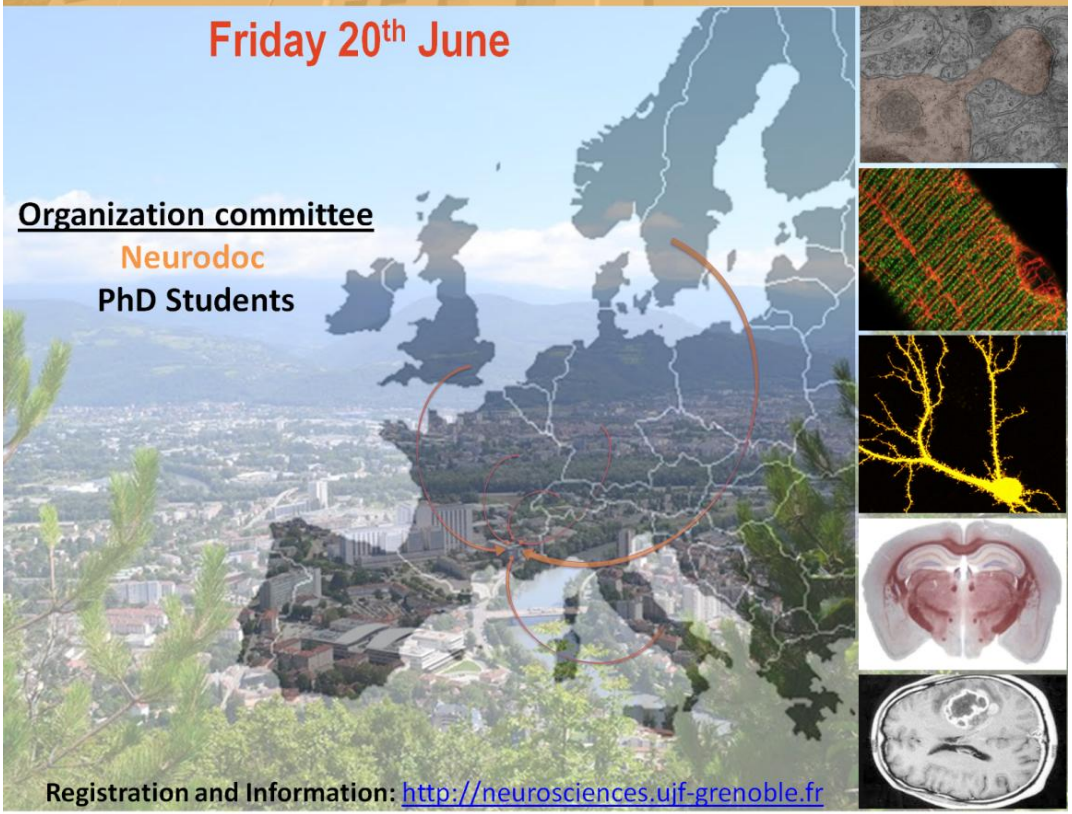
European Meeting of Neurosciences by PhD students

Friday 20th June



Organization committee

Neurodoc

PhD Students

Registration and Information: http://neurosciences.ujf-grenoble.fr

UNIVERSITE JOSEPH FOURIER SCIENCES.TECHNOLOGIE.SANTÉ

Institut des Neurosciences GRENOBLE

Inserm

dominique Dutscher

cea

EDISCE

EDCSV

CHU GRENOBLE

covalab R&D in Biotechnology

Acknowledgements

Partners

The committee relies on the generosity of the Grenoble Institute of Neurosciences, the University of Joseph Fourier and both Doctoral school EDCSV (*Ecole Doctorale Chimie et Science du Vivant*) and EDISCE (*Ecole Doctorale Ingénieurie pour la Santé, la Cognition et l'Environnement*) to make this 1st Edition of the meeting possible. We thank them for their financial support.

Sponsors and exhibitors

We welcome and thank Covalab, Dutscher and IseDesign for their services and support for the event.

Special thanks to…

The Director of the Grenoble Institute of Neurosciences Frédéric Saudou for his support and his enthusiasm for this 1st Edition of the European Meeting of Neurosciences by PhD students.

We thank Amélie Danfossy for fees and traveling organization. We are really grateful for the kind help of Karine Mora, Christine Chatellard-Causse, Béatrice blot, Yasmina Saoudi and Fiona Hemming.

Then we thank Karin Pernet-Gallay who was the first to trust and support this meeting.

Organization committee

Alexis Osseni, Astrid Kibleur, Charlotte Javalet, Chrystelle Aillaud, Elea Prezel, Julie Jonckheere, Lucie Dardevet, Marc Dollmeyer, Marine Laporte, Melina Bouldi, Muriel Sébastien, Stephen Ramanoël and Vincent Mercier.

Thank you to all the people involved in the organization of The 1st Edition of the European Meeting of Neurosciences by PhD Students.

Friday, June 20, 2014
Programme at a glance
Grenoble Institute of Neurosciences

European Meeting of Neurosciences by PhD students

WELCOME	
08:30 – 09:00	Welcome Coffee and Posters Installation
09:00 – 09:15	Opening Word by Dr Frédéric Saudou, GIN Director

PLENARY LECTURES SESSION 1	
	SESSION CO-CHAIRS: Marine LAPORTE and Alexis OSSENI
09:15 – 09:40	Simultaneous Imaging and Patch Clamp Recordings in Live Cells to Visualise and Interfere with Endocytic Vesicle Formation Morgane ROSENDALE (Bordeaux, France)
09:40 – 10:05	Could RNAi be a Therapeutic Approach for Fronto Temporal Dementia and Parkinsonism Linked to Chromosome 17? Kavitha SIVA (Trento, Italy)
10:05 – 10:30	An FTD-Linked CHMP2B Mutant Triggers Behavioral Defects and Motor Dysfunction in Mice Aurélia VERNAY (Strasbourg, France)

COFFEE BREAK AND POSTER SESSION	
10:30 – 11:10	Refreshment Break, Poster in the Hall

PLENARY LECTURES SESSION 2	
	SESSION CO-CHAIRS: Mélina BOULDI and Astrid KIBLEUR
11:10 – 11:35	PNEUMATK: A Python Tool Kit for the Analysis, Comparison, and Classification of Neuronal Morphologies Lida KANARI (Lauzanne, Switzerland)
11:35 – 12:00	Different Time Courses and Distribution of Aβ Deposition, Astrocytosis, and Microgliosis in Alzheimer APPswe Mice Ruiquing NI (Stockholm, Sweden)

LUNCH BREAK	
12:00 – 13:40	Lunch Break in the Cafeteria

PLENARY LECTURES SESSION 3	
	SESSION CO-CHAIRS: Charlotte JAVALET and Marc DOLLMEYER
13:40 – 14:05	Amyloidβ-Induced NMDA-Receptor Signaling to the Nucleus Katarzyna GROCHOWSKA (Madgeburg, Germany)
14:05 – 14:30	Functional Remodeling of Presynaptic Active Zone induced by Picomolar Levels of Endogenous Amyloid-β Maria ANDRES-ALONSO (Magdeburg, Germany)

COFFEE BREAK AND POSTER SESSION	
14:30 – 15:10	Refreshment Break, Poster in the Hall

