolism in the brain may therefore have value for treating disorders of excitability, particularly epileptic seizures. This is also evident from the anticonvulsant effect of dietary or pharmacologic manipulations that reduce glucose utilization and promote ketone body metabolism. We have found that alterations in BAD, a protein with dual functions in apoptosis and glucose metabolism, impart striking resistance to epileptic seizures in mice. Using a combination of genetic models and multiple experimental approaches ranging from mitochondrial respirometry in primary neural cultures and slice electrophysiology to behavioral and electrographic seizure monitoring *in vivo*, we provide a vertical analysis of BAD's effect on neural carbon substrate utilization, neuronal excitability, and seizure susceptibility. Our observations suggest that BAD imparts reciprocal effects on glucose and ketone body consumption through a phospho-regulatory mechanism that modifies S155 within its BH3 domain. An electrophysiologic consequence of this metabolic shift is a marked change in potassium conductance. Moreover, genetic studies indicate that this link between BAD-dependent programming of neural mitochondrial metabolism and neuronal excitability extends to reductions in electrographic and behavioral seizures, including the near absence of generalized tonic-clonic seizures. Our findings reveal BAD as a novel molecular player in the metabolic control of neuronal excitation that imparts robust changes in susceptibility to both behavioral and electrographic seizures.

ST 6

# Amyloid-β peptide induces mitochondrial dysfunction by inhibition of preprotein maturation[#]

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Alzheimer's disease (AD) is a severe neurodegenerative disease affecting millions of people worldwide. Although already described in 1907 by Alois Alzheimer, clear diagnostic markers and medical treatment are still missing. In recent years it became evident that mitochondrial dysfunction is one of the earliest hallmarks in the progression of AD. Several lines of evidence suggest that the amyloid-β peptide (Aβ) is imported into mitochondria, where it causes a variety of organellar dysfunctions. In order to fulfill their essential functions, mitochondria have to be equipped with proteins that are nuclear-encoded, synthesized in the cytosol and thus have to be sorted into the correct mitochondrial subcompartments. The majority of mitochondrial preproteins undergo N-terminal processing upon import into the organelle carried out by the mitochondrial processing peptidase MPP.
We found that preprotein processing by MPP and peptide turnover - mediated by the peptidasome PreP/Cym1 - are functionally coupled to ensure mitochondrial proteostasis. Surprisingly, Aβ competes with presequence peptides for degradation by PreP/Cym1 leading to feed-back inhibition of MPP. As consequence preproteins accumulate within mitochondria and are rapidly degraded ultimately leading to an imbalanced mitochondrial proteome. Using an *in vivo* yeast AD model, we show that impaired preprotein maturation by Aβ precedes mitochondrial dysfunctions (e.g. decrease in membrane potential, elevation of reactive oxygen species levels and decrease in mitochondrial respiration). Furthermore, we observed accumulation of several mitochondrial preproteins *in vivo* in mitochondria of our yeast AD model and in AD patient brain samples. For the first time now, we can explain how Aβ can induce the multiple different mitochondrial dysfunctions accompanying AD. Therefore our findings provide an exciting possibility for the development of new diagnostic markers and therapeutic interventions.

[#]Mossmann et al. (2014) Cell Metab. (in press)

ST 4

# Mitochondrial carrier related neurological disorders

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Which could be the common cause of global cerebral hypomyelination, agenesis of corpus callosum and optic nerve hypoplasia? The common cause could reside on an impaired metabolite translocation from the mitochondria to the cytosol and *vice versa* [1]. Recently for the first time it was observed a global cerebral hypomyelination [2] in a patient who carried a mutation in the tissue specific Aspartate/Glutamate antiporter (AGC1) binding site. AGC1 catalyses the exchange of cytosolic glutamate for aspartate produced in the mitochondrial matrix. The mutation R590Q completely abolished aspartate supply from the mitochondria to the cytosol impairing normal myelin formation. The novel syndrome affecting a 3 yo girl is characterized by arrested psychomotor development, hypotonia, and seizures and global hypomyelination in the cerebral hemisphere. Similarly for the first time agenesis of corpus callosum was recently related to two mutations impairing the function of another mitochondrial carrier[3], the citrate carrier (CIC). CIC catalyses the exchange of mitochodrial citrate for the cytosolic malate. The accumulation of citrate within mitochondria induced a prominent excretion of 2-hydroxyglutaric acid and Krebs cycle intermediates in urine. The mutations G130D and R282H impaired severely protein function, affecting either substrate binding to the transporter or its translocation mechanism. The 18-month-old girl showed marked hypotonia, a near total lack of psychomotor development with a generalised epilepsy. Brain MRI demonstrated a complete agenesis of corpus callosum and ophtalmologic examination revealed hypoplastic optic nerves. It is necessary to recognize as soon as possible symptoms that could be related to AGC1 or CIC affected function because to date no cure is available for the above cited diseases. It appears that only gene therapy or enzyme replacement could be used in next future to limit the damage produced from the above cited mutations to patients. The described mitochondrial carrier related diseases were diagnosed thanks to exom sequencing that one more time reveals to be the fastest and for many aspects cheapest approach for obtaining a correct diagnosis. A dedicated scoring function based on evolutionary and structural information has been developed to predict pathogenicity of mitochondrial carrier mutations [4].

[1] Palmieri F. The mitochondrial transporter family SLC25: identification, properties and physiopathology. Mol Aspects Med. 2013
[2] Wibom R, Lasorsa FM, Töhönen V, Barbaro M, Sterky FH, Kucinski T, Naess K,Jonsson M, Pierri CL, Palmieri F, Wedell A. AGC1 deficiency associated with global cerebral hypomyelination. N Engl J Med. 2009 Jul 30;361(5):489-95.

[3] Edvardson S, Porcelli V, Jalas C, Soiferman D, Kellner Y, Shaag A, Korman SH, Pierri CL, Scarcia P, Fraenkel ND, Segel R, Schechter A, Frumkin A, Pines O, Saada A, Palmieri L, Elpeleg O. Agenesis of corpus callosum and optic nerve hypoplasia due to mutations in SLC25A1 encoding the mitochondrial citrate transporter. J Med Genet. 2013
[4] Pierri CL, Palmieri F, De Grassi A. Single-nucleotide evolution quantifies the importance of each site along the structure of mitochondrial carriers. Cell Mol Life Sci. 2014

ST 2

# Mfn2 downregulation in excitotoxicity causes mitochondrial dysfunction and delayed neuronal death

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Mitochondrial fusion and fission is a dynamic process critical for the maintenance of mitochondrial function and cell viability. During excitotoxicity neuronal mitochondria are fragmented but the mechanism underlying this process is poorly understood. Here we show that Mfn2 is the only member of the mitochondrial fusion/fission machinery whose expression is reduced in *in vitro* and *in vivo* models of excitotoxicity. Whereas in cortical primary cultures Drp1 recruitment to mitochondria plays a primordial role in mitochondrial fragmentation in an early phase that can be reversed once the insult has ceased, Mfn2 downregulation intervenes in a delayed mitochondrial fragmentation phase that progresses even when the insult has ceased. Downregulation of Mfn2 causes mitochondrial dysfunction, altered calcium homeostasis and enhanced Bax translocation to mitochondria, resulting in delayed neuronal death. We found that transcription factor MEF2 regulates basal Mfn2 expression in neurons, and that excitotoxicity-dependent degradation of MEF2 causes Mfn2 downregulation. Thus, Mfn2 reduction is a late event in excitotoxicity and its targeting may help to reduce excitotoxic damage and increase the currently short therapeutic window in stroke.

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# ABSTRACTS

## P1.01

## OPA1 loss of function affects in vitro neuronal maturation and functioning through mitochondrial alterations

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**Co-seniorship*

Mitochondrial dynamics control the organelle's morphology, through a balance between a fusion process resulting in the formation of elongated tubules and a fission process leading to isolated puncta. This dynamic balance participates in the regulation of mitochondrial functions. Recent reports have shown that disruption of mitochondrial dynamics contributes to neurodegenerative diseases. Mutations of the inner membrane fusogenic GTPase OPA1 are responsible for dominant optic atrophy, by mechanisms not fully understood. We showed that in rodent cortical primary neurons, down-regulation of the OPA1 protein led to fragmented mitochondria that become less abundant along the dendrites and axons. Furthermore, this inhibition resulted in reduced expression of mitochondrial respiratory complexes as well as mitochondrial DNA, decreased mitochondrial membrane potential, and diminished respiratory levels. The onset of synaptogenesis was markedly impaired through reductions in pre- and postsynaptic structural protein expression and synapse numbers. Accordingly, preliminary data, by electrophysiological recordings, show that OPA1 depletion could be associated to a decrease in glutamatergic synaptic transmission. With longer time in culture, OPA1 extinction led to a



major restriction of dendritic growth. Altogether, our findings suggest a new role for OPA1 in synaptic maturation and dendritic growth through maintenance of proper mitochondrial oxidative metabolism and distribution, highlighting the role of mitochondrial dynamics in neuronal functioning and providing insights into dominant optic atrophy pathogenesis, as OPA1 loss affecting neuronal maturation could lead to early synaptic dysfunction.

Synaptic alterations have been demonstrated to lie at the early onset of the pathological mechanisms leading to neuronal apoptosis in various neurodegenerative diseases, including Alzheimer's Disease. We have demonstrated that OPA1 down-regulation *per se* did not result in neuronal apoptosis but however led to ROS over-production, which was buffered by increasing antioxidant defenses. Our preliminary data suggest that increasing oxidative imbalance, through application of the pro-oxidant rotenone, potentiates neuronal death rate. Thus, mitochondrial defects due to the loss of OPA1 could pre-sensitize neurons to further insults and, in conjunction with supplemental subthreshold stress, would finally induce apoptosis in late-onset diseases.

## P1.02

# Neuron-Astrocyte metabolic coupling and brain imaging: contribution of astrocytes to the signal

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Brain possesses intrinsic mechanisms that allow the regulation of its major substrate, i.e. glucose, both for its contribution and its utilization depending on local cerebral activity variations. Despite the general recognition of this principle, the cellular and molecular mechanisms that underlie such a tight relationship between neuronal activity and energetic metabolism are still unknown and highly debated. More than 100 years ago, Camillo Golgi suggested, at the sight of their cyto-architectural connexions with endothelium cells and neurons, that astrocytes could play a central role in the distribution of energetic substrates between blood vessels and neurons. During the last 10 years, series of studies have been performed, both *in vitro* and *in vivo*, and have led to the recognition of the major role that astrocytes seem to play in brain energetic metabolism and have revealed a part of the mechanisms that come into play; astrocytes being more glycolytic and neurons more oxidative. These results give us not only a glimpse on the way the neuronal energetic needs could be satisfied during cerebral activity but also let us foresee how astrocytes participate to the emergence of some metabolic signals used in functional brain imaging that are applied for the study of cognitive functions. Experiments performed i*n vitro, ex vivo* and *in vivo* using nuclear magnetic resonance (NMR) spectroscopic studies to address this issue are presented. Results obtained support the idea that astrocytic lactate could be a supplementary oxidative fuel for neurons under physiological conditions and during brain activation.

## P1.03

# Cannabinoid-induced behavioral impairments depend on mitochondrial $CB_1$ receptors

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Understanding the specific mechanisms of cannabinoid effects is mandatory for their safe therapeutic use. The G protein coupled cannabinoid type-1 ($CB_1$) receptor is widely expressed in the brain, where it is present both at plasma and mitochondrial membranes ($mtCB_1$). This study shows that important behavioral effects of cannabinoids can be ascribed to activation of brain $mtCB_1$ receptors. Soluble adenylyl cyclase (sAC) mediates the effects of intracellular $mtCB_1$ receptor signaling and local brain inhibition of sAC activity blocks cannabinoid-induced catalepsy, but not antinociception. Moreover, we generated a mutant $CB_1$ protein (DN22-$CB_1$) lacking $mtCB_1$ signaling. Hippocampal inhibition of sAC activity and the viral expression of DN22-$CB_1$ *in vivo* blocked cannabinoid-induced decrease of glutamatergic neurotransmission and impairment of long-term memory. These data show that $mtCB_1$ receptors mediate important cannabinoid-induced adverse effects, such as catalepsy and amnesia, and provide potential therapeutically-relevant approaches for discriminating between desired and undesired effects of cannabinoid drugs.

## P1.04

# Cross-talk between the PINK1/Parkin pathway and the ER-mitochondria interface: role in the regulation of mitochondrial physiology

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The mitochondrial kinase PINK1 and the E3 ubiquitin ligase Parkin, products of two genes responsible for familial Parkinson's disease (PD), participate in the same mitochondrial quality control pathway, orchestrating the clearance of damaged organelles by mitophagy. Upon loss of mitochondrial membrane potential, the PINK1/Parkin pathway gets activated, leading to increased fission, outer membrane rupture and finally complete elimination of damaged organelles by mitophagy.
Interestingly, several mitochondrial targets of the PINK1/Parkin pathway physically and functionally associate with ER-mitochondria contacts. This interface is not only responsible for lipid and $Ca^{2+}$ exchanges between the two compartments, but also seems to play a crucial role in Parkin-dependent mitochondrial clearance.
Using a subcellular fractionation-based approach, we observed that the ER-mitochondria tether Mfn2 is increased in abundance in tissue from Parkin-deficient mice, specifically at ER-mitochondria contacts. In keeping with this finding, we demonstrated a significant increase in ER-mitochondria contacts in different cellular models of Parkin dysfunction, including fibroblasts of PD patients with *PARK2* mutations. Increased proximity between mitochondria and ER was accompanied by enhanced ER-to-mitochondria $Ca^{2+}$ transfer and mitochondrial $Ca^{2+}$ overload in Parkin-deficient cells. Normal $Ca^{2+}$ transients could be restored in Parkin-deficient fibroblasts by ectopic expression of Parkin, suggesting a direct involvement of this E3 ligase in the regulation of ER-mitochondria contacts, possibly *via* the tether Mfn2.
Current work aims at further elucidating the involvement of Parkin in the regulation of ER-mitochondria contacts and their function. In particular, we are focusing on the analysis of $Ca^{2+}$ homeostasis and its relation to cell death mechanisms in human dopaminergic neurons. Overall, these studies should provide new insight into how PINK1 and Parkin regulate mitochondrial physiology and how loss of their function contributes to the development of Parkinsonism.

## P1.05

# Role of the mitochondrial import complex TOM and the PINK1/Parkin pathway in mitochondrial quality control in neurons

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Mutations in genes encoding Parkin and PINK1 cause autosomal recessive forms of Parkinson's disease. These proteins regulate jointly several processes relevant to maintenance of mitochondrial quality. Using FRET microscopy to explore local physical proximities between

proteins, we showed that Parkin interacts with PINK1 at the TOM machinery, a protein complex responsible for the mitochondrial import of the vast majority of the mitochondrial proteins. The data accumulated suggest that after massive mitochondrial depolarization, the degradation of key subunits of TOM initiates Parkin-dependent mitophagy. These results were obtained in different cell lines, such as COS7, HEK293 and PC12.
Here, we explored the relevance of these findings in primary cortical neurons from wild type and Parkin-deficient mice. By confocal and FRET microscopy, we show that following mitochondrial depolarization triggered by the protonophore CCCP, PINK1 accumulates on the outer mitochondrial membrane (OMM) and recruits Parkin in proximity of TOM. These events are impaired in Parkin-deficient cells, suggesting that Parkin plays a role in stabilizing PINK1 on the OMM. Using an image-based quantitative analysis of markers of different mitochondrial subcompartments at various time points after CCCP treatment, we confirm significant mitochondrial loss in wild type but not Parkin-deficient cells. Components of the TOM machinery are lost earlier than other mitochondrial markers, confirming that this machinery is an early target for Parkin-dependent degradation. In parallel, we investigated the relationship between PINK1/Parkin-mediated mitochondrial degradation and mitochondrial biogenesis by exploring the expression of master genes of this process, as well as nuclear and mitochondrial genes encoding key mitochondrial components. Our results indicate that mitochondrial biogenesis is slowly activated following CCCP treatment in neurons, and that this process is compromised in the absence of Parkin.
Altogether our results confirm that the TOM machinery acts as a molecular switch in the PINK1/Parkin pathway, coupling loss of mitochondrial protein import efficiency with different quality control mechanisms, including mitochondrial degradation and biogenesis.

## P1.06

# Miro1- Dependent Mitochondrial Distribution is Critical for Dendritic Development and Maintenance and Protects Against Neurodegeneration

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Correct mitochondrial distribution is critical to satisfy local energy demands, calcium buffering requirements and supports key cellular processes. The mitochondrially targeted proteins Miro1 and Miro2 are key components of the mitochondrial transport machinery but their specific roles in mitochondrial trafficking and distribution have barely been addressed. Furthermore, the role played by mitochondrial positioning in the regulation of neuronal development remains unclear. Using a genetic knockout-out based approach we explore the functional similarities and differences between Miro1 and Miro2 in mitochondrial trafficking and hence in



establishing a correct mitochondrial distribution. We show that Miro1 is the main regulator of mitochondrial trafficking in neurons both in axons and dendrites. Defects in trafficking due to the lack of Miro1 are concomitant with a disruption of mitochondrial distribution. In these neurons mitochondria accumulates in proximal regions of the dendrites while distal dendritic segments appear depleted of these organelles. We also evaluate the consequences of disrupting correct mitochondria localization on the induction and maintenance of dendritic complexity. Miro1 knockout neurons show an altered pattern of branch points in a way that strictly correlates with distribution of mitochondrial density supporting a model whereby local availability of mitochondrial mass is critical to induce and sustain correct dendritic arborization. Furthermore, using a conditional system, we show that disrupting postnatal mitochondrial distribution *in vivo* by deleting Miro1 in mouse mature neurons, leads to a progressive loss of dendritic complexity in a process that ultimately compromises neuronal survival suggesting that ensuring mitochondrial distribution in neurons is critical to sustain dendritic complexity, neuronal function and protects against neurodegeneration.

## P1.07

# Virtual Mitochondrion

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Introduction: Virtual Mitochondrion is a project of a multilevel modelling of mitochondrial bioenergy metabolism. It involves:
- A molecular/atomic level with stochastic modelling (Gillespie) of electrons and protons transfers in respiratory chain complexes and super complexes of respiratory chain. It allowed us to predict a natural bifurcation of electrons in complex III (proof of Q-cycle hypothesis of Mitchell) to clarify the antimycin inhibition constraints and to simulate the ROS production in complex I and III. It also permits to jump to the upper level of enzyme kinetics.
- A mitochondrial level with the global modelling of the respiratory chain using simple but thermodynamical correct kinetics equations developed for the respiratory chain complexes (Michaelis-Menten like equations with the introduction of the proton gradient). The aim is to understand how local changes (pathological mutations for instance, drug effect, competition between respiratory substrates) in respiratory complexes influence the global behaviour of the oxidative phosphorylation. (In collaboration with Edda Klipp, Berlin).
- A cell level with the description of simple(s) model(s) of central energy metabolism easy to manipulate and to understand. The aim is to coherently integrate various types of data, metabolomics, fluxomics, transcriptomics and to follow the reroutings of metabolism, their regulations and controlling steps/targets (Metabolic Control Analysis).

Conclusion: We would like to emphasize the connection between lower and upper levels: how the functioning at a given level explains (or does not explain) the functioning at the upper (more integrated) level? Thus, in this work, the purpose of a model is not only to fit the experimental results accurately but rather to evidence inconsistencies that will lead to unveil mechanisms or properties which were hitherto not taken into account or even unknown.

**Ref.**
- Jean-Pierre Mazat, Stéphane Ransac, Margit Heiske, Anne Devin and Michel Rigoulet (2013),: Mitochondrial Energetic Metabolism – Some general principles IUBMB Life 65: 171-179.
- Meunier, B., Fisher, N., Ransac, S., Mazat, J. P. and Brasseur : Respiratory complex III dysfunction in human and use of yeast as a model to study mitochondrial diseases. Biochim. Biophys. Acta. (2013); 1827:1346-61

## P1.08

# Effect of mitochondrial ATP synthase subunits e and g (*ATP5I* and *ATP5L*) down-regulation in HeLa cells

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Mitochondrial morphogenesis is a key process of cell physiology as it ensures the proper function of this double membrane-delimited organelle. As the inner membrane surface is bigger than that of the outer membrane, it has to be packed in a very ordered way called *cristae*. The *cristae* morphology is known, since the late sixties, to be regulated by the energetic state of mitochondria but it is only in 2002 that the F1F0 ATP synthase was proposed to be involved in this process.
In yeast, the mitochondrial ATP synthase is able to form dimers that can assemble into oligomers. Two ATP synthase subunits (*e* and *g*) are involved in this supramolecular organization. The absence of subunits *e* and *g* and thus, of ATP synthase supercomplexes, promotes the modification of mitochondrial ultrastructure indicating that ATP synthase oligomerization is involved in *cristae* morphogenesis. To date, it is still not known if the oligomerization of the ATP synthase is involved in the morphological plasticity of the organelle under physiological conditions or if its impairment would have any physiopathological consequences in multicellular organism (see [1] for review).
We report here for the first time that in mammalian cells in culture, the shRNA-mediated down-regulation of subunits *e* and *g* affects the stability of ATP synthase and unexpectedly results in a 50% decrease of the available functional enzyme. As previously described in yeast, when subunits *e* and *g* expression are repressed, ATP synthase dimers and oligomers are less abundant when assayed by native electrophoresis. Mammalian ATP synthase

dimerization/oligomerization impairment has functional consequences on the respiratory chain leading to a decrease in OXPHOS activity. These alterations have a strong impact on the organelle itself leading to the fission of the mitochondrial network and the disorganization of mitochondrial ultrastructure.
The findings presented in this work may be of a great interest as they suggest that a defect in ATP synthase oligomerization has strong mitochondrial repercussions. Although it has to be formally investigated, we may speculate that ATP synthase oligomerization defect could be at the origin (or concomitant to) mitochondrial pathologies [2].