PLENARY LECTURES SESSION 4	
	SESSION CO-CHAIRS: Lucie DARDEVET and Vincent MERCIER
15:10 – 15:35	The Role of Prion Protein in Neurodegenerative Disorders Agnese De MARIO (Padova, Italy)
15:35 – 16:00	The Bacterial Protein Toxin Cytotoxic Necrotizing Factor 1 (CNF1) Provides Long-Term Survival in a Murine Glioma Model Eleonora VANNINI (Pisa, Italy)

CLOSURE REMARKS	
16:00 – 17:00	GIN Presentation by PhD Students

EUROPEAN PHD STUDENTS MEET "G.I.N"	
17:00 – 19:00	Discussions and Drinks
19:30 – 22:00	European PhD Students meet GIN PhD Students Dinner

Summary

ORAL COMMUNICATIONS.....8

OC1: Morgane Rosendale

Simultaneous Imaging And Patch Clamp Recordings In Live Cells To Visualise And Interfere With Endocytic Vesicle Formation 9

OC2: Kavitha Siva

Could Rnai Be A Therapeutic Approach For Fronto Temporal Dementia And Parkinsonism Linked To Chromosome 17?.....11

OC3: Aurélia Vernay

An Ftd-Linked Chmp2b Mutant Triggers Behavioral Defects And Motor Dysfunction In Mice 13

OC4: Lida Kanari

Pneumatk: A Python Tool Kit For The Analysis, Comparison, And Classification Of Neuronal Morphologies 15

OC5: Ruiquing Ni

Different Time Courses And Distribution Of Aβ Deposition, Astrocytosis, And Microgliosis In Alzheimer Appswe Mice 17

OC6: Katarzyna Grochowska

Amyloidβ-Induced Nmda-Receptor Signaling To The Nucleus 19

OC7: Maria Andres-Alonso

Functional Remodeling Of Presynaptic Active Zone Induced By Picomolar Levels Of Endogenous Amyloid-B 21

OC8: Agnese De Mario

The Role Of The Prion Protein In Neurodegenerative Disorders 23

OC9: Eleonora Vannini

The Bacterial Protein Toxin Cytotoxic Necrotizing Factor 1 (Cnf1) Provides Long-Term Survival In A Murine Glioma Model 25

POSTERS 28

P1: Aine Rubikaite
Arl8, Autophagy And Axon Branching **29**
P2: Marta Costa
Bipolar Disorder And Cluster Headache: Microarray Study For The Identification Of Genes And Pathway Potentially Involved In Lithium Response **31**
P3: Julie Jonckheere
Repeated Electroconvulsive Seizures Increase Adult Neurogenesis In An Animal Model Of Psychiatric Disorders: Map6 Null Mice. **33**
P4: Charlotte Javalet
Neuronal Exosomes Contain Selective Micrornas And Can Be Endocytosed By Recipient Neurons **35**
P5: Marwa Dagshni
Role Of The Cacnb4 Subunit Of Voltage Gated Calcium Channels In The Regulation Of Gene Expression **37**
P6: Muriel Sébastien
Dynamics Of Triadic Proteins In Skeletal Muscle **39**
P7: Stephen Ramanoël
Effect Of Rms Contrast Normalization On The Retinotopic Processing Of Spatial Frequencies During Scene Categorization **41**
P8: Paul Gimenez
Radiotherapy By Photoactivation Of Nanoparticles And Mössbauer Effect **43**
P9: Florian Studer
Radiosurgery Of Epilepsy Using Synchrotron X-Ray Microbeams **45**
P10: Damien Benis, Astrid Kibleur
Stn Stimulation And Motor Inhibition **47**
P11: Marc Dollmeyer
Studying Aβ-Oligomers Induced Synaptic Plasticity Perturbations In Dendritic Spines **49**
P12: Auréliane Elie, Elea Prezel
Tau Mediates The Cross-Talk Between Microtubules And Actin Filaments **51**
P13: Attya Omer
Interested In Knocking-In, Knocking-Out, Knocking-Down ? Do Crispr! **53**
P14: Amandine Virlogeux
Regulation Of Huntingtin Localization And Function In Axonal Transport By Palmitoylation **55**

ORAL COMMUNICATIONS

Interdisciplinary Institute for Neuroscience, CNRS and Bordeaux University, Bordeaux, France

SIMULTANEOUS IMAGING AND PATCH CLAMP RECORDINGS IN LIVE CELLS TO VISUALISE AND INTERFERE WITH ENDOCYTIC VESICLE FORMATION

Morgane Rosendale, Dolors Grillo-Bosch, Isabel Gauthereau, Daniel Choquet, Matthieu Sainlos and David Perrais.

The formation of clathrin-coated vesicles is a fundamental process of all eukaryotic cells controlled by extensive protein-protein interactions. Among others, peptides have been described as inhibitors of endocytic activity. The most widely used of these peptides, known as D15, mimics the proline rich domain of dynamin. However, its low affinity for SH3 domains imposes to work routinely at rather high concentrations, *i.e.* ~1-2mM. We propose rational-based optimisation strategies of the D15 and characterise the obtained peptides by *in vitro* and live cell experimentation. Extension and engineering of the D15 sequence enable stronger binding of the peptides to their target, as defined by K_d determination. Specifity is also improved over the original sequence as shown by pull-down and proteomics experiments. In parallel, a quantitative functional assay has been developed to detect the formation of single endocytic vesicles in live cells while dialysing peptides by patch clamp. NIH-3T3 cells transfected with transferrin receptor fused to super-ecliptic pHluorin (TfR-SEP) are observed by time-lapse TIRF microscopy. By alternating extracellular pH every 2 seconds between 7.4 and 5.5, newly internalised vesicles can be observed in isolation with high temporal resolution (Merrifield C.J. & Perrais D. *et al.* Cell, 2005). Meanwhile, the frequency of vesicle formation is monitored: first in 'cell-attached' configuration (internal control), then in 'whole-cell' configuration to allow cell dialysis. A 'control' solution with buffered calcium, ATP and GTP enables such monitoring of the endocytic activity with minimal run-down. On the other hand, addition of optimised D15-derived peptides efficiently blocks endocytic activity within minutes.

NOTES

Centre for Integrative Biology, University of Trento, Italy

COULD RNAi BE A THERAPEUTIC APPROACH FOR FRONTO TEMPORAL DEMENTIA AND PARKINSONISM LINKED TO CHROMOSOME 17?

Kavitha Siva, Giuseppina Covello, and Michela Alessandra Denti.