*[1] J. Habersetzer, W. Ziani, I. Larrieu, C. Stines-Chaumeil, M.-F. Giraud, D. Brèthes, A. Dautant, P. Paumard, ATP synthase oligomerization: From the enzyme models to the mitochondrial morphology, Int. J. Biochem. Cell Biol., 45 (2013) 99–105.*
*[2] J. Habersetzer, I. Larrieu, M. Priault, B. Salin, R. Rossignol, D. Brèthes, P. Paumard, Human F1F0 ATP Synthase, Mitochondrial Ultrastructure and OXPHOS Impairment: A (Super-)Complex Matter?, PLoS ONE, 8 (2013) e75429.*

## P1.09

# Procedures and controls for an accurate $CB_1$ receptor localization in brain mitochondria

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It is well established that $CB_1$ receptors are localized on neuronal axon terminal membranes throughout the brain, where they tightly control neuronal activity and synaptic functions. In addition to this presynaptic localization, our team has recently demonstrated that $CB_1$ receptors are also present in mitochondria ($mtCB_1$) of CA1 hippocampus and susbstantia nigra of the rodent brain.
We showed by a highly sensitive pre-embedding immunogold method for electron microscopy that about 30% of $CB_1$-WT CA1 mitochondria displayed $CB_1$ immunolabeling. Importantly, only ~3% of CA1 mitochondria in $CB_1$-KO mice showed nonspecific labeling (Benard et al., 2012). So far in our hands, this method has proven to be the best one for $mtCB_1$ localization. For example, the use of a more sensitive pre-embedding immunoperoxidase (ABC) method with Ni-intensified 3,3´-diaminobenzidine-4HCl (DAB-Ni) as a chromogen, revealed that 27.8% ± 2.2% of $CB_1$-WT mitochondrial sections showed $CB_1$ immunoreactivity, whereas 9.5% ± 1.0% displayed unspecific labeling in $CB_1$-KO. Because of diffusion of the black immunoreaction product, DAB-Ni staining prevented discrimination between different cellular membrane elements. In

addition, the DAB-Ni method yielded higher background staining levels (~9%) than the immunogold procedure applied. The unspecific $CB_1$ immunoreactivity was also evaluated in *$CB_1$*-KO tissues without primary antibody: 6.3% ± 1.0% of *$CB_1$*-KO mitochondria were still labeled using this procedure. These results indicate that, due to its diffuse staining and its higher levels of background staining, the DAB-Ni method is less suitable to evaluate the detection of the low levels of mt$CB_1$ receptors as compared with the immunogold technique used in our laboratory (Hebert-Chatelain et al., 2014).
However, doubts may arise as to silver-intensified gold particles might be anatomically related to other subcellular compartments (e.g., plasma or endoplasmic reticulum membranes) rather than mitochondrial membranes. Thus, we performed a more strict quantification, considering only mitochondrial gold particles located at more than 80 nm (the average diameter of silver-intensified gold particles is ~30 nm) from other membrane compartments. This approach revealed that about 20% of *$CB_1$*-WT mitochondrial sections were $CB_1$ immunopositive, whereas only 3% displayed $CB_1$ receptor labeling in *$CB_1$*-KO mice (Hebert-Chatelain et al., 2014).
Our results show that the use of $CB_1$ antibodies in combination with the appropriate immunocytochemical methods, controls and quantifications allows the detection of $CB_1$ receptors in brain mitochondria.

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## P1.10

# *$bc_1$* Complex Mechanism

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Using a stochastic simulation without any other hypotheses, we demonstrated the natural emergence of the Mitchell Q-cycle in the functioning of the *$bc_1$* complex, with few short-circuits and a very low residence time of the reactive semiquinone species in the $Q_o$ site [1]. However, this simple model fails to explain both the inhibition by antimycin of the *$bc_1$* complex and the accompanying increase in ROS production.
To obtain such an inhibition, we recently showed that it could be necessary to block the electron transfer from haem $b_L$ to semiquinone [2]. The protons transfers could play a major role in this mechanism. However, construction of such a model was not convincing because a too high increase in semiquinone lifetime and a high residual activity (GRC bioenergetics poster 2011).
In fact, to obtain an inhibition by antymicin, it is necessary to trap a molecule in the $Q_o$ site. This molecule could be neither a quinone (it will freely leave the active site) neither a semiquinone since semiquinone has not be evidenced in $Q_o$ site because its lifetime is too low. So the only molecule which can be trapped in the $Q_o$ site is the quinol. Based on this hypothesis, we propose here a new model which explains both the antimycin inhibition and the accompanying

increase in ROS production. This model takes into account the dimeric structure of $bc_1$ complex and is compatible with the half-of-the-site reactivity.

[1] S. Ransac, N. Parisey, J.-P. Mazat, The loneliness of the electrons in the $bc_1$ complex, Biochim. Biophys. Acta, 1777 (2008) 1053–1059.
[2] S. Ransac, J.-P. Mazat, How does antimycin inhibit the $bc_1$ complex? A part-time twin, Biochim. Biophys. Acta, 1797 (2010) 1849–1857.

## P1.11

# Bcl-2 family proteins are dispensable for catalysis and modulation of mitochondrial fusion

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Mitochondria are dynamic organelles that move, fuse and divide. The fusion of outer and inner mitochondrial membranes by distinct machineries enables molecular exchanges between them and is relevant for mitochondrial biogenesis, function and degradation. The mutation of genes encoding key fusion factors (dynamin-related proteins Mfn2 or OPA1) is associated to severe neuropathies (Charcot Marie Tooth Disease type 2A/CMT2A and Dominant Optic Atrophy/DOA, respectively).
The majority of fusion factors identified to date appear specific to mitochondria and localize to the mitochondrial membranes or compartments where they exert their functions. In addition, several Bcl-2 family proteins (BCL2FPs) known to regulate and/or mediate the apoptotic permeabilization of mitochondrial membranes (Bax, Bak, Bcl-xL and Bcl-2), have been involved in the modulation of mitochondrial fusion (refs. 1-3). Although debated (ref. 4), these findings challenge the prevailing view on the role of this protein family. Mitochondrial fusion is regulated by energy metabolism and various stresses as well as during development and cell cycle. The identification and characterization of the signaling cascades and of upstream (cytosolic?) modulators remains a major challenge that may open to therapeutic strategies for the treatment of neuropathies linked to fusion defects.
To investigate this, we setup a novel fusion assay enabling the quantitative analysis of mitochondrial fusion *in vitro*. We show that *in vitro* fusion (1) recapitulates fusion *ex vivo*, notably the requirement of nucleotides and inner membrane potential, and (2) proceeds efficiently in the absence of cytosol. We further find that crude cytosolic fractions, but also unrelated control proteins, can modulate the efficacy of *in vitro* fusion and that cytosols derived from wild-type, Bax -/- or Bcl xL -/- MEFs (Mouse Embryonic Fibroblasts) exert similar

effects. The latter finding being in contradiction with results reported by others (refs. 1-3), we analyzed mitochondrial morphology and dynamics *ex vivo* in MEF-lines devoid of anti-apoptotic (Bcl-2, Bcl-xL) or pro-apoptotic BCL2FPs (Bax, Bak) as well as in MEFs overexpressiong Bcl-2 or Bcl-xL. The absence of any significant phenotype confirmed our *in vitro* results and argues against a direct role of BCL2FPs in mitochondrial fusion. We pursue the quantitative analysis of mitochondrial fusion and seek to identify novel factors and/or molecules able to modulate this process.

**Ref.:**

***1.*** *Karbowski et al. (2006). Role of Bax and Bak in mitochondrial morphogenesis. Nature* ***443****, 658.*

***2.*** *Rolland et al. (2009). The BCL-2-like protein CED-9 of C. elegans promotes FZO-1/Mfn1,2- and EAT-3/Opa1-dependent mitochondrial fusion. J.Cell Biol.* ***186****, 525.*

***3.*** *Hoppins et al. (2011). The Soluble Form of Bax Regulates Mitochondrial Fusion via MFN2 Homotypic Complexes. Mol. Cell* ***41****, 150.*

***4.*** *Breckenridge et al. (2009). Bcl-2 Proteins EGL-1 and CED-9 Do Not Regulate Mitochondrial Fission or Fusion in C. elegans. Curr. Biol.* ***19****, 768.*

## P1.12

# Loss of the MTCH2 mitochondrial protein in the forebrain affects neuronal regulation of whole body homeostasis and behaviour

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The brain is the centre of the nervous system and its function is to exert centralized control over the other organs of the body. Mitochondria are key organelles that allow efficient energy production to sustain neuronal membrane potential and firing activity. When mitochondrial functionality is compromised, changes in cellular energetics are observed and the whole-body energy balance is affected.

Mitochondrial carrier homolog 2 (MTCH2) is a novel outer membrane mitochondrial protein, previously identified in our laboratory as a receptor-like protein for the pro-apoptotic BID protein. Recently, MTCH2 was identified as one of six new neuronal gene loci associated with increased body mass index and obesity in humans, suggesting that MTCH2 may play a role in metabolism.

To determine the functional importance of MTCH2 in the brain, we generated a MTCH2 forebrain-conditional knockout mouse (BKO) using the CamKII-Cre deleter mice. BKO mice gain 5%-to-10%-less weight compared to the WT littermates on regular chow diet. In addition, BKO mice are hyperactive during the night cycle and, probably as a result of increased locomotor activity, they display higher oxygen consumption as well as increased $CO_2$ and heat production. BKO mice also eat more, possibly due to their increased activity and/or the lower leptin levels

detected in their serum (especially in the BKO females). Interestingly, no differences in insulin and glucose serum levels were observed. Finally, BKO mice seem to have impaired motor planning and coordination as well as learning and spatial memory disability.

All together our findings are consistent with the idea that proper mitochondrial function mediated in part by the MTCH2 protein is critical for multiple neuronal activities, especially locomotor activity. Our future studies are aimed to clearly determine how MTCH2 – via its function at the mitochondria – regulates these events.

## P1.13

# The mitochondrial cannabinoid receptor 1 (mtCB1) regulates mitochondrial axonal motility

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Neurons strongly rely on mitochondrial energy metabolism with particular high energy demands at locations distant from the cell body. For this reason, mitochondria have to be placed correctly through mechanisms involving fusion, fission and transport. Emerging evidence indicates a strong link between mitochondrial trafficking and neurodegenerative diseases. The cannabinoid receptor CB1 regulates neuronal activity and deregulations of its signaling are centrally involved in brain diseases. This function is exerted at least at two sites: (i) at the plasma membrane, where CB1 (pmCB1) acts as a classical G-protein coupled receptor (GPCR) and (ii) at the mitochondria, where it was recently described as the first GPCR associated to these organelles (mtCB1), regulating the mitochondrial activity in the brain. Here we show that in the central nervous system mtCB1 is implicated in the trafficking of mitochondria. The percentage of transported mitochondria both, anterograde and retrograde, is increased in CB1 KO axons compared to control WT neurons. Moreover, the mitochondrial transport is reduced in CB1-overexpressing neurons when CB1 is activated by a specific CB1-agonist (HU), but not when a mutant lacking the predicted signal to mitochondria (DN22CB1) is expressed. Confirming these results, when a non-permeable version of HU (HU-Biotin that can only act in the pmCB1) is used, the reduction in mitochondrial transport is not observed. Our data show that mtCB1 participates in the regulation of mitochondrial trafficking and positioning in axons, indicating and additional mode of cannabinoid regulation of brain bioenergetics processes.

P1.14

# Carbon monoxide improves astrocytic metabolism and mitochondrial function preventing neural cell death

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The strategy modulation of cerebral cell metabolism for improving the outcome of hypoxia-ischemia and reperfusion is a strategy poorly explored. Because carbon monoxide (CO) is known to prevent cerebral cell death; herein the role of CO in the modulation of astrocytic metabolism, in particular, at the level of mitochondria is investigated. Still, the non-cell autonomous effect of CO in astrocytes-to-neuron communication and their purinergic signaling is also assessed.
Low concentrations of CO enhanced intracellular ATP generation, which was accompanied by an increase on specific oxygen consumption, a decrease on lactate production and a reduction of glucose use, indicating an improvement of oxidative phosphorylation rate. Accordingly, CO increased cytochrome c oxidase (COX) enzymatic specific activity and mitochondrial population. In astrocytes, COX interacts with Bcl-2, which was verified by immunoprecipitation; this interaction is superior after 24 h of CO treatment. Furthermore, CO enhanced Bcl-2 expression in astrocytes. By silencing Bcl-2 expression with siRNA transfection, the CO effects in astrocytes were prevented, namely: (i) inhibition of apoptosis, (ii) increase on ATP generation, (iii) stimulation of COX activity and (iv) increase on mitochondrial population. Thus, Bcl-2 expression is crucial for CO-modulation of oxidative metabolism and for conferring cytoprotection. CO-induced cell oxidative metabolism improvement confers not only a cell-autonomous, effect by directly decreasing astrocytic cell death, but also a non-cell autonomous effect by promoting indirect neuroprotection, whereas CO-pre-treated astrocytes reduced neuronal cell death in the co-culture systems. Indeed, in this system there is no physical contact between neurons and astrocytes, and CO induces the release of ATP from astrocytes, which was assessed by luciferase assay. While, suramin a chemical inhibitor of the purinergic receptor P2 reverted CO-induced neuroprotection *via* astrocytes in co-cultures. Furthermore, in monoculture of neurons, the addition of ATP (αβmeATP resistant to degradation) into the media increased neuronal survival. Therefore, the CO-induced astrocytic metabolic improvement and ATP increased production promotes neuroprotection. Finally, *in vivo,* low concentrations of CO exposure decrease tissue lesion and cell death in a model of perinatal cerebral ischemia. Overall, one can conclude that CO neuroprotective role is not limited to a direct action into a single cell type. This gasotransmitter has the ability to manipulate the complexity of the brain organization, modulating cell metabolism and purinergic signalling in order to increase astrocytic protection against neuronal cell death.

P2.01

# Mitochondria in glutamatergic nerve terminals are selectively affected by exposure to $A\beta_{1-42}$ modeling early Alzheimer's disease

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Alzheimer's disease (AD) is defined by a loss of cognitive function associated with an abnormal processing and accumulation of amyloid β-peptide (namely $A\beta_{1-42}$) and by a hypometabolism and hypofunction of brain cortical regions, mainly the hippocampus. With the aim to arrest the evolution of AD, particular attention has been devoted to synaptic dysfunction and loss, which are one of the precocious modifications accompanying cognitive dysfunction[1]. Mitochondria plays a key role in the maintenance of adequate synaptic function and mitochondrial structural and functional abnormalities are well characterized features of AD[2]. Additionally, mitochondrial accumulation of Aβ was shown to occur before extracellular Aβ deposition[3]. On the other hand, a predominant susceptibility of glutamatergic synapses was already described, using an Aβ-based model of AD[4]. This prompts us to post the hypothesis that mitochondria located in glutamatergic terminals are particularly affected in AD, leading to modifications of calcium balance and energy power supply that underlie the synaptic degeneration in AD.

Hippocampal nerve terminals obtained in a discontinuous Percoll gradient[5] were plated and incubated with oligomeric $A\beta_{1-42}$ peptide (500 nM for 2 hours). Qualitative analyses through live imaging experiments were done to measure the changes between final and initial baseline of the mitochondrial membrane potential Δ ($\Delta\psi_m$), using a fluorescent probe, $TMRM^+$. The plasma membrane potential of the nerve terminals was also measured using the fluorescent probe PMPI. Oligomycin and FCCP were used as stimuli.

We report a reduction of 23.0% ± 5.2% (n=6) of $\Delta\psi_m$ after incubation of the nerve terminals with $A\beta_{1-42}$ peptide, without any modification of the plasma membrane potential. This reduction was mostly observed in the glutamatergic nerve terminals (immunopositive for vGluT 1), as confirmed by immunocytochemistry.

These results are in agreement with the contention that synaptic mitochondria are an important trigger of "synaptic apoptosis", contributing to synaptic dysfunction and degeneration in AD and further indicate glutamatergic terminals as primary targets of $A\beta_{1-42}$-induced toxicity. This prompts the correction of synaptic mitochondria dysfunction as an increasingly a justifiable candidate to therapeutically alleviate early AD.

1. Selkoe, D. J. Alzheimer's disease is a synaptic failure. *Science* **298,** 789–91 (2002).
2. Du, H. *et al.* Early deficits in synaptic mitochondria in an Alzheimer's disease mouse model. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 18670–5 (2010).
3. Caspersen, C. *et al.* Mitochondrial Aβ: a potential focal point for neuronal metabolic dysfunction in Alzheimer's disease. *FASEB J.* **19,** 2040–1 (2005).

4. Canas, P. M., *et al*. Predominant loss of glutamatergic terminal markers in a β-amyloid peptide model of Alzheimer's disease. *Neuropharmacology* **76,** 51-6 (2014).
5. Dunkley, P.R., *et al*. A rapid method for isolation of synaptosomes on Percoll gradients. *Brain Res.* **372,** 115–29 (1986).

## P2.02

# Modulating AMPK's activity has neuroprotective effects in an α-synuclein model of Parkinson's disease

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Mitochondrial dysfunction and significant decrease in the efficacy of protein clearance are two major hallmarks of the aging brain. According to a growing body of clinical and laboratory evidence, these phenomena are likely to be centrepieces in the pathogenesis of Parkinson's disease (PD). Hence, strategies targeting the mitochondrial/lysosomal axis may prevent neurodegeneration and provide prospective therapies for PD.

AMPK is a heterotrimeric protein complex that senses energy levels and controls mechanisms to restore proper metabolic balance in the cell. AMPK is therefore a promising target for PD therapy, as it controls mitochondrial function and protein clearance in both direct and indirect manners. Various factors including drugs, diet and exercise can modulate AMPK's activity, which has already been found to have a role on neuronal loss in conditions such as ALS and stroke.

Here, we used AAV vectors to overexpress variants of the catalytic α2 subunit of the AMPK complex in both primary cortical neurons and in the rat substantia nigra. Our aim was to genetically determine how AMPK activity controls the degeneration of neurons exposed to the mitochondrial toxin rotenone and to α-synuclein accumulation. We observed that expression of the wild-type α2 subunit reduces the overall catalytic activity of the AMPK complex in neuronal cells, which predominantly express the α1 subunit. This effect was further pronounced by expressing a dominant negative (DN), inactive form of the α2 subunit. In cortical neurons treated with rotenone, basal oxygen consumption is increased in neurons expressing the wild-type and DN α2 subunits, and wild-type α2 prevents mitochondrial load from being reduced following toxin exposure. *In vivo*, we found that over-expression of the same two forms of AMPK's α2 subunit in the rat *substantia nigra* significantly protected dopaminergic neurons against α-synuclein-induced toxicity.

Overall, our data indicate that modulating AMPK activity may have neuroprotective effects in various conditions that are relevant to PD. They also support further studies to elucidate the effects of both the α1 and α2 subunits in the context of metabolic perturbations associated with disease progression.

P2.03

# Selenoprotein T exerts an essential thioredoxin reductase-like activity that prevents early and severe motor deficits in a mouse model of Parkinson's disease

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Oxidative stress is central to the pathogenesis of Parkinson's disease, but the mechanisms involved in the control of this stress in dopaminergic neurons have not been fully elucidated. Here, we demonstrated that the newly identified selenoprotein T (SelT) is a key thioredoxin reductase-like enzyme indispensable for embryonic development that controls oxidative stress in the nigrostriatal pathway and protects dopaminergic neurons in a mouse model of Parkinson's disease. Indeed, genetic disruption of SelT in mice is lethal *in utero* and its conditional knockout in the brain provoked a reduction in the size and the dopamine content of the striatum. Treatment with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rapidly triggered severe parkinsonian-like motor defects in brain specific SelT-deficient mice, which were associated with elevated oxidative stress after mitochondrial complex I impairment and decreased tyrosine hydroxylase activity and dopamine levels in the striatum. In contrast, MPTP administration to wild-type littermates increased SelT expression levels in the nigrostriatal pathway, preventing thereby the otherwise induced motor deficit. In a dopaminergic cell model, both silencing and overexpression of SelT affected oxidative stress and cell survival. These findings reveal a novel thioredoxin reductase-like enzyme that protects dopaminergic neurons and prevents movement impairment, providing clues for the understanding of the molecular underpinnings of oxidative stress in Parkinson's disease.