Abnormalities of microtubule associated protein tau (*MAPT*) have been shown to be linked to pathogenesis of neurodegenerative diseases collectively termed as “Tauopathies". About half of the mutations in *MAPT* lead to perturbation of RNA splicing and tau fibrillization leading to formation of tau aggregates. A missense mutation in exon 10 at codon 279, results in an asparagine to lysine substitution (N279K). This impinges alternative splicing of exon 10 of the tau mRNA, and amends the normal ratio of 4Rtau/3Rtau causing an increased expression of 4R tau causing agglomeration of tau proteins. RNA interference has proven to be an efficient strategy for silencing mutant alleles of dominant disease genes as in Alzheimer’s disease, Machado-Joseph disease, Spinocerebellar ataxia type 3 and tau mutation (V337M) that causes fronto-temporal dementia. This project explores the feasibility of a siRNA-based gene therapy to enable post-transcriptional gene silencing of Exon10-containing MAPT mRNA in FTDP-17. A panel of siRNAs targeting Tau Exon 10 have been synthesised, which will be further tested upon CHP212 cells and SH-SY5Y cell lines. The outcome of RNA interference will be tested by both RT-PCR and western blot analysis. Based on these results, the small interfering RNA sequences will be embedded in siRNA expressing vector (psiUx) relying on strong and ubiquitous pol II dependent promoter of human U1 small nuclear RNA (U1 snRNA) gene. Allele specific silencing effects of these constructs will be monitored and analysed in the neuroblastoma cell lines. The effect on human tau pre-mRNA, will be monitored via a mini-gene reporter system, recapitulating to a large extent the behaviour of exon 10 in the context of tau gene. Further work will be directed to test the therapeutic efficacy of the AAV-vectored sh-RNAs in the animal model of FTDP-17 (T-279 mouse), which recapitulates the disease from a histopathological and behavioural point of view.

Key words: siRNA, Gene Regulation, FTDP-17, Alzheimer’s Disease

NOTES

Inserm U1118, Peripheral and Central Mechanisms of Neurodegeneration, UDS, Strasbourg, France

AN FTD-LINKED CHMP2B MUTANT TRIGGERS BEHAVIORAL DEFECTS AND MOTOR DYSFUNCTION IN MICE

Aurélia Vernay, Ludivine Therreau, Sylvie Grosch, Rémy Sadoul, Laurent Schaeffer, Jean-Philippe Loeffler, and Frédérique René.

Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are two connected neurodegenerative diseases. ALS is characterized by muscle weakness evolving to paralysis, due to the degeneration of upper and lower motoneurons. FTD is characterized by language and behavioral changes associated with an atrophy of the frontal and/or temporal cortices. Approximately 15% of FTD patients develop ALS and 20% of ALS patients also develop FTD. In addition to this clinical overlap, ALS and FTD share common histopathological hallmarks and a genetic continuum. Among the genes implicated in this syndrome, mutations in CHMP2B are found in ALS, FTD and ALS-FTD cases. In order to examine the role of the mutated protein in the ALS-FTD pathology we generated a transgenic mouse line that expresses the FTD linked CHMP2B^{intron5} mutant in neurons. The CHMP2B^{intron5} mice developed gait abnormalities, cramping, muscle weakness and a loss of motor coordination. A distal degeneration of the motor nerve and partial muscle denervation occurred. Moreover, we investigated dementia in the Chmp2B^{intron5} mice and found FTD-relevant behavioral alterations, such as changes in food preferences, restlessness and social interactions impairments. Like in humans, the CHMP2B^{intron5} protein formed inclusions in cortical neurons and spinal motoneurons, accompanied with astrocytosis in both tissues. These data indicate that the CHMP2B^{intron5} neuronal expression induces a motor phenotype associated with dementia symptoms and with histopathological hallmarks of ALS and FTD.

NOTES

Blue Brain Project , École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

PNEUMATK: A PYTHON TOOL KIT FOR THE ANALYSIS, COMPARISON, AND CLASSIFICATION OF NEURONAL MORPHOLOGIES

Lida Kanari, Joe W. Graham, Guy Atenekeng, Julian Shillcock, Felix Schuermann, and Henry Markram.

Neurons are not shapeless computational units but complex information processors containing detailed arborizations that define their function. Dendrites reach for inputs and define local microcircuits while axons communicate the neuronal output to other neurons in a local and global scale. Neuronal computation takes place along the dendrites, stressing the importance of accurate neurite morphology. It is hence no surprise that researchers are interested in morphologically realistic neuron models, rendering their use more and more widespread. Morphologies are at the core of many neuroscientific endeavors because they are used, amongst other things, for classification of neuronal types, discrimination of pathological cases, and in electrophysiological and network simulations. There is an abundance of software tools that each support some aspects of morphology processing in neuroscience, but an open-source, generic tool supporting multiple functionalities is missing. In this work we present PNEUMATK (the Python NEUronal Morphology Analysis Tool Kit). Pneumatk is a Python module supporting multiple post-reconstruction steps in the analysis, comparison and classification of populations of neuronal morphologies. Pneumatk can load neuronal morphology data files in the common SWC format, HDF5 format and Neurolucida ASCII format. Pneumatk computes standard morphometrics as well as as a range of more exotic features describing the complexity of a neuron and its spatial embedding. Pneumatk has the ability to statistically and graphically compare different groups of neuronal morphologies. Pneumatk includes basic machine learning functionality to explore the classification of morphologies into different types. All of these functions can be used in scripting more complex analyses. The main advantage of Pneumatk over existing tools is that all the morphometric functionality is

consistently accessible in one Python package, which increases its ease of use. It also allows for a central repository of tools that prevents constant reinvention of the wheel among different labs and researchers. As such, Pneumatk is the first open-source software package that offers the neuroscience community a large suite of tools supporting the visualization and analysis of neuronal morphologies.

Keys words: Python, Neuron, Morphology, Analysis, Classification

Alzheimer Neurobiology Center, Department of Neurobiology, Care Sciences and Society, Karolinska Institute, Stockholm, Sweden

DIFFERENT TIME COURSES AND DISTRIBUTION OF Aβ DEPOSITION, ASTROCYTOSIS, AND MICROGLIOSIS IN ALZHEIMER APPSWE MICE

Ruiqing Ni, Elena Rodriguez-Vieitez, Larysa Voytenko, Amelia Marutle, and Agneta Nordberg.

Emerging evidence suggests a link between neuroinflammation and beta-amyloid (Aβ) deposition in Alzheimer's disease (AD). Our recent *in vivo* microPET study showed higher ^{11}C-AZD2184 binding in 18-24 months APPswe mice, while elevated ^{11}C-deuterium-L-deprenyl binding in 6 months APPswe mice, suggesting different time courses of pathological events (Rodriguez-Vieitez, AAIC 2013). Here we investigate further the temporal and regional relation of astrocytosis, microgliosis and Aβ deposition in APPswe mice *in vitro* by autoradiography along with immunostaining. Binding characteristics of ^{3}H-AZD2184,^{3}H-PIB,^{3}H-L-deprenyl and ^{3}H-PK11195 were evaluated in APPswe mice brain tissue. Distribution of Aβ, astrocytosis and microgliosis in APPswe mice (6,8-15,18-24 months, n=3-6/group) and wild-type mice (8-12,18-24 months, n=3-6/group) were analyzed by *in vitro* autoradiography using the aforementioned ligands and immunostaining performed with antibodies for $A\beta_{42}$, GFAP, Iba-1 in sagittal brain slides.^{3}H-AZD2184,^{3}H-PIB,^{3}H-L-deprenyl and ^{3}H-PK11195 autoradiography revealed presence of high affinity binding sites (nM range) in APPswe mice cortex. Both the ^{3}H-AZD2184 and ^{3}H-PIB binding were elevated in the cortex and hippocampus of 18-24 months APPswe mice compared to wild-type mice, corroborated with increased $A\beta_{42}$ plaque deposition observed in aged APPswe mice. The ^{3}H-L-deprenyl binding was high already in young APPswe mice and demonstrated no changes with age, while the ^{3}H-PK11195 binding was increased in the hippocampus of aged APPswe mice. A greater number of hypertrophic GFAP+ astrocyteswere surrounding and distant to Aβ plaques, whereas Iba1+ activated microglia were in close proximity to Aβ plaques in aged APPswe mice. Our findings demonstrate that astrocytosis precedes amyloid deposition, and that microgliosis is more closely associated

with Aβ plaques deposition in APPswe mice brain, supporting our *in vivo* microPET finding of early astrocytosis in APPswe mice.