*Key words: Selenoproteins, Parkinson's disease, Oxidative stress*
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P2.03-b

# A novel *in vitro* brain slice model of pharmacoresistant mitochondrial epilepsy: 'a dual neuronal – astrocytic hit hypothesis'

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Up to a third of patients with mitochondrial disease develop epilepsy – 'mitochondrial epilepsy'. This epileptic phenotype is extremely difficult to control with very poor prognosis. Drug development has been lagging due to a lack of good functional models. Post-mortem neuropathological studies of temporal neocortex from patients with mitochondrial epilepsy have shown deficiency in mitochondrial respiratory chain complexes I and IV in both GABAergic interneurons and astrocytes, with a pattern of astrogliosis. Building on these observations, we aim to develop a novel model of mitochondrial epilepsy utilizing various mitochondrial inhibitors; rotenone (complex-I inhibitor), potassium cyanide-KCN (complex-IV inhibitor), and fluorocitrate (astrocytic specific aconitase inhibitor). Epileptic activity in the pattern of interictal and ictal activity was readily generated in both the hippocampus (CA3) and temporal neocortex by adding fluorocitrate (0.1 mM) followed by co-application of rotenone (500nM) and KCN (10µM). Applying either fluorocitrate or rotenone-KCN alone did not generate any epileptic activity. We have also replicated these experiments in surgically resected human temporal neocortical slices (n=6). Of the six antiepileptics tested (levetiracetam, lamotrigine, carbamazepine, midazolam, pentobarbital, and sodium valproate), only sodium pentobarbital was able to suppress this epileptiform activity. Post-hoc immunohistochemistry of these epileptic brain slices showed a similar pattern of astrogliosis as seen in the human neuropathological studies. Interestingly, there was also a significant reduction of parvalbumin-expressing interneurons in the hippocampus CA3 (n=6).
In conclusion, we have successfully developed a novel *in vitro* brain slice model for mitochondrial epilepsy. The model replicates most of the features seen in the human neuropathology and also show signs of pharmacoresistance as is what is observed in the clinical setting. We hypothesized that there is a selective vulnerability of inhibitory interneurons towards respiratory chain inhibition that when coupled with astrocytic mitochondrial impairment can lead to epileptic activity generation.

P2.04

# Loss of OPA1 induces oxidative metabolism perturbation and antioxidant defences activation

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Dominant Optic Atrophy (DOA) is a common cause of inherited visual failure affecting at least 1 in 50 000 of the general population. OPA1 mutations are the main genetic cause of DOA, causing bilateral symmetrical optic atrophy due to specific loss of retinal ganglion cells. Although optic nerve degeneration remains the hallmark of DOA, a syndromic form called "DOA plus", including deafness, ataxia, myopathy, peripheral neuropathy and progressive external ophtalmoplegia, was recently reported to affect up to 20% of all mutations carriers. More than 200 mutations have been listed, the major part are truncations therefore supporting haploinsufficiency as the major pathogenic mechanism of DOA. To assess the consequences of OPA1 mutations on energetic metabolism and redox states, two supposed pathological mechanisms leading to DOA, we mimicked haploinsufficiency phenomenon, using a siRNA strategy to down-regulate OPA1 expression in human cells and in embryonic rat neurons in primary culture. The existence of energetic defects in DOA patients are indeed controversial: (i) defective muscle mitochondrial oxidative metabolism was described in vivo (ii) coupling defect of oxidative phosphorylation and reduction of complex I or IV activities were observed in skin fibroblasts (iii) while no perturbation occurred in lymphoblastoid cells. On the other hand, while mutations in invertebrate OPA1 orthologs were shown to increase ROS levels and to lead to exogenous ROS hypersensitivity, nothing was demonstrated in mammals. In this work, we showed that the mitochondrial respiratory chain quantity is perturbed, without changes in the intracellular ROS level. Nevertheless, the aconitase activity is decreased, suggesting an increase of the intra-mitochondrial ROS level. We then explored the antioxidant machinery and revealed that the NRF2 pathway is activated and some target proteins are increased. Similar analyses are nowadays performed on patients fibroblasts. Altogether, these results suggest that the antioxidant defenses are activated to circumvent oxidative imbalance induced by OPA1 down-regulation, thus opening a novel way of reading DOA-1 pathogenesis.

P2.05

# Alpha-synuclein/mitochondria interaction alters mitochondrial protein import

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Alpha-synuclein (α-Syn) accumulation and mitochondrial dysfunction play prominent roles in the pathology of Parkinson's disease (PD). A fraction of α-Syn interacts with mitochondria, but the consequences of α-Syn–mitochondria interactions in the normal and pathologic effects of α-Syn remain poorly defined. Protein import represents a critical pathway for mitochondria because, among the ~1,400 estimated proteins in mitochondria, a mere 13 are encoded by mitochondrial DNA. The other ~1,387 proteins are delivered to mitochondria through the import pathways; thus deficits in such pathways may contribute to neurodegenerative disease such as PD.

In neuroblastoma BE-M17 cells, our results show that α-Syn is translocated to mitochondria after complex I inhibition. Interestingly enough, induction of complex I inhibition by the mitochondrial toxin $MPP^+$, in cells overexpressing α-Syn, diminishes the interaction of α-Syn with mitochondria. To test for direct effects of complex I inhibition and α-Syn accumulation in mitochondria on protein import, we performed mitochondrial import assays in isolated mitochondria from neuroblastoma BE-M17 cells overexpressing or not wt-α-Syn. Complex I inhibition and α-Syn overexpression markedly increased protein import of OTC (~50%) $^{35}S$-labeled proteins.

In vitro as well as in vivo, in the midbrain of MPTP-intoxicated brain of mice, the integrity of the mitochondrial protein import is affected. Indeed, our data indicate that the system of mitochondrial import of the outer membrane, measured by the expression levels of specific outer membrane mitochondrial proteins Tom40, is not affected after treatment with MPTP. However, the level of expression of Tim 23, a protein involved in the mitochondrial import system of the inner membrane was decreased by 50% as early as 1 day after the last MPTP injection and maintained until 7 days after the last injection. Our data indicate that the import system through the inner membrane of mitochondria, after inhibition of complex I, is affected and can contribute to mitochondrial dysfunction associated with dopaminergic neurodegeneration in Parkinson's disease. Although further experiments are necessary to understand the link between decreased expression of Tim 23 and an increase of protein import, we hypothesis that altered mitochondrial protein content accompanied by selective increases in protein import into mitochondria, which may be associated with increased aggregates inside the mitochondria and mitochondrial quality control dysfunction, might be part of the mitochondrial damage arising from complex I inhibition and α-Syn accumulation in mitochondria.

## P2.06 - *Cancelled*

## P2.07

# Silencing of CMT-associated gene GDAP1 reduces store-operated Ca2+ entry (SOCE) and results in a lower SOCE-stimulation of respiration in intact neuroblastoma cells

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GDAP1 (ganglioside induced differentiation associated protein 1) gene causes Charcot-Marie-Tooth neuropathy, the most frequent hereditary neuromuscular disorder. GDAP1 is an outer mitochondrial membrane protein that interacts with the vesicle-organelle trafficking proteins RAB6B and caytaxin, which suggests that GDAP1 may participate in the mitochondrial movement within the cell. GDAP1 silencing in neuroblastoma SH-SY5Y cells induces abnormal distribution of the mitochondrial network, decreases the contact between mitochondria and endoplasmic reticulum (ER) and reduces 1) $Ca^{2+}$ inflow through store-operated $Ca^{2+}$ entry (SOCE) following mobilization of ER-$Ca^{2+}$ and 2) SOCE-driven $Ca^{2+}$ entry in mitochondria.

Any $Ca^{2+}$ signal causes ATP breakdown by $Ca^{2+}$ pumps or exchangers in order to restore $Ca^{2+}$ levels. In addition, Calcium regulates oxidative phosphorylation via two different methods: a) calcium entry in mitochondria (through the mitochondrial calcium uniporter, MCU) and activation of mitochondrial dehydrogenases or b) extramitochondrial calcium activation of metabolite transport. As SOCE-driven $Ca^{2+}$ entry in mitochondria is reduced in GDAP1-KD neuroblastoma cells, we have investigated mitochondrial respiration during SOCE mechanism in intact neuroblastoma cells. We have found that SOCE activation induces a marked stimulation of respiration in neuroblastoma cells, which is reduced (by 41%) in the GDAP1–KD cell line.

We have next investigated the effect of different pathological GDAP1 missense mutantions in SOCE activity, after transient expression on the GDAP1-silenced cells. Recessive mutations in GDAP1 located inside the α-loop (protein-protein interactor domain, amino acids 106-152) fail to restore SOCE activity in GDAP1 KD cells. This is the case of the recessive GDAP1 mutation p.S130C. In agreement with this, we have found that GDAP1 silencing in HEK293T reduces SOCE-driven stimulation of respiration and that the recessive mutation pS130C fails to revert the stimulation of respiration. These findings suggest that the pathophysiology of GDAP1-related CMT neuropathies may be associated with an impaired SOCE-driven $Ca^{2+}$ uptake and/or signaling in mitochondria, which could impair neuronal respiration and bioenergetics.

P2.08

# Olesoxime, a novel mitochondrial targeted compound provides neuroprotection for treatment of spinal muscular atrophy

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Olesoxime (cholest-4-en-3-one, oxime; TRO19622) is a novel mitochondrial targeted neuroprotective compound that was recently validated in a clinical trial as a neuroprotective therapeutic in spinal muscular atrophy (SMA), a pathology with no effective treatment. This small molecule identified by Trophos from an original phenotypic screening program on primary motor neurons displays remarkable neuroprotective and neuroregenerative properties in various *in vitro* and *in vivo* models. Indeed, olesoxime increased motor neuron survival following facial nerve axotomy, delayed disease onset and prolonged survival in a transgenic model of familial ALS and increased life span and motor function in transgenic models of SMA. Both binding and functional data indicate that olesoxime interacts with two outer mitochondrial membrane proteins, the 18 kDa translocator protein (TSPO, formerly named peripheral benzodiazepine receptor) and the voltage-dependent anion channel (VDAC). Using a fluorescent derivative of olesoxime, we confirmed the mitochondrial tropism of olesoxime. About 80% of the compound is found in a mitochondrial-enriched subcellular fraction. Results from multiple cell death models converge on an effect of olesoxime to counteract intrinsic and extrinsic mitochondrial apoptotic pathways. In that sense, only Fas-L-induced mitochondrial-mediated death of primary motor neurons could be rescued by olesoxime while it had no impact on mitochondrial-independent LIGHT-mediated cell death. Similarly, olesoxime conferred neuroprotection against DNA-damaging agent camptothecin in primary cortical neurons. Neuronal death induced by this topoisomerase inhibitor occurs through p53 activation mediating Bax translocation, cytochrome c release and caspase activation. We showed that olesoxime dose-dependently delayed camptothecin-induced cell death preventing cytochrome c release and caspase activation but it had no impact on upstream p53-dependent pathways or on pro-survival pathways ERK and PI3K/Akt. Additionally, olesoxime protected differentiated human neuroblastoma cells from neurite retraction, cytoplasmic shrinkage and cell death induced by alpha-synuclein overexpression. This protection was also associated with a mitochondrial protection, a reduction in cytochrome c release from mitochondria and caspase-9 activation. In conclusion, olesoxime promotes neuronal survival by reducing the risk of activating mitochondrial pro-apoptotic pathways. Whether this process involves a physical mitochondrial permeability transition pore complex is still an open question; however, olesoxime and other members of the cholesterol oxime family appear to be promising drug candidates to treat a variety of neurodegenerative diseases.