Keys words: Positron Emission Tomography, Amyloid Plaque, Inflammation

Research Group Presynaptic Plasticity, Leibniz Institute for Neurobiology, Magdeburg, Germany

AMYLOIDβ-INDUCED NMDA-RECEPTOR SIGNALING TO THE NUCLEUS

Katarzyna Grochowska

It is widely believed that amyloidβ oligomers cause synaptic disruption via alterations of NMDAR signaling leading to onset of Alzheimer's disease and cognitive impairment. Jacob is a protein messenger that encodes the origin of synaptic vs. extrasynaptic NMDAR signals and delivers them to the nucleus. The phosphorylation state of Jacob determines whether it induces cell death or promotes cell survival and enhances synaptic plasticity. Upon the stimulation of synaptic NMDARs, Jacob is phosphorylated by ERK and translocates to the nucleus promoting expression of pro survival genes. On the contrary, upon stimulation of GluN2B containing extrasynaptic NMDARs, non-phosphorylated Jacob translocates to the nucleus causing CREB shut-off, stripping of synaptic contacts and retraction of dendrites. We show that application of Aβ oligomers induces extrasynaptic GluN2B activation and results in Jacob accumulation in the nucleus and CREB shut-off, shRNA knockdown of Jacob prevents Aβ- induced CREB shut-off and Jacob entering the nucleus following Aβ-application is not phosphorylated by ERK. We next tested different types of Aβ oligomers. $A\beta_{3(pE)-42}$ (truncated Aβo with an aminoterminal pyroglutamate), was reported to be more toxic than conventional $A\beta o_{1-42}$. Surprisingly, we observed that $A\beta o_{1-42}$ causes more severe spine loss and CREB shut-off than $A\beta_{3(pE)-42}$. The morphological effects of $A\beta o_{1-42}$ induced extrasynaptic GluN2B signaling are strongly linked to nuclear import of Jacob and Jacob-induced CREB shut-off. Moreover, we observed that $A\beta_{3(pE)-42}$ shows increased detrimental effects on neuronal cell morphology and function only in the presence of astroglia, indicating different mechanisms of action of Aß-oligomers.

Keys words : Amyloidβ, NMDAR, Signaling, Jacob

NOTES

Research Groupe Neuroplasticity, Leibniz Institute for Neurobiology, Magdeburg, Germany

FUNCTIONAL REMODELING OF PRESYNAPTIC ACTIVE ZONE INDUCED BY PICOMOLAR LEVELS OF ENDOGENOUS AMYLOID-β

Maria Andres-Alonso, Vesna Lazarevic, Eckart Gundelfinger, and Anna Fejtova.

Amyloid-beta (Aβ) is involved in the development of Alzheimer's disease (AD), mainly characterized by severe cognitive decline and memory loss. However, the physiological function of this peptide and its role in pathophysiology are still not fully clarified. In this regard, many studies claim that Aβ has an effect at postsynaptic level, where it induces NMDA receptor-mediated neurotoxicity. Lately, a work made by Abramov et al (2009)[1] suggested that Aβ could act at presynaptic level by increasing the probability of neurotransmitter release (Pr). In order to study the molecular and cellular mechanisms underlying the modulation of Pr by Aβ, we established an experimental model based on rat and mouse primary hippocampal neurons in which we added picomolar amounts of Aβ1-42, or pharmacologically modulated endogenous levels of Aβ by interfering with its synthesis or degradation. By using this approach, we confirmed that picomolar Aβ induces an enhancement of the presynaptic function by mainly modulating two presynaptic processes within the presynaptic compartment: the distribution of synaptic vesicles between ready-releasable, recycling and resting presynaptic pools and the molecular organization and composition of the release machinery. According to our experiments, Aβ induces an enlargement of the ready-releasable pool and the recycling pool, the whole set of releasable vesicles. Moreover, increase of physiological levels of endogenous Aβ also affects the composition of the release apparatus, causing an increase in the expression levels of most proteins composing it. Most importantly, our experiments suggest that this remodeling induces functional recruitment of N-type voltage-dependent calcium channel at release sites, facilitating the neurotransmission upon action potential, and requires of functional presynaptic α7 nicotinic acetylcholine receptors.

Keys words: Active zone, Amyloidβ, Synaptic Plasticity, Presynapse

NOTES

Dept. of Biomedical Sciences and CNR Institute of Neuroscience, University of Padova, Padova, Italy.

THE ROLE OF THE PRION PROTEIN IN NEURODEGENERATIVE DISORDERS

Agnese De Mario, C. Peggion, A. Bertoli, M. Massimino and M.C. Sorgato.

The cellular prion protein (PrP^C) is a cell surface glycoprotein mainly expressed in the central nervous system. A β sheet-reach abnormal conformer of PrP^C generates the prion, the infectious particle causing fatal neurodegenerative disorders, called prion diseases. Despite intensive research, the physiological function of PrP^C remains enigmatic, although it has been suggested that it could participate in different cell functions. It has also been proposed that PrP^C may be part of the complex system controlling cell Ca^{2+} homeostasis, and we have recently found that indeed this occurs in primary neuronal cultures (Lazzari et al., 2011). Another recent proposal entails that PrP^C serves as a high-affinity receptor for amyloid-β (Aβ) fragments of the amyloid precursor protein implicated in Alzheimer's disease (AD), and that PrP^C-Aβ interactions could be crucial for AD-related impairment of synaptic plasticity (Lauren et al., 2009). In the present study, utilizing the Ca^{2+} probe aequorin targeted to different neuronal compartments and delivered by lentiviral vectors, we have measured Ca^{2+} fluxes in the plasma membrane, cytosol and the mitochondrial matrix of cerebellar granule neurons (CGN) derived from PrP-knockout (PrP-KO) mice, and, as controls, CGN from Tg46 mice in which normal PrP^C levels were rescued over a PrP-KO genotype mice. We found that, compared to controls, PrP-KO neurons have significant increased Ca^{2+} transients in all the above domains after activating both store-operated Ca^{2+} channels (SOCC) and glutamate, and/or NMDA-AMPA-Kainate receptors (R) and higher levels of active Fyn. To assess whether Aβ fragments deranged Ca^{2+} metabolism in a PrP^C-dependent manner, we carried out similar experiments but in the presence of oligomeric Aβ (1-42) peptides. On this, we have obtained results suggesting that, at least in part, Aβ affects neuronal local Ca^{2+} fluxes and Fyn activation in a PrP^C-dependent way.