P2.09

# Activity and density of mitochondrial enzymes in several tissues of parkinsonian monkeys using cell membranes microarrays

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Mitochondrial dysfunction has been implicated in the pathogenesis of Parkinson ´s disease (PD). The first evidence appeared when it was found that long exposure to the inhibitor of complex I of mitochondrial electron transport chain (ETC), MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), generated parkinsonism in humans and monkeys. In this context, taking into account the continuing increase of the aging population and neurodegenerative diseases prevalence, we have developed cell membrane microarrays from control and MPTP-treated non-human primate (*Macaca fascicularis)*. This animal model has been well characterized in the past and clearly reproduces the main pathological and clinical features of PD. Using cell membrane microarrays we have found changes in some principal complexes of ETC such as complex II and IV as well as in complex I. Thus, MPTP reduces the activity of ETC in liver, heart and cerebellum, while increases it in hippocampus and hypothalamus. Moreover, cytochrome c oxidase (complex IV) activity is decreased in cerebral cortex, substantia nigra and amygdala, whereas succinate dehydrogenase (complex II) activity is increased in olfactory bulb, putamen, caudate and substantia nigra.

Our results show that a single miniaturized assay provides a powerful tool for identifying and validating new therapeutic targets that might accelerate the drug discovery process and also predict the occurrence of possible side effects with animals, time and cost savings.

## P2.10

# Wfs1$^{-/-}$ mice: phenotyping and gene therapy against Wolfram Syndrome disease

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The Wolfram Syndrome (WS) is an early onset genetic disease (1/200 000) featuring diabetes mellitus and progressive optic neuropathy ensuing mutations in the *WFS1* gene. We investigated mice with deleted exon 8 of the gene to imitate the visual aspects of the disease.
The model has been known to exhibit pancreatic β-cell atrophy but the visual function has not yet been investigated. Therefore, we focused on assessing it via *in vivo* and *post mortem* studies of Wfs1$^{+/+}$ and Wfs1$^{-/-}$ mice at 3 and 7 months of age.
Firstly, we examined the visual acuity via changes in the optokinetic relfex, the retinal ganglion cell (RGC) function via the post-scotopic treshold responce (pSTR), and the eye physiology via the optical coherence tomography (OCT). We also determined the proportion of retinal ganglion cells (RGC) and the axonal loss at the age of 7 months, with anti-Brn3a immuno-labeling of retinal sections and electron microscopy of optic nerve (ON) sections, respectively. We observed progressive loss of visual acuity accompanied by a loss of axons in the ON.
Secondly, we performed an intravitreal gene therapy (GT) with AAV-2/2-CMV-*WFS1* at 1 month old Wfs1$^{+/+}$ and Wfs1$^{-/-}$ mice. The assessment of the visual acuity and the RGC function at 3 and 6 months of age showed no worsening of the vision coming from the GT nor the injections themselves. Moreover, WFS1$^{-/-}$ mice after the GT exhibited an improved visual acuity and slower deterioration of RGC function with age. The study is ongoing for histological analysis.
The presented data qualify the murine model for researching the visual aspects of the WS. Additionally, the promising preliminary results of the gene therapy encourage further studies under a treatment for the Wolfram Syndrome patients.

## P2.11

# Quantitative intravital microscopic analysis of neuronal mitochondria morphology under injury

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Using intravital two-photon microscopy, we imaged the dynamics of neuronal mitochondrial morphology under traumatic conditions in the brain of anesthetized mice expressing mitoCFP under Thy1 promoter and quantified these dynamics we developed an automated analysis method based on supervised learning.

To tested automated analysis method sensitivity we compare images prior to and after cardiac arrest, and found it to be sensitive to significant changes in mitochondrial morphology, and thus useful in detecting abnormalities in mitochondria. Further, to examine dynamics of mitochondria morphology within cortical layer 1 to injury, we measured the degree of mitochondrial fragmentation in three models: i) mild photodamage (MPD); ii) focal laser-lesion (FLL); and iii) Rose Bengal photosensitization (RBPS). To access the time course, changes in mitochondrial morphology were analyzed during acute (3 hours), subchronic (2, 4, 7 days) and chronic (3 weeks) phases after injury. MPD was induced in a small volume (typically 165x165x100µm) by exposing the tissue to approximately 50-fold higher light energy than during imaging; FLL was produced by targeted laser light eliminating individual branches of the apical dendrites in a controlled volume (typically 50-100 µm3) of somatosensory cortex; RBPS was achieved by i.v. injection of Rose Bengal followed by exposure to green light, after which mitochondrial morphology was monitored sequentially in the core and remote areas. We found that MPD resulted in a rapid mitochondrial fragmentation in the high-exposure region. Surprisingly, the damage did not extend to the surrounding tissue and recovery of mitochondria morphology occurred only 4 days after MPD. FLL resulted in a complete loss of the CFP fluorescence at the lesion site and induced mitochondria fragmentation at the perilesion site that recovered during one week. Spontaneous dendritic regrowth into the lesion site occurred during 7-14 days and was accompanied by recovery in mitochondrial morphology. RBPS rapidly produced a core with severe mitochondrial fragmentation that did not show any recovery over 3 weeks. We observed some recovery of mitochondrial morphology after 3 weeks but only in relatively small parts of the remote area. Finally, we used dual TG mouse line Thy1-MitoCFP×Thy1-EGFP-M to evaluate the temporal relationship between mitochondrial morphology and the dendritic structure during the first 3h after RBPS. Interestingly, structural changes in neurons, such as blebbing, were always observed with some delay after the mitochondrial fragmentation occurred first in response to RBPS.

We propose that microscopic imaging with supervised learning-based analysis of neuronal mitochondrial morphology in vivo reveals a pivotal role of mitochondria in neuronal survival or death.

## P2.12

# Role of Receptor Expression-Enhancing Protein 1 (REEP1) in mitochondrial structure and energetic function

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Receptor accessory protein 1, REEP1, is a membrane protein with ubiquitous intracellular localization as the protein is present in the mitochondria, endoplasmic reticulum and lipid droplets. However, REEP1 function in these organelles is not clearly elucidated. Mutations in this gene cause spastic paraplegia autosomal dominant type 31, SPG31, a neurodegenerative disorder. Recently, we described a mitochondrial hyperfusion and a decrease in mitochondrial energy production in a muscle biopsy and skin fibroblasts derived from a patient having a truncative mutation in the gene of REEP1 (c.106delG ; p.V36Sfs*4) (Goizet C. et al . Hum Mut., 2011). We now confirme an alteration of mitochondrial function and architecture using fibroblasts from additional four patients with missense mutations (one patient: c.166G>A, p.D56N and three related patients: c.124T>C, p.W42R). Interestingly, we also find that SPG31 patients reveal higher level of mitochondrial DNA (around 3-fold increase) compared to controls. To analyze whether REEP1 is involved in mitochondrial physiology, we have expressed different isoforms of REEP1 in HeLa cells and we demonstrate different localization profiles for REEP1. We observed that one pool of REEP1 strongly locates to mitochondria inducing the organelle fragmentation. All together, our results validate the role of REEP1 in mitochondrial functions.

## P2.13

# Impaired mitochondrial dynamics in SCA28 M665R-Knock-in MEFs

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SCA28 is one of the 31 known subtypes of autosomal dominant Spinocerebellar Ataxias (SCA). It is caused by mutations in the *AFG3L2* gene, encoding for an ATP-dependent metalloprotease belonging to the AAA-superfamily (ATPases Associated with a variety of cellular Activities). AFG3L2 can form homo- or hetero-oligomeric complexes with paraplegin: both reside in Inner Mitochondrial Membrane and exert protein quality surveillance and mediate protein processing.
SCA28 patients mainly have missense mutations in the peptidase domain of *AFG3L2*, but the function altered by the mutations is still unclear. To further evaluate the role of missense changes, we generated a knock-in (KI) mouse model carrying the p.M665R mutation (human p.M666R). It should be noted that two SCA28 mouse models are described in literature, but both carry a loss-of-function mutation in *Afg3l2*. SCA28 M665R-KI heterozygous mice showed a phenotype onset between 12 and 14 months, while homozygous M665R gave a perinatal lethality. We studied mitochondrial dynamics in Mouse Embryonic Fibroblasts (MEFs) from KI and WT animals: (i) AFG3L2 protein levels were comparable; (ii) OPA1, a protein found in the inner membrane of mitochondria, involved in mitochondria fusion process, showed an increase of the short forms and absence of the long forms; (iii) preliminary results on mitochondrial network morphology by mitotrackerRED staining, suggested an altered fission/fusion pathway.
Taken together, these data show an impairment of OPA1 processing that results in increased mitochondrial fragmentation. This is in accordance with the cellular phenotype seen in *Afg3l2* knock-out mouse model, corroborating the idea that SCA28 mutations hitting the peptidase domains negatively impact on m-AAA complex function.

## P2.14

# Rescue of synaptic loss and mitochondrial alterations of adult-born neurons in a mouse model of Alzheimer's disease

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A large number of studies indicate that mitochondria dysfunctions are involved in neurodegenerative diseases and particularly in Alzheimer's disease (AD). Moreover, alterations of hippocampal adult neurogenesis have been described in AD patients and in several mouse models of AD, suggesting that reduced neurogenesis could be implicated in the inability to acquire and store new information. Our work examines, for the first time, the mitochondrial network of adult-born hippocampal neurons in a mouse model of AD. We describe a dramatic perturbation of the mitochondrial network in the new granule neurons of AD mice. Moreover, we demonstrate that selective overexpression of a transcription factor was able to increase mitochondrial biogenesis and to promote neuritic maturation and spinogenesis in primarily cultured neurons. *In vivo*, targeted expression of this transcription factor rescues the mitochondrial content of hippocampal adult-born neurons in our AD mouse model.

## P2.15

# Prolactin modulates mitochondrial proteins involved in apoptosis in hippocampal neurons in an *in vitro* excitoxicity model

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It has been reported recently that prolactin (PRL) exerts a neuroprotective effect against excitotoxicity in hippocampus of the rat, and play an important role in the hippocampus functions[1,2]. However the mechanism by which prolactin mediates this effect remains unknown[3]. Bcl2 family proteins participate in apoptosis mechanism via mitochondrial pathway. In this study we assess BcL2, BAX and capase-3 expression pattern by Western blot analysis, in primary cultures of hippocampal neurons, under excitotoxicity induced by glutamate (Glu) and PRL treatments. The cell viability was assessed by MTT method.

The results demonstrated that cells treated with PRL/Glu have a significant higher viability than neurons treated onl with Glu. The protein content of mitochondrial anti-apoptotic protein BCL-2 presented a two fold increase in neurons treated with PRL/Glu as compared to neurons treated only with Glu. Interestingly, neurons treated with PRL overexpress BCL-2 content. BAX and Caspase-3 protein content were also evaluated; neurons treated with Glu overexpress the protein content of BAX whereas the groups with PRL and PRL/Glu have lower expression, while Caspase-3 has no changes between all treated groups. Our results suggest that PRL may induce neuroprotection against excitotoxicity on hippocampal neurons, partially by inducing

antiapoptotic balance of Bcl-2/BAX, inhibiting apoptosis. Further studies are required to elucidate the changes induced by this pleiotropic hormone in apoptosis pathway.

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*[1]* Vanoye-Carlo, A; Morales, T; Ramos, E; Mendoza-Rodrìguez, A; Cerbòn, M. *Neuroprotective effects of lactation against kainic acid treatment in the dorsal hippocampus of the rat.* Horm Behav. 2008. 53(1):112-23.
*[2]* Ross, CA; Poirier, MA. *Protein aggregation and neurodegenerative disease.* 2004. Nature Medicine. 10:S10-S17.
*[3]* Cabrera, V; Cantù, D; Ramos, E; Vanoye,A; Cerbòn, M; Morales, T. *Lactation is a natural model of hippocampus neuroprotection against excitotoxicity.* 2009. Neuroscience Letter. 461:136-39.

## P2.16

# Mitochondrial ATP governs neuronal response to NMDA, and is maintained by $Ca^{2+}$ activation of ATP-Mg/Pi carrier, SCaMC-3

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Glutamate excitotoxicity is caused by sustained activation of neuronal NMDA receptors causing elevations in cytosolic $Ca^{2+}$ and $Na^{+}$, activation of PARP-1, fall in cytosolic $NAD^{+}$ and ATP, and delayed $Ca^{2+}$deregulation (DCD) followed by neuronal death. Mitochondria undergo early changes in membrane potential during excitotoxicity but their relation with these events is still controversial. SCaMC-3/Slc25a23 is a mitochondrial ATP-Mg/Pi carrier which transports ATP or ADP in a strictly $Ca^{2+}$-dependent way and is a candidate to play a role in the initial mitochondrial response to NMDA. We have studied the early responses to NMDA in cortical neurons including a rapid increase in oxygen consumption rate (OCR) which was found to be due to $Ca^{2+}$-dependent upregulation of respiration. NMDA exposure resulted in a rapid fall in mitochondrial ATP ($[ATP]_{mit}$) in SCaMC-3 KO neurons, but not in control neurons, in which $Ca^{2+}$-dependent adenine nucleotide uptake through the carrier maintained $[ATP]_{mit}$. The fall in $[ATP]_{mit}$ in the KO neurons was associated with a blunted increase in respiration and a further decrease in cytosolic ATP levels, indicating that maintenance of $[ATP]_{mit}$ was required to upregulate OXPHOS upon NMDA exposure. The rapid fall in $[ATP]_{mit}$ and blunted respiratory response were

prevented by PARP-1 inhibitors, indicating a rapid NMDA-induced PARP-1 activation as cause. A second consequence of the lack of SCaMC-3 was an early appearance of DCD. This was associated with reduced $Ca^{2+}$ retention capacity (CRC) and increased $Ca^{2+}$-dependent swelling in mitochondria from SCaMC-3 KO mice incubated with physiological millimolar concentrations of ADP or Mg-ATP, suggesting that failure to maintain matrix AdN is responsible for both the impaired CRC in mitochondria and earlier neuronal DCD. SCaMC-3 KO neurons were more vulnerable to glutamate excitotoxicity in vitro and SCaMC-3 KO mice were more susceptible to kainate-induced seizures, showing that SCaMC-3 is an early player in excitotoxic cascade both in vitro and in vivo.

## P2.17

# Abnormal mitochondrial dynamics play a role in axonal degeneration in a model of inflammatory neuropathy

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The role of impaired mitochondrial trafficking is increasingly recognised in the pathogenesis of peripheral neuropathies, but their behaviour *in vivo* is very poorly understood. To study the effect of inflammation on mitochondrial function and transport, mitochondria were observed by time lapse confocal imaging in the exposed saphenous nerves of anaesthetised mice with experimental autoimmune neuritis (EAN). We found that, in comparison with naive animals, the mitochondrial trafficking was increased in both EAN and adjuvant-only control animals. However, the number of mobile mitochondria was significantly lower in animals with EAN compared with adjuvant controls ($p<0.001$) or asymptomatic animals ($p<0.001$). There were no differences in the speed of mitochondrial transport between the groups, indicating that the function of molecular motors is not impaired in EAN. Interestingly, at the onset of EAN, but not in matched asymptomatic or adjuvant-only control animals, we observed a number of small to medium diameter fibres (3.34+/-0.61µm) which contained focal accumulations of stationary mitochondria. The accumulations started abruptly at the proximal end, but 'tailed off' gradually distally, over several tens of microns. Mitochondrial movement was absent at this tail end, and further distally. The accumulated mitochondria were polarised, thus seemingly healthy. Time-matched, asymptomatic animals with EAN showed few, if any such accumulations, but we were able faithfully to reproduce these accumulations by laser damaging (photo-bleaching) the mitochondria in such axons. The damaged mitochondria became depolarised, fragmented and immobile, presumably depleting the energy supply of the affected portion of the axons. Interestingly, the more proximal mitochondria in these axons started to move towards the damaged region in significantly increased numbers than before photo-bleaching ($p=0.007$). Upon arriving they slowed or stopped moving, seemingly obstructed by the damaged mitochondria. The increase in proximal mitochondrial movement occurred in all axons, but it was only in small axons (2.7+/-0.45 µm) that the accumulations occurred. Thus in

the larger axons alone the mitochondria passed unobstructed into the damaged field, repopulating it with healthy mitochondria. We suggest that failure of mitochondria to re-populate small axons may help to explain the selective loss of smaller axons in some peripheral neuropathies.

## P2.18 - *Cancelled*

## P2.19

# A viral peptide that targets mitochondria protects against neuronal degeneration in models of Parkinson's Disease

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Most neurodegenerative disorders involve distal mitochondrial dysfunction leading to progressive and specific loss of neuronal populations. Therefore, suggestion was recently raised that protection of mitochondrial function represent a promising therapeutic approach. On another hand, control of cellular apoptosis is a strategy often used by viruses to limit cellular damage and allow persistence in their host. Given the poor renewal capacity of neurons, it is even a key element for persistent neurotropic viruses. To achieve this goal, many viruses express proteins able to limit or prevent cell death, leading to the tantalizing hypothesis that some of these viral encoded proteins - especially those targeting mitochondrial functions - might delay or block neurodegenerative processes outside of the viral context.

Borna disease virus (BDV) is a prototypic neurotropic virus, whose persistence in the central nervous system involves expression of a small viral 10 kDa protein called X in the mitochondria. Using an innovative oriented primary neuronal culture based on microfluidics, we observed that X protein is endowed with neuroprotective properties *in vitro* against toxins that target the mitochondrial respiratory chain, even when expressed alone independently of the viral context. We showed that X protein stabilizes mitochondrial chaperone proteins and induces modifications in the mitochondria network dynamics of neurons, thereby preventing the marks of mitochondrial oxidative stress and initial stages of neurodegeneration. We also demonstrated that X protein expression *in vivo* in the nigrostriatal pathway almost completely blocked neuronal loss in murine models of Parkinson's disease. Finally, we developed a non-invasive strategy for delivery of therapeutic X-derived peptides, which settles a new lead for the development of novel therapies against neurodegeneration by targeting mitochondria morphology and preventing the earliest events of degenerative processes.

## P2.20

# Quantification of mitochondrial DNA in diabetes and Alzheimer's disease

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Introduction: Mitochondrial dysfunction has been shown to play a central role in the pathology of both diabetes mellitus and Alzheimer's disease (AD). The long-term effects of diabetes on brain function have not been extensively studied despite evidence that diabetic patients are at a ~2-fold increased risk of cognitive decline and developing AD in comparison to healthy individuals. We have previously found that patients with diabetes have altered levels of circulating mitochondrial DNA (MtDNA) which is precursor to mitochondrial dysfunction.[1] Mitochondrial dysfunction and inflammatory changes have also been observed in the peripheral blood of AD patients.[2] In the current project we examine the hypothesis that mitochondrial dysfunction is present in conditions of hyperglycaemia and AD and can be detected in peripheral blood and brain samples.

Methods and biological samples: Mitochondrial dysfunction was assessed by measuring MtDNA content, measured as mitochondrial to nuclear genome ratio (Mt/N). Using real-time quantitative polymerase chain reaction (qPCR), regions in nuclear DNA and unique regions in the mitochondrial genome, not duplicated in the nuclear genome, were amplified. In a pilot study we assessed Mt/N, inflammation and mitochondrial biogenesis in brain tissue of a streptozotocin-induced diabetic mouse model.

Results: In the diabetic mouse brain, we found no evidence of changes in Mt/N or biogenesis but there was a trend for increased inflammation when compared to control brains. We are currently measuring Mt/N and inflammation in peripheral blood and brain tissue of human AD patients. A subset of these patients is also diabetic.

Conclusions: Due to the progressive nature of diabetic complications, it is possible that the duration of diabetes <4 weeks was too short to result in glucose-induced changes in the mouse brain. Analysis of MtDNA content and mitochondrial function in these experimental systems and patients will enable us to determine the effect of hyperglycaemia on mitochondria in the brain and in the blood, and whether changes in MtDNA contribute to AD.

(1) Malik, A.N., Shahni, R., Iqbal, M.M. (2009) Increased peripheral blood mitochondrial DNA in type 2 diabetic patients with nephropathy. 86(2):e22-4. *Diabetes Res ClinPract*.
(2) Lunnon, K. et al. (2012) Mitochondrial dysfunction and immune activation are detectable in early Alzheimer's disease blood. J Alzheimers Dis. 30(3):685-710.

## P2.21

# Striatal mtDNA depletion potentiates dyskinetogenic changes in dopaminergic signal transduction

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Parkinson's Disease (PD) is a neurodegenerative disorder in which the dopaminergic neurons of the substantia nigra pars compacta (SNc) deteriorate, causing a decrease in dopamine (DA) stimulation in the striatum. Individuals with the disorder have difficulty initiating movements and suffer from rigidity and tremors. Current pharmacologic treatment replaces DA with L-3,4-dihydroxyphenylalanine (L-DOPA), a DA precursor that crosses the blood-brain-barrier. Although treatment with L-DOPA is initially beneficial, over time it leads to the development of L-DOPA Induced Dyskinesia (LID), characterized by excessive and uncontrolled movements that decrease the quality of life of patients.