Keys words: Calcium, Aβ, Oligomers, Neurons

NOTES

Institute of Neuroscience of the National Research Council of Italy, Pisa, Italy

THE BACTERIAL PROTEIN TOXIN CYTOTOXIC NECROTIZING FACTOR 1 (CNF1) PROVIDES LONG-TERM SURVIVAL IN A MURINE GLIOMA MODEL

Eleonora Vannini

Glioblastomas are largely unresponsive to all available treatments and there is therefore an urgent need for novel therapeutics. We have probed the antineoplastic effects of a bacterial protein toxin, cytotoxic necrotizing factor-1 (CNF1) in the syngenic GL261 glioma cell model. CNF1 produces a long-lasting activation of Rho GTPases, with consequent blockade of cytodieresis in proliferating cells and promotion of neuron health and plasticity. In cell culture experiments, we found that CNF1 was very effective in blocking proliferation of GL261cells, leading them to death within 15 days. CNF1 had a similar cytotoxic effect in human glioma cells obtained from surgical specimens. In in vivo experiments, we injected GL261 glioma cells into the adult mouse visual cortex, and five days later we administered either a single intracerebral dose of CNF1 or vehicle. To further compare CNF1 with a canonical antitumoral drug, we infused temozolomide (TMZ) via minipumps for 1 week in an additional animal group. Low dose (2 nM) CNF1 produced a survival effect, comparable to that of continuous TMZ infusion (median survival 35 days vs. 28 days in vehicle controls). Remarkably, increasing CNF1 concentration to 80 nM resulted in a dramatic enhancement of survival with no obvious toxicity. Indeed, 57% of the treated animals survived upto 60 days following GL261 glioma cell transplant. Tumor volume was also significantly decreased, while we found an enhanced recruitment of both astroglial and microglial cells at the border of the tumor. We are currently investigating whether CNF1 preserves neuronal response in the peritumoral area, and we have preliminary evidence that CNF1 administration results in functional sparing. Altogether, our data demonstrate that activation of Rho GTPases by CNF1 represents a novel potential therapeutic strategy for the treatment of central nervous system tumors.

Keys words : Glioma, CNF1, Mouse

NOTES

POSTERS

Medical Research Council for developmental Neurobiology, King's College, London, United Kingdom

ARL8, AUTOPHAGY AND AXON BRANCHING

Aine Rubikaite and Uwe Drescher.

Autophagy is a lysosome- dependent degradation pathway by which cells remove and recycle proteins or organelles. It is required for cell's maintenance and function, including responses to nutrient deprivation, oxidative stress, infection and accumulation of misfolded proteins. The homeostatic role of autophagy is particularly important for the long- lived postmitotic cells such as neurons, where disregulation of autophagic clearance usually manifests in neurodegenerative disorders. However, the role of autophagy in neuronal development is less clear. Here we ask if autophagy plays a role in remodelling of neuron architecture and how it may relate to establishing the precise connections during the nervous system development. By using chick retinotectal system as a model, we analyse autophagy processes in retinal ganglion cell (RGC) axon and how they control axon's morphology. We show that small GTPase Arl8 present in RGC axons is involved in autophagy pathway and that it regulates RGC axon branching. We also look at how manipulations of autophagic processes affect axon and branch morphology.

Key words: Retinal Ganglion Cell, Axon Branching, Autophagy, Arl8, Lysosomes

NOTES

Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Monserrato, Cagliari, Italy

BIPOLAR DISORDER AND CLUSTER HEADACHE: MICROARRAY STUDY FOR THE IDENTIFICATION OF GENES AND PATHWAY POTENTIALLY INVOLVED IN LITHIUM RESPONSE

Marta Costa, Alessio Squassina, Donatella Congiu, Paola Niola, Raffaella Ardau, Erminia Stochino, Arianna Deidda, Maria Del Zompo

Cluster headache (CH) and bipolar disorder (BD) are pathological conditions affecting 1% and 1.5% of the general population, respectively. Albeit the pathogenesis has not yet been completely elucidated, family and twin studies have suggested a genetic basis for both disorders, with an estimated heritability of 80% for BD and up to 60% for CH. Although BD and CH are very different diseases, they show important clinical similarities, such as a temporal pattern of disturbances, dysregulation of the wake-sleep cycle, neuroendocrine derangements, and more important positive clinical response to lithium and valproate treatments in a significant proportion of patients. In the present study, we sought to explore whether BD and CH patients responders to lithium share common molecular pathways potentially involved in predisposing to positively respond to prophylactic lithium treatment. To this aim, we carried out a transcriptome study in lymphoblastoid cell lines from 10 BD type I patients and 8 CH patients, all responders to lithium treatment, as well as 10 healthy subjects (CO) that had never been exposed to lithium therapy. Expression profiles were measured by Affymetrix GeneChip Human Gene ST 1.0, which interrogates 36,079 transcripts. Expression levels of BD and CH patients were compared with CO to identify commonly dysregulated genes and pathways. Pathway analysis was performed based on the hypergeometric test for over representation of specific *Kyoto Encyclopedia of Genes and Genomes* (KEGG). A total of 544 and 1172 genes were differentially expressed in BD versus CO and CH versus CO respectively. Filtering criteria were based on corrected p value < 0.05 and Fold Change (FC) $\geq |1.3|$. Among the filtered genes, 314 were commonly altered in both CH and BD when compared to CO. The most significant differentially expressed gene in both samples was RNA binding motif (RNP1, RRM) protein 3

(RBM3), implicated in sleep regulation and in the temperature entrained circadian gene expression (corrected p value of 6,30x 10^{-09} in BD vs CO and 1,88x 10^{-09} in CH vs CO). Pathway analysis showed that *Protein processing in endoplasmic reticulum* was one of the most significantly enriched in BD and CH when compared to CO. In conclusion, data from preliminary analyses of this microarray study may provide useful and relevant information for a better understanding of the molecular underpinnings of lithium response and on the neurobiology of BD and CH.

Keys words: ACCN1, Bipolar Disorder, Lithium, Genetics

Team Physiopathology of the Cytoskeleton, GIN

REPEATED ELECTROCONVULSIVE SEIZURES INCREASE ADULT NEUROGENESIS IN AN ANIMAL MODEL OF PSYCHIATRIC DISORDERS: MAP6 NULL MICE.

Julie Jonckheere, Gaëlle Dall' Igna, Nicolas Chauliac, Jean-Christophe Deloulme, Annie Andrieux, Jérôme Holtzmann, Marie-Françoise Suaud-Chagny, Sylvie Gory-Fauré.

The exact mechanisms underlying the actions of electroconvulsivotherapy (ECT) are not yet understood. The current hypotheses come from results obtained with electroconvulsive stimulation (ECS), the animal counter part of ECT. However, results are often inconclusive, mainly because data are based on unchallenged animals. Thus, we aim to develop a translational approach in MAP6-KO mice to decipher the biological effects underlying ECT. This mice exhibit some behavioral and biological features, and pharmacological response relevant to some aspect of the major depressive disorder. The first objective was to study the effects of ECS in MAP6-KO mice compared to WT mice or Sham-treated animals (handled the same way but no current delivered) at the behavioural level. We had shown that ECS applied in the MAP6-KO mice improved behavioral disorders such as social withdrawal or lack of motivation. The second objective was to explore the regulatory effect of ECS on adult neurogenesis in the dendate gyrus as well as on neuronal morphology with a particular interest for dendritic spines. After ECS treatment the proliferation and the survival/integration of newborn neurons is enhanced in the MAP6-KO mice hippocampus. We also observed an increase of spines density in the cortex after ECS treatment. Altogether results obtained after ECS in MAP6-KO mice recapitulate some features observed after ECT in humans such as behavioural restoration. We believe that these results validate MAP6-KO mice as a pertinent model to investigate molecular and cellular changes triggered by ECS treatment with the big advantage of the use of an animal model where extended characterisation is available.

Key words: MAP6-KO Mice, Electroconvulsive-Stimulation, Depression, Neuroplasticity

NOTES

Team Neurodegeneration and Plasticity, GIN

NEURONAL EXOSOMES CONTAIN SELECTIVE microRNAs AND CAN BE ENDOCYTOSED BY RECIPIENT NEURONS

Charlotte Javalet, Mathilde Chivet, Karine Laulagnier, Fiona J. Hemming, Béatrice Blot, Amy Buck and Rémy Sadoul.

Exosomes are vesicles of endocytic origin released by cells into their environment up on fusion of multivesicular endosomes with the plasma membrane. Exosomes represent a novel mechanism of cell communication through intercellular transfer of proteins, lipids and nucleic acids, including microRNAs. MicroRNAs are known regulators of neuronal physiology. We have demonstrated that exosomes are released from cortical neurons and that this release is regulated by synaptic glutamatergic activity. Our aims are to identify microRNAs contained in neuronal exosomes and to show interneuronal transfer of microRNAs by way of exosomes. Exosome release from primary cortical neurons (15 DIV) was stimulated by bicuculline. Exosomes were purified by ultracentrifugation. RNAs were extracted from exosomes and exosome releasing neurons. Deep sequencing revealed expression profiles of 250 microRNAs. Confocal microscopy was also used to investigate uptake of fluorescently labeled exosomes by receiving neurons. We found that exosomes contain only small RNAs. 154 of the microRNA were represented differently in exosomes and neurons: 105 were significantly underepresented in exosomes and 49 were significantly enriched in exosomes. Using fluorescently labeled exosomes, we found that purified exosomes bind to and are endocytosed by hippocampal neurons. **Conclusion:** We show that upon glutamatergic stimulation, neurons secrete exosomes containing selective microRNA which are endocytosed by receiving neurons. Further experiments will allow us to test how microRNAs can modify the physiology of receiving neurons.

Key words: Exosomes, Neurons, Tetanus Toxin, microRNAs

NOTES

Team Calcium Channels, Functions and Pathologies, GIN

ROLE OF THE CACNB4 SUBUNIT OF VOLTAGE GATED CALCIUM CHANNELS IN THE REGULATION OF GENE EXPRESSION

Marwa Dagshni, Mohammad Rima, Juan Bruses, Michel Ronjat, and Michel De Waard.

Voltage-gated calcium channels (VGCC) play a key role in neuronal communication and excitability. We have recently shown that cacnb4 (β4) an auxiliary subunit of VGCC interacts with the thyroid hormone receptor (TRalpha1) and modify its transcription factor activity in a way similar to the thyroid hormone T3. In the present study we investigated the interaction of β4 with another transcription factor, Kaiso. This transcription factor is an important actor of Wnt signaling pathway that control the expression of several genes involved in cell proliferation and differentiation. Using heterologus expression of β4 together with different proteins of the Wnt signaling pathway in HEK 293 and CHO cells we show that i) β4 interacts with p120-catenin known to interact with Kaiso, and ii) interaction of β4-p120-catenin regulates the nuclear translocation of β4. We are currently characterizing the interaction of β4 with p120 and its effect on the formation of p120-catenin/Kaiso complex. P120-catenin/Kaiso complex regulates activity of different genes among which genes regulated by β-catenin. We are currently studying the effect of β4 on this regulation.

Key words: β4 subunit, p120-catenin, β-catenin, Wnt Signaling Pathway, Voltage-gated Calcium Channels

NOTES

Team Muscle and Pathologies, GIN

DYNAMICS OF TRIADIC PROTEINS IN SKELETAL MUSCLE

Muriel Sébastien, Eric Denarier, Julie Brocard, Oriana Sarrault, Didier Grunwald, Isabelle Marty, and Julien Fauré.

Skeletal muscle cells are very organized cells, highly structured and entirely dedicated to contraction. Muscle stimulation at the neuromuscular junction results in massive intracellular calcium releases which induce contraction. This calcium release, mediated by the calcium release complex (CRC), occurs in a very specific part of muscle cells : Triads. Triads are formed by the close apposition of two sarcoplasmic reticulum (SR) terminal cisternae (TC) on both sides of an invagination of the plasma membrane, the transverse-tubule (TT). All CRC proteins are exclusively localized in the triad. The molecular mechanisms leading to the traffic and exclusive localization of CRC proteins at the triads are so far unknown. Triadin is a transmembrane protein of the CRC residing in SR membrane and proposed to anchor other members of the complex at the triad. To investigate the mechanisms responsible for Triadin localization in skeletal muscle we have expressed a photoactivatable version of the protein in primary myotubes cultivated from triadin KO mice. Video recording of the traffic of activated pools of triadin show it diffuses in SR and progressively accumulates in triads. Moreover, the traffic of photoactivatable triadin seems isotropic and able to span distances representing several microns. Our data suggest synthesis of triadin can occur in all SR, and that the molecule is able to traffic over long range before specific molecular determinants triggers its immobilization in triads.

Key words: Muscle, Photoactivation, Traffic, Anchoring, Triads

NOTES

Team Functional Neuroimaging and Brain Perfusion, GIN

Effect of RMS contrast normalization on the retinotopic processing of spatial frequencies during scene categorization

Stephen Ramanoël, Louise Kauffmann, Nathalie Guyader, Alan Chauvin, Cédric Pichat, Michel Dojat and Carole Peyrin.

Since there is considerable evidence suggesting that visual perception is based on spatial frequencies (SF) processing, a growing number of studies investigate the cerebral regions involved in the processing of low and high SF (LSF and HSF) information in complex visual stimuli (such as scenes). LSF and HSF stimuli are created using low- and high-pass filters that respectively attenuates signals with frequencies higher and lower than a cutoff frequency. The contrast is reduced in HSF relative to LSF images. Thus, recent fMRI studies normalized root mean square (RMS) contrast in image in order to avoid that differential cortical activations in LSF and HSF processing might be due to contrast differences. In the present fMRI study, we investigated whether RMS contrast normalization induced change in retinotopic processing of SF during scene categorization. For this purpose, participants performed a categorization task using large black and white photographs of natural scenes filtered in LSF, HSF and non-filtered (NF) scenes, in eight block-designed functional scans. In four functional scans, both mean luminance and RMS contrast of LSF, HSF and NF scenes were equalized, while in the other four functional scans only the mean luminance was equalized. When RMS contrast was not normalized, results showed that LSF (relative to HSF) scenes elicited activation in the anterior half of the calcarine fissures linked to the peripheral visual field, whereas HSF (relative to LSF) scenes elicited activation in the posterior part of the occipital lobes, which are linked to the fovea, according to the retinotopic property of visual areas. However, RMS contrast normalization drastically increased activation for HSF scenes only, such as no significant activation was obtained for LSF scenes compared to HSF scenes. Our study suggests that RMS contrast normalization should be used with caution when investigating the neural basis of SF processing in retinotopic areas.