While ninety percent of PD patients treated with L-DOPA develop LID within ten year[1], some patients develop LID more rapidly than others. Our lab examined molecular differences in the brain putamen of "dyskinetic" and "nondyskinetic" patients, which had been matched for disease duration and L-DOPA dosage. Strikingly, mitochondrial DNA (mtDNA) was significantly decreased in the dyskinetic group compared to the control and nondyskinetic groups. No significant change was observed in the cerebellum[2]. We therefore hypothesize that decreased mtDNA in the putamen of dyskinetic patients plays a critical role in accelerating the development of LID.

We explored the impact of decreased mtDNA levels on DAergic signaling in the putamen using mouse primary striatal cultures treated with ethidium bromide (EtBr). The striatum is the murine equivalent of the human putamen. In this model, we show that EtBr-treated cultures recapitulate several of the signaling pathway changes observed in rodent models of DA deprivation and LID. EtBr-treated cultures produce significantly more cFos mRNA in response to DA stimulation, which may be analogous to the increase in cFos in the hypersensitive DA-deprived striatum in 6-OHDA treated rodent models[3]. Upon stimulation with DA, ERK1/2 is hyperphosphorylated and Histone 4 is deacetylated in EtBr-treated cultures, both phenotypes that have been repeatedly associated with L-DOPA treatment and the development of LID[4]. We hypothesize that decreased mtDNA potentiates the development of LID in PD patients by altering the DAergic signaling pathway.

*1. Ahlskog JE, Muenter MD (2001) Frequency of Levodopa-Related Dyskinesias and Motor Fluctuations as Estimated From the Cumulative Literature. Movement Disorders 16:448-458*
*2. Naydenov AV, Vassoler F, Luksik AS, Kaczmarska J, Konradi C (2010) Mitochondrial abnormalities in the putamen in Parkinson's disease dyskinesia. Acta neuropathologica 120:623-631.*
*3. Nadjar A, Gerfen CR, Bezard E (2009) Priming for L-dopa-induced dyskinesia in Parkinson's disease: A feature inherent to the treatment or the disease? Progress in Neurobiology 87:1-9*

*4. Nadjar A, Gerfen CR, Bezard E (2009) Priming for L-dopa-induced dyskinesia in Parkinson's disease: A feature inherent to the treatment or the disease? Progress in Neurobiology 87:1-9*

## P2.22

# Parkin and PGC-1α functionally interact to control mitochondrial activity in neuronal cells

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Mitochondrial dysfunction is central in the pathology of Parkinson's disease (PD). The Parkin protein, which is directly associated to recessive forms of PD, was recently found to promote mitophagy and other mechanisms controlling the quality of mitochondria. In addition, PD has been linked to a decrease in the activity of PGC-1α, a transcription co-regulator, which is considered to be a master regulator of mitochondrial biogenesis. Our goal was to explore the functional interaction between Parkin and PGC-1α in neuronal cells, and determine how these two factors may together control mitochondrial function.

In primary cortical neurons, we find that Parkin overexpression further increases mitochondrial biogenesis induced by PGC-1α. In addition, the combined overexpression of Parkin and PGC-1α leads to the rapid recovery of mitochondrial membrane potential following exposure to the protonophore CCCP, which is not observed with any of these factors alone. This effect coincides with a significant increase in the mitochondrial reserve respiratory capacity, as measured by oxygen consumption in presence of CCCP.

*In vivo*, we unilaterally overexpressed PGC-1α and Parkin in the adult rat substantia nigra using AAV vectors. As previously shown, chronic overexpression of PGC-1α selectively affects the expression of dopaminergic markers, as well as the survival of nigral dopaminergic neurons. In this condition, we found a significant effect of Parkin on amphetamine-induced rotations, which indicates a partial recovery of the dopaminergic function. Although the survival of neurons expressing PGC-1α was not significantly increased by human Parkin expression with respect to a control non-coding vector, there was a significant difference between wild-type Parkin and the PD-associated R42P and K161N mutants. This result indicates that Parkin mutations may affect neuronal survival when PGC-1α is overexpressed.

Overall, these results highlight the concerted role of Parkin and PGC-1α on mitochondrial function, suggesting that the combined action of these two proteins may have a critical role in the survival and activity of nigral dopaminergic neurons in the normal and diseased conditions.

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# General Information

## MEETING VENUE

## AGORA

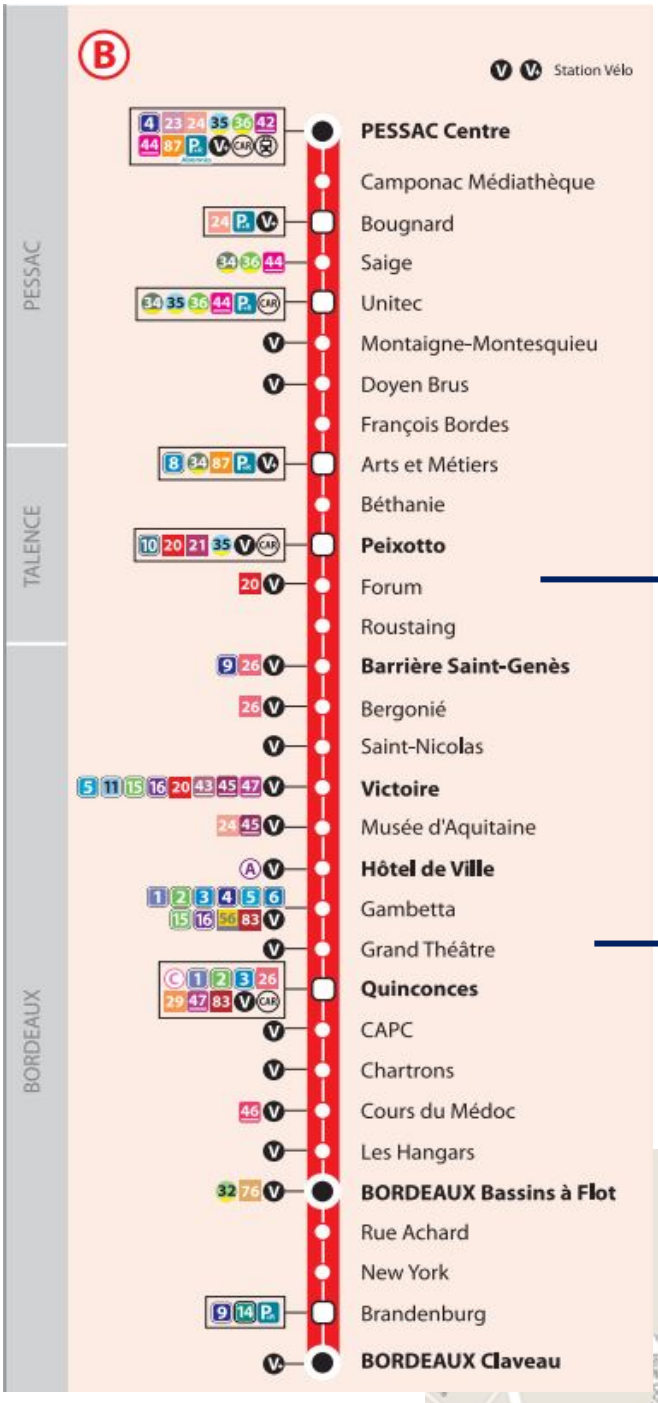
Domaine du Haut-Carré
43 rue Pierre de Noailles - TALENCE

**GPS**: Latitude 44.810012 - Longitude -0.59645

**How to get there:**
**Tram**: line B – Forum station (recommended)
**Bus**: line 20 – Forum station

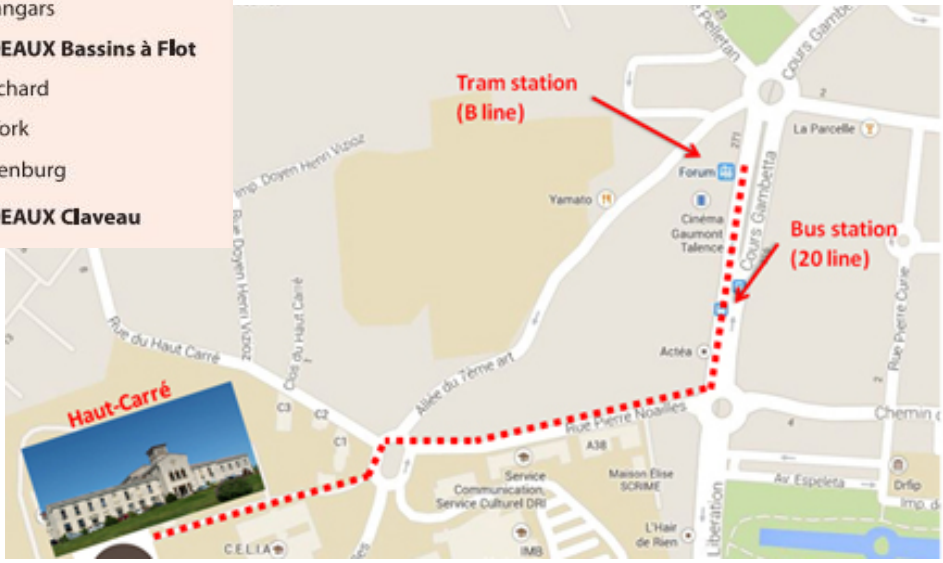
… and then a short 5 min walk uphill!!! (see next page for the map)

# TBC Transportation Map (Tram, B Line)




**Forum station:**
**AGORA Haut-Carré**

**Grand Théâtre station**

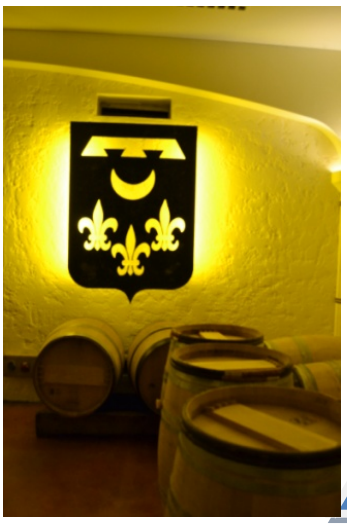


## SOCIAL EVENT: GALA DINNER
## Thursday, October 2nd

## Château Smith Haut Lafitte

Martillac

**Château Smith Haut Lafitte**, located **20 minutes** from Bordeaux city centre, train station and airport, nestled in a **78-hectare** vineyard, will unveil for you its 16th century tower, its underground cellar where **1 000 barrels** are resting, its cooperage and its unique reception rooms.



# Wifi access at the Haut-Carré: user guide

- Choose the REAUMUR wireless network
- Start your internet browser and try to access a web site
- Follow the instructions.
- Choose "Conferences/Invites"
- Identify yourself with :
- Login: **mito**
- Password: **brain**

EDUROAM access is also provided

MitoBrain institutional and academic partners are

Our industrial partners are