NOTES

Team Synchrotron Radiation and Medical Research, GIN

RADIOTHERAPY BY PHOTOACTIVATION OF NANOPARTICLES AND MÖSSBAUER EFFECT

Paul Gimenez, Hélène Elleaume and Jean-Luc Ravanat.

The Mössbauer effect has been discovered in 1958. The resonant and recoil-free absorption and subsequent re-emission of γ rays by Mössbauer isotopes (e.g. Fe57) leads to the emission of many nanometric range Auger electrons. These high LET electrons can induce clustered DNA damages (Double Strand Breaks (DSB) and Locally Multiple Damaged Sites (LMDS)) if emitted in the direct vicinity of the DNA. Dose (in J/kg) enhancement using Mössbauer effect has been first described in vitro by Mills et al. (1988). We propose to evaluate the efficiency of iron nanoparticles (NPFe) enriched in ^{57}Fe to enhance dose deposition during radiotherapy. A sharp increase is expected in presence of the nanoparticles, due to Mössbauer effect at the resonance energy in comparison to off resonance. In order to measure this dose enhancement in liquid samples, a chemical dosimeter (Coumarin) quantifying the OH° production *in situ* will be used. Experiments at ID18 show that Mössbauer interactions can happen at room temperature in NPFe in a material with tissue-like rigidity. In parallel to this study on the Mössbauer effect, the photoelectric effect is also tested on these NPFe. Cells incubated with NPFe and irradiated at different energies on the Beamline ID17 present a decreased survival rate because of photoelectrons produced by the interaction of low energy radiations (30-80 keV) with heavy atoms. To conclude, the experiments done until now refine our comprehension of the physics of Mössbauer interactions and its challenges, as well as confirm the theoretical basis for its use to enhance radiotherapy with X-Rays.

Key words: Mössbauer, Iron Nanoparticles, X-Rays, Radiotherapy, Dosimetry, Coumarin

NOTES

Team Dynamics of Epileptic Synchronous Networks, GIN

RADIOSURGERY OF EPILEPSY USING SYNCHROTRON X-RAY MICROBEAMS

Florian Studer, Antoine Depaulis and Brigitte Piallat.

Despite development of new molecules, about a third of epileptic patients cannot be controlled by anti-epileptic drugs. Among them, only a limited number may benefit from resective surgery after invasive monitoring. Radiosurgery has proven to be an efficient alternative, especially for the treatment of small/circumcised epileptic foci but require further development to avoid unacceptable functional deficits that is often the risk with current methods. Synchrotron-generated X-rays allow the delivery of focalized radiation doses to discrete brain volumes via interlaced arrays of microbeams (IntMRT) of 50 µm, that have limited tissular damages. Our group showed that such synchrotron radiations abolish electroencephalographically (EEG) recorded seizures arising from the somatosensory cortex in a genetic model of epilepsy in the rat (GAERS). The objective of my PhD project is to further investigate the efficacy, optimal parameters and mechanisms of action of synchrotron X-ray microbeams in a focal and chronic epilepsy rat model, focal epilepsies being therapeutically the most challenging form of epilepsy nowadays. To this aim I am developing a new model of cortical focal seizures by injecting tetanus toxin or kainic acid in the motor cortex of Wistar rats. Using this model, I will evaluate the antiepileptic effects of synchrotron microbeams irradiations of different powers, widths and intervals, using electrophysiological techniques (EEG, local field potentials), immunohistochemistry and behavioral tests. This work will allow to define the most efficient conditions for synchrotron X-rays microbeams antiepileptic effects and will be then adapted to non-human primates using a similar model of focal epilepsy.

Key words: Focal Epilepsy, Animal Models, Radiosurgery, EEG, Tetanus Toxin, Kainic Acid, Rat, Non-Human Primate

NOTES

Team Brain Function and Neuromodulation, GIN

STN STIMULATION AND MOTOR INHIBITION

Damien Benis, Astrid Kibleur, Mircea Polosan, Jean-Philippe Lachaux, Eric Seigneuret, Paul Krack, Valérie Fraix, Stéphan Chabardès, Julien Bastin and Olivier David.

As a direct input structure of the Basal Ganglia system, the STN is thought to play a key role in executive functions, but the way these processes are implemented in the STN and how STN stimulation modulates them remains unclear. To investigate the neural correlates of inhibition in the STN and the cortical modulations triggered by STN DBS, 2 distinct studies were conducted in patients performing a Stop Signal Task (SST) which is a cognitive task designed to study inhibition. In the first study, direct neural recordings were performed on Parkinson patients undergoing implantation of DBS electrodes at two distinct scales: at the single neuron scale and at the Local Field Potential (LFP) scale (synchronous neuronal population). We isolated at the single neuron scale two neuronal subpopulations coding respectively for movement and stopping. Furthermore, we observed at the LFP scale a modulation of beta band activity associated with Reactive and Proactive inhibitory processes. In a second study, scalp EEG of stimulated OCD patients were registered ON DBS and OFF DBS. Source reconstruction showed that the right inferior frontal gyrus and the pre superior motor areas were modulated by the stimulation associated with decrease in inhibitory performance. STN stimulation also modulated inhibition monitoring ERPs. Finally, Dynamical Causal Modelling showed that DBS affects the bilateral cortical connections to basal ganglia structures. In conclusion, our studies show the direct involvement of the STN in inhibition, and that disruption of STN activity by DBS alters cortical activity in the inhibition network.

Key words: Subthalamic Nucleus, Deep Brain Stimulation, Inhibition, EEG, LFP

NOTES

Team Neuropathologies and Synaptic Dysfunctions, GIN

STUDYING AB-OLIGOMERS INDUCED SYNAPTIC PLASTICITY PERTURBATIONS IN DENDRITIC SPINES

Marc Dollmeyer, Marie-Lise Frandemiche, Travis J. Rush, Eve Borel and Alain Buisson.

Alzheimer's disease is the primary cause of senile dementia in the world and is associated with neuronal dysfunction and degeneration and by the presence of Amyloid-β oligomers (Aβo). Dendritic spines constitute the post-synaptic structure, and are subject to morphological modifications proportional to their activity; the stronger the synapse activity, the bigger the spine head will be. Surprisingly, our first results show that Aβo treatment increases the spine head size without inducing any activation. Moreover, Aβ-treated spines no longer resemble mature spines, and they present protrusions from the spine head. Given that the spine head shape is dictated by its actin cytoskeleton, the aim of our study is to identify the potent actin regulatory pathways disturbed by the presence of Aβo, which may be involved with these spine shape alterations.

Key words: Alzheimer, Synapse, Dendritic Spine, Actin, Cofilin, Aβ-oligomers

NOTES

Team Regulation of Cytoskeleton Dynamics and Structure, GIN

TAU MEDIATES THE CROSS-TALK BETWEEN MICROTUBULES AND ACTIN FILAMENTS

Auréliane Elie, Elea Prezel, Christophe Guerin, Sacnicte Ramirez-Rios, Ninon Zala, Leticia Peris, Annie Andrieux, Anne Fourest-Lieuvin, Laurent Blanchoin, and Isabelle Arnal.

The cooperation between microtubule and actin cytoskeletons is essential for neuronal differentiation and synaptic function, but the underlying molecular mechanisms remain poorly characterized. Tau is a neuronal Microtubule-Associated Protein that might be involved in this cytoskeletal crosstalk. Indeed, several recent but controversial studies have suggested that tau is able to bind actin filaments. Furthermore, besides its main localization in axons where it stabilizes microtubules, tau has been recently found in actin-rich regions like dendritic spines, wherein microtubules enter transitorily to remodel the actin network. Using purified cell-free systems, we investigated whether tau mediates the direct interaction between microtubules and actin filaments. We first demonstrated that tau binds to actin filaments and organizes them into bundles. Tau is also able to simultaneously interact with pre-polymerized actin filaments and microtubules, and to induce the bundling of both polymers. We next designed conditions to visualize in real-time by using TIRF microscopy the co-assembly of tubulin and actin in the presence of tau. Results revealed that the neuronal MAP coordinates both networks by co-aligning growing microtubules and actin filaments. We expanded our *in vitro* findings to primary neuron cultures, in which we confirmed a direct interaction between endogenous actin and tau by FRET microscopy. Furthermore tau co-localizes with microtubules and actin in growth cones of developing neurons. Altogether our results demonstrate that tau acts as a molecular linker between microtubule and actin cytoskeletons, leading to a co-organisation of both cytoskeleton networks that might be essential during the neuronal development.

Key words: Tau, Microtubules, Actin, TIRF Microscopy

NOTES

Team Neuronal Progenitors and Brain Pathologies, GIN

INTERESTED IN KNOCKING-IN, KNOCKING-OUT, KNOCKING-DOWN ? DO CRISPR!

Attya Omer, and Sandrine Humbert.

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR Associated (Cas) system success story started just few years ago. In 2007, scientists from Danisco found a way to boost the phage defenses of this workhouse microbe. They exposed the bacterium to a phage and showed that this essentially vaccinated it against that virus. It revealed something fundamental: Bacteria have a kind of adaptive immune system, which enables them to fight off repeated attacks by specific phages. By 2011 the details of the Cas9-based CRISPR system were becoming clear. In bacteria, the endogenous CRISPR/Cas system targets foreign DNA with a short, complementary single-stranded RNA that localizes the Cas9 nuclease to the target DNA sequence. The crRNA can bind on either strand of DNA and the Cas9 will cleave both strands. The key proof-of-principle work showed that an engineered "guide RNA" was enough to target specific DNA sequences with excellent specificity. Papers have now reported gene modifications using the CRIPSR technology in cell lines, mouse, zebrafish, drosophila. Methods for enhancing expression as well as shutting it down have already been described. Latest study showed that this technology is in off to do genome editing at one-cell-stage embryos in monkey. My PhD project is to use this technology to modify the *htt* gene in cell lines and in mouse. This gene encodes the huntingtin (HTT) protein, that is the protein mutated in Huntington's disease. I will generate knock-in variants of *htt* with a C-terminal GFP tag or with an N-terminal mCherry tag or with both as well as *htt* with point mutations of post-translational modifications. I am also planning to delete large regions of this gene.

Key words: Huntingtin, Genome-Editing, Knock Out, Double Tag, CRISPR

NOTES

Team Intracellular Dynamics and Neurodegeneration, GIN,

REGULATION OF HUNTINGTIN LOCALIZATION AND FUNCTION IN AXONAL TRANSPORT BY PALMITOYLATION

Amandine Virlogeux, Frédéric Saudou, and Diana Zala.

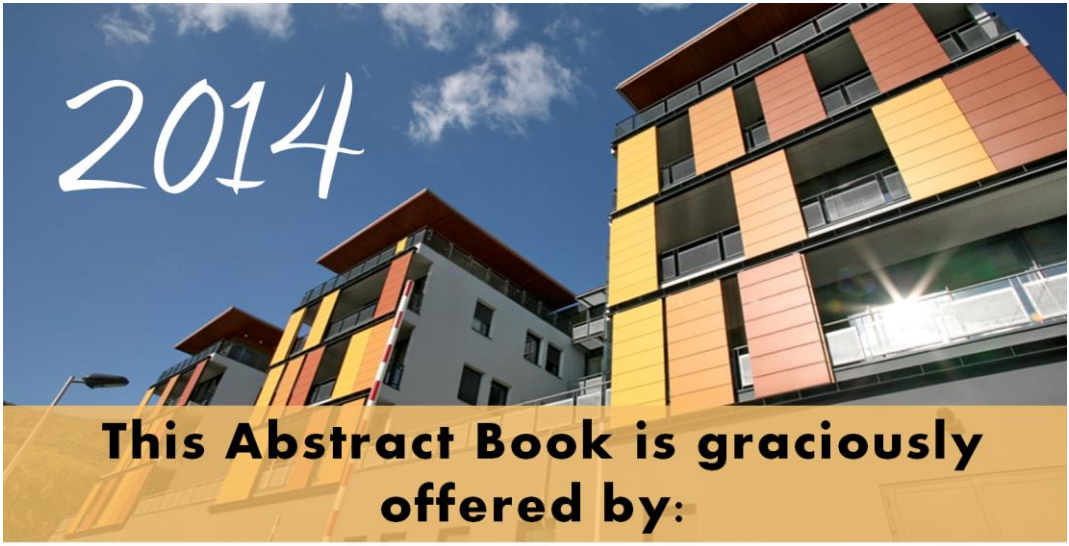
Huntington Disease (HD) is a devastating mid-life onset inherited neurodegenerative disorder. The defective gene in HD contains an unstable trinucleotide CAG repeat which encodes a polyglutamine stretch (polyQ) in the corresponding protein huntingtin (HTT). When the gene encodes a protein with more than 35 glutamines, it triggers a neuronal death preferentially in the striatum and the cortex. HTT is widely expressed and involved in numerous functions, including fast axonal transport. Palmitoylation is a reversible post-translational modification of cysteine residues with a lipid palmitate, which has recently emerged as an important mechanism for regulating protein function and location. Palmitoylation is catalyzed by 23 Palmitoyl-acyl-transferase (PATs) in mammalian. Two PATs are specific of palmitoylation of HTT: Huntingtin-Interactor-Protein 14 (HIP14 or DHHC17) and HIP14Like (HIP14L or DHHC13). HIP14 is dysfunctional in presence of mutation HD, and HIP14 and HIP14L-deficient mice develop features of HD. HIP14 is dysfunctional in presence of mutation HD, and HIP14 and HIP14L-deficient mice develop features of HD. In this study, we will assess the role of HTT palmitoylation at cysteine 214 on HTT vesicular localization and fast axonal transport in vitro and in vivo. To do this, we have set-up a protocol allowing the analyses of FAT in micro-fluidically isolated cortical neurons and in drosophila wings. We have generated a non-palmitoylable HTT (C214A) and analyzed the localization of HTT at vesicles and the effect on FAT. Our preliminary data suggest that palmitoylation is important for the vesicular targeting of HTT and for its role in axonal transport.

Key words: Huntingtin, Fast Axonal Transport, Palmitoylation, HIP14, HIP14L

NOTES

NOTES

NOTES



This Abstract Book is graciously offered by